Challenges and Solutions for the Next Generation of Vaccines:
Development of Cell Culture-based Live Attenuated Influenza Vaccine

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Influenza

A Major health problem worldwide, each year
- 5-15% of the population are affected with upper respiratory tract infections
- 3-5 million cases of severe illness
- 250,000 – 500,000 deaths

Pandemic and seasonal flu

Vaccination - the principal measure to prevent the disease and reduce the impact

Caused by influenza virus
- 8 segmented negative sense RNA genome
- encode 11 proteins including 2 surface antigens: HA and NA
- Antigenic differences in HA and NA determine virus type (influenza A viruses) and lineage (influenza B viruses)

http://www.who.int/mediacentre/factsheets/fs211/en/index.html

Kaiser et al., Science, Vol 312, p. 380
FluMist® (Influenza Virus Vaccine Live, Intranasal)

- Cold adapted, live attenuated vaccine
- Innovative technology (nasal administration)
- Antigen sparing (high yield)
- Durable mucosal and systemic immunity
- High efficacy*

*Belches et al., NEJM, 356 (7), p. 685-696
Manufacture of FluMist® Influenza Vaccines

Wt

Donor strain
ca A/Ann Arbor/6/60 or
cb B/Ann Arbor/1/66
(MedImmune)

Selection (1/2⁸ or 1/256) Cloning

6:2 reassortant
vaccine

Transfection

Plasmids
Containing genes for vRNA

PB1 NP
PB2 M
PA NS
MDV WT

HA
NA

Manufacture

Eggs

Blending of three strains (ca, ts, att)

FluMist®
(Influenza Virus Vaccine Live, Intranasal)
Old and New Influenza Vaccine Production Technologies

- Challenges with *egg production platform*
  - Egg stock vulnerability
  - Production capacity limited
  - Less defined biological starting material and significant operator intervention
  - Egg allergies

- Advantages of *cell culture-based production platform*
  - Susceptibility to a broad spectrum of influenza virus strains
  - Better defined production substrate
  - More controlled manufacturing process sequestered from the environment
  - Surge capacity flexibility
  - Rapid scale-up
Production Cell:

**Madin-Darby Canine Kidney (MDCK) cells**
Selection of Host Cell for Cell Culture Flu Vaccine Production

- Thirteen cell lines (9 mammalian and 4 avian host cells) were tested
  - MRC-5, WI-38, VERO, FRhL-2, 293, NIH 3T3, CHO, MDCK and other human cell lines
  - CEF, CEK, DF-1 and avian embryonic stem cell line

- Only Vero and MDCK cells produced viruses >6.0 log_{10} TCID_{50}/ml for FluMist strains

- Only MDCK cells produced viruses with titer >7.0 log_{10} TCID_{50}/ml for all types and families of seasonal strains tested
Challenges Related with Use of MDCK cells for Vaccine Production

- Original line of MDCK cells was non-tumorigenic
- Some MDCK derivatives have been found to be highly tumorigenic
- Highly tumorigenic cell substrates have never been used to manufacture viral vaccines
- Highly tumorigenic cell substrates pose significant regulatory challenges

Krause, VRBPAC 2005

Product Safety and Regulatory Concerns!
MedImmune’s Approach to Minimize Risk of MDCK Cells

- Produced cell bank with low tumorigenic potential from biologically cloned cells

- Extensive testing strategy developed. Process developed to deliver vaccine with the following characteristics:
  - Sterile product
  - Acellular
  - Reduction of host cell DNA quantity
  - Reduction of host cell DNA size
  - Minimal exposure to animal derived components (ADCs)

- with guidance from CBER for:
  - Adventitious agents
  - Tumorigenicity of live, intact cells
  - Oncogenicity of host cell DNA and lysate
Produced Low Tumorigenic Cell Bank

- Development of Serum-free MDCK Cells

- Numerous in-house SFM formulations developed

- Cells evaluated for several primary factors
  - Maintenance of cell line growth
  - Potency (influenza virus yield)
  - Karyology
  - Tumorigenicity in athymic nude mouse model
## Tumorigenicity of $10^7$ Uncloned MDCK Cells in Adult Nude Mice

<table>
<thead>
<tr>
<th>Treatment Group (n=10)</th>
<th>Animals with Tumors</th>
<th>Tumor Formation Rate</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDCK Cells in Serum medium</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>MDCK Cells in SFM A</td>
<td>6</td>
<td>60%</td>
<td>tumors at injection site</td>
</tr>
<tr>
<td>MDCK Cells in SFM B</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Negative Cell Control</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Positive Cell Control</td>
<td>10</td>
<td>100%</td>
<td>tumors at injection site</td>
</tr>
</tbody>
</table>
Produced Low Tumorigenic Cell Bank

- MDCK Cells Cloning

- Began after development of SFM that maintained cell karyology and low potential tumorigenic nature of the ATCC MDCK cells
- Initiated from a new vial of ATCC MDCK cells
- Performed in serum-containing media
- Two rounds of limiting dilution cloning completed
- Clones initially selected based on productivity
### Initial Cloning of MDCK Cells

#### Cloning Data

<table>
<thead>
<tr>
<th>Range</th>
<th>&lt; 7.6</th>
<th>7.6-7.9</th>
<th>8.0</th>
<th>8.1</th>
<th>8.2</th>
<th>8.3</th>
<th>8.4</th>
<th>8.5</th>
<th>8.6-8.8</th>
<th>&lt; 7.6 to 8.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone No.</td>
<td>1014</td>
<td>105</td>
<td>38</td>
<td>35</td>
<td>16</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1228</td>
</tr>
<tr>
<td>Percentage</td>
<td>82.6%</td>
<td>8.6%</td>
<td>3.1%</td>
<td>2.9%</td>
<td>1.3%</td>
<td>0.9%</td>
<td>0.5%</td>
<td>0.1%</td>
<td>0.2%</td>
<td>100%</td>
</tr>
</tbody>
</table>

#### Graph

- **X-axis:** Range of Virus Titer (log FFU/mL)
- **Y-axis:** Number of Clones

- The graph shows the distribution of clones across different ranges of virus titer.
- The range < 7.6 has the highest number of clones, followed by the range 7.6-7.9.
- The percentage of clones for each range is also indicated in the table.
Not All High-Producing Clones in SFM Maintained Cell Growth

- Gradually reduced growth rate in SFM
- No longer growing after 11 passages
- Eliminated from clone selection

![Graph showing cell density over cell passage number for MDCK Subclone A](Image)
# Tumorigenicity and Oncogenicity of Cloned MDCK Cells

<table>
<thead>
<tr>
<th>Study</th>
<th>Test Sample</th>
<th>Animal with Tumors/Total Animals</th>
<th>Location of Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumorigenicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>adult nude mice</strong></td>
<td>DPBS</td>
<td>2/33</td>
<td>lymph node, spleen, liver</td>
</tr>
<tr>
<td></td>
<td>Hela cells</td>
<td>38/41</td>
<td>site of injection</td>
</tr>
<tr>
<td></td>
<td>$10^5$ MDCK cells</td>
<td>1/44*</td>
<td>spleen, liver, lung</td>
</tr>
<tr>
<td></td>
<td>$10^1$, $10^3$, $10^7$ MDCK cells</td>
<td>0/132</td>
<td></td>
</tr>
<tr>
<td><strong>Tumorigenicity</strong></td>
<td>Hela cells</td>
<td>44/44</td>
<td>site of injection</td>
</tr>
<tr>
<td><strong>NB nude mice</strong></td>
<td>$10^1$, $10^3$, $10^5$, $10^7$ MDCK cells</td>
<td>0/176</td>
<td></td>
</tr>
</tbody>
</table>

* tumors confirmed by histology examination, not sharing MDCK cell morphology, not located at SOI and not related with MDCK cells as verified by immunohistochemistry analysis

<table>
<thead>
<tr>
<th>Test Samples</th>
<th>Newborn Animals</th>
<th>non-injected</th>
<th>Saline</th>
<th>MDCK cell lysate</th>
<th>MDCK cell DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDCK Cell Lysate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Oncogenicity</td>
<td>mice</td>
<td>1/25</td>
<td>0/45</td>
<td>0/45</td>
<td>n.a.</td>
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<tr>
<td></td>
<td>hamsters</td>
<td>0/25</td>
<td>0/45</td>
<td>0/45</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>rats</td>
<td>0/25</td>
<td>0/45</td>
<td>1/45*</td>
<td>n.a.</td>
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<tr>
<td><strong>MDCK Cell DNA</strong></td>
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<tr>
<td>Oncogenicity</td>
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<td>rats</td>
<td>0/25</td>
<td>1/45</td>
<td>n.a.</td>
<td>0/45</td>
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</table>
Production Platform:

A Flu Vaccine Manufacture Process without Extensive Process Development
Challenges Related with Annual Flu Vaccine Production

<table>
<thead>
<tr>
<th>Surveillance</th>
<th>JAN</th>
<th>FEB</th>
<th>MAR</th>
<th>APR</th>
<th>MAY</th>
<th>JUN</th>
<th>JUL</th>
<th>AUG</th>
<th>SEP</th>
<th>OCT</th>
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<th>DEC</th>
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<tbody>
<tr>
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<td>Distribution</td>
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<td>Immunization</td>
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</tbody>
</table>

### Virus Strain

**A (H3N2)**
- A/Guizhou/54/1989
- A/Beijing/253/1989
- A/Beijing/32/1992
- A/Beijing/39/1993
- A/Shandong/32/1992
- A/Johannesburg/33/1994
- A/Wuhan/359/1995
- A/Sydney/5/1997
- A/Shanghai/10/1999
- A/Fujian/411/2002
- A/California/7/2004
- A/Wisconsin/67/2005
- A/Brisbane/10/2007 like

**A (H1N1)**
- A/Singapore/6/1986
- A/Bayern/7/1995
- A/Beijing/262/1995
- A/New Caledonia/20/1999
- A/Solomon Islands/3/2006
- A/Brisbane/99/2007 like

**B**
- B/Yamagata/16/1988
- B/Panama/45/1990
- B/Beijing/184/1993
- B/Sichuan/379/1995
- B/Hong Kong/330/2001
- B/Shanghai/361/2002
- B/Malaysia/2506/2004
- B/Florida/4/2006

- Frequent vaccine strains and manufacture process changes
- Short (often 1-3 weeks) Process Development Time
MedImmune’s Approach to Address Process Development Challenges

- Understand process parameters to all strains
- Develop a true “platform process” capable of production of all strains
- Conduct DOE and reduce pre-production PD time to approx. 2-4 weeks
Time of Infection and Virus Yield

**Virus:** A/Wisconsin/67/05

Data collected using shake flasks

Virus titer estimated using FFA and viable cell density estimated using nuclei counts

Error bars correspond to assay replicates and biological replicates

Peak titer increases with the time of infection (e.g., 4dps vs 3dps) irrespective of the virus input at the time of infection

Trends are similar among different sub-types
Input Virus and Virus Yield

Virus: A/Wyoming/03/03

Data collected using shake flasks

Virus titer estimated using FFA and viable cell density estimated using nuclei counts

Error bars correspond to assay replicates

Lower virus input improves virus titer
Spread of Virus Productivity Using Phase I Platform Process

Virus titer (Log_{10} FFU/ml)

B strains

H1N1

H3N2

Pandemic

Cold adapted vaccine strains
Risks Posed by Virus Productivity Variation

Cold adapted vaccine strains

Doses/ml increases

~ 60 doses/ml

~ 1 dose/ml
Improvement in Process Performance and Robustness

Pre-optimization

Post-optimization

Cold adapted vaccine strains

Average productivity improvement = 0.5 log$_{10}$FFU/ml

Lowest virus titer observed 7.9 log$_{10}$FFU/ml (up from 7.1 log$_{10}$FFU/ml)

Reduced variability in yield, increasing process robustness
Fully Disposable Small Scale Platform Process

- Fully disposable process implemented in GMP Pilot Plant
  - No need for cleaning/validation with disposable culture vessels
  - Shortened timeline for implementation in clinical production
- Quick turned-around between batches (in a few hours), making possible to re-start production very rapidly.
Projected Large-scale Manufacturing Process

- Vial Thaw
- Cell Factories
- 75 cm² Flask
- 225 cm² Flasks
- 50 L Bioreactor
- 500 L Bioreactor
- Production Bioreactor ~2000 L

Cell expansion
Virus production
Theoretical Bulk Vaccine Dose Output

<table>
<thead>
<tr>
<th>Purification Yield (%)</th>
<th>Number of Trivalent Doses (x10^6)</th>
<th>Harvest Titer (FFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.0</td>
<td>8.1</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
<td>58.7</td>
</tr>
<tr>
<td>20</td>
<td>78.2</td>
<td>98.5</td>
</tr>
<tr>
<td>35</td>
<td>97.8</td>
<td>123.1</td>
</tr>
</tbody>
</table>

Trivalent doses for 2x2000L bioreactors, 6 month campaign, 7.0 log\(_{10}\) FFU dose
Summary and Outlook

- With a proprietary serum-free medium and through biological cloning, MedImmune has prepared a MDCK cell bank that is shown to be
  - Low in tumorigenicity and oncogenicity
  - High in virus productivity for both pandemic and seasonal influenza vaccine strains

- By identifying and optimizing critical process parameters, MedImmune has developed a flu vaccine platform manufacture process that is
  - Highly productive for seasonal (H1N1, H3N2 and B) and pandemic (H5N1, H7N3, H9N2) virus strains with yield >8 log_{10} FFU/mL
  - Used to complete Phase I GMP clinical production campaigns successfully in spite of a last minute change in H1N1 virus

- Phase II Process Development is on-going with focuses on process scale up, robustness and shorter development time
Poster #27. Development of a Cell Culture Production Platform for Cold-Adapted Live Attenuated Influenza Vaccine (CAIV) Strains of FluMist®: Effects and Interactions of Medium Components, Trypsin, and Influenza Virus Family/Type in Process Productivity

Poster #28. Development of a Cell Culture Production Platform for Cold-Adapted live attenuated Influenza Vaccine (CAIV) strains of FluMist®: Accelerated Development of a Fully Disposable Phase I Clinical Manufacturing Process