MDCK as a substrate for influenza vaccine production

Solvay Pharmaceuticals
Presentation to VRBPAC
November 16, 2005
Agenda

- Background Solvay and influenza
- Background Solvay’s MDCK project
- Safety analysis of MDCK-based vaccine
- Conclusions
Solvay Pharmaceuticals

- Division of Solvay Group
- Global – top 40 pharma
- 12,800 employees
- 2004 sales: $2.8 billion
- Major R&D sites
  - Marietta, GA
  - Dijon and Paris, France
  - Hanover, Germany
  - Weesp, The Netherlands
Solvay Pharmaceuticals & Influenza

• Egg-based vaccine
  – First influenza vaccine in Europe (1950)
  – Uninterrupted supply for 55 years
  – Total > 250 million doses
  – Fourth supplier worldwide; over 50 countries
Solvay Pharmaceuticals & Influenza

US Headquarters
Marietta, GA

Influenza vaccine production facilities –
Weesp, The Netherlands

Countries supplied with Solvay’s egg-based influenza vaccine
Why a cell-based vaccine project?

- Eggs are open production system prone to contamination
- Availability of eggs is vulnerable to avian diseases
Why MDCK?

- Broad susceptibility to influenza viruses
- Substantial experience in influenza research and surveillance
- High virus yields
  - Economically feasible
  - Favorable ratio of virus to impurities
Solvay Pharmaceuticals’ MDCK-based influenza vaccine project

- More than 10 years experience
- Microcarrier, serum-free
- Preclinical and clinical development program
- License granted in The Netherlands
- Commercial scale facility
- License to be updated to commercial scale product, followed by worldwide licensing, including US
Solvay Pharmaceuticals’ MDCK-based vaccine facility

Solvay Pharmaceuticals, Weesp, The Netherlands
MDCK safety assessment

• Identify and quantify potential risks of cell line
• Quantify elimination of these potential risks by downstream processing (DSP)
• Quantify potential risks of cell substrate remaining for the vaccine recipient

CBER’s Defined-Risks Approach
MDCK safety assessment

1. Cell line characterization
2. Downstream processing
3. Final product safety
MDCK safety assessment

1. Cell line characterization
   a. Passage history
   b. Adventitious agents
   c. Tumorigenicity
1a. Cell characterization - History

- 1958: Isolation from dog kidney
- 1964: Deposition at American Type Culture Collection (ATCC)
- No introduction of Bovine Spongiform Encephalopathy-like agents
- 1991: Preparation of ATCC working stock
1a. Cell characterization - Passage history

- 0: Isolation from dog kidney
- 49: Deposition at American Type Culture Collection (ATCC)
- 52: Preparation of ATCC working stock
- 56: Preparation of Master Cell Bank
- 57: Preparation of Working Cell Bank

passages used for vaccine manufacture

- 97: Preparation of Extended Cell Bank

passages used for safety assessment
1b. Cell characterization - Adventitious agents

- Mycoplasmas
- Bacterial and fungal sterility
- *In vitro* co-cultivation in detector cell lines
- *In vivo* testing in various species
- Electron microscopy
- Retrovirus testing

No adventitious agents found in Solvay’s MDCK
1b. Cell characterization - Adventitious agents

- Specific testing for naturally-occurring canine viruses
- Specific testing for viruses to which MDCK is susceptible

No adventitious agents found in Solvay’s MDCK
1c. Cell characterization – Tumorigenicity

• Phenotypic characteristic; tumor formation in animal models

• Concern of exposure of vaccine recipient to
  – Intact cells
  – Cellular components
  – Residual cellular DNA

• Unlikely in vaccine recipient due to allograft rejection
1c. Cell characterization – Tumorigenicity

- **Tumorigenic potential of intact cells**
  - 4 week and 6 month study in adult immune-deficient nude mice

- **Tumorigenic potential of cell lysates**
  - 6 month study in adult and newborn nude mice, newborn rats and newborn hamsters

- **Oncogenic potential of DNA**
  - 6 month study in adult and newborn nude mice, newborn rats and newborn hamsters
## 1c. Cell characterization – Tumorigenicity

- **Intact cells**: 6 month study in 4 week nude mice

<table>
<thead>
<tr>
<th></th>
<th>Incidence</th>
<th>Nodule size (mm²)</th>
<th>Complete regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>40 days</td>
<td>6 mos</td>
</tr>
<tr>
<td>Negative control</td>
<td>0/26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$10^1$ MDCK, p98</td>
<td>0/26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$10^3$ MDCK, p98</td>
<td>0/26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$10^5$ MDCK, p98</td>
<td>18/26</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>$10^7$ MDCK, p98</td>
<td>30/30</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>$10^7$ MDCK, p56 (ATCC)</td>
<td>23/26</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Positive control (10^7 HeLa)</td>
<td>25/26</td>
<td>109</td>
<td>sac</td>
</tr>
</tbody>
</table>
1c. Cell characterization – Tumorigenicity

- **Intact cells**: 6 month study in 4 week nude mice

<table>
<thead>
<tr>
<th></th>
<th>Nodule at injection site</th>
<th>Tumor at injection site (MDCK adenocarcinoma)</th>
<th>Tumor in spleen (hystiocytic tumor)</th>
<th>Tumor in lung (adenoma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0/26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10^1 MDCK, p98</td>
<td>0/26</td>
<td>-</td>
<td>1*</td>
<td>-</td>
</tr>
<tr>
<td>10^3 MDCK, p98</td>
<td>0/26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10^5 MDCK, p98</td>
<td>18/26</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10^7 MDCK, p98</td>
<td>30/30</td>
<td>16</td>
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<td>1</td>
</tr>
<tr>
<td>10^7 MDCK, p56 (ATCC)</td>
<td>23/26</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

* Non-MDCK
# 1c. Cell characterization – Tumorigenicity

- **Lysate of $10^7$ cells:** 6 month oncogenicity study

<table>
<thead>
<tr>
<th>Test group</th>
<th>N</th>
<th>Nodules at injection site</th>
<th>Tumors in other tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4w + 0-4d nude mice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cell lysate</td>
<td>68</td>
<td>0</td>
<td>0</td>
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<tr>
<td>neg. controls</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>0-7d hamsters</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>cell lysate</td>
<td>98</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>neg. controls</td>
<td>54</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>0-7d rats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cell lysate</td>
<td>91</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>neg. controls</td>
<td>58</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
# 1c. Cell characterization – Tumorigenicity

- **0.1mg MDCK-DNA**: 6 month oncogenicity study

<table>
<thead>
<tr>
<th>Test group</th>
<th>N</th>
<th>Nodules at injection site</th>
<th>Tumors in other tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>4w + 0-4d nude mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1mg DNA</td>
<td>48</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>neg. controls</td>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0-7d hamsters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1mg DNA</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>neg. controls</td>
<td>48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0-7d rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1mg DNA</td>
<td>69</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>neg. controls</td>
<td>39</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
1c. Cell characterization – Tumorigenicity

• Characterization of tumors in DNA study
  – Histopathology
    • One mouse: histiocytic tumor in liver
    • One mouse: lymphoma in thoracic cavity
  – Spontaneous tumors not unexpected in immune-deficient nude mice
1c. Cell characterization – Tumorigenicity

Follow-up studies with MDCK cell DNA

- Larger study in nude mice
- Fetal and neonatal safety study in rats
1c. Cell characterization – Tumorigenicity summary

- Moderate tumorigenic potential in immune-deficient animals shown at dose levels $\geq 10^5$
- Majority of nodules partially regress; complete regression in 5/25 at $10^5$ and in 4/28 at $10^7$
- Increases with passage level and/or adaptation to serum-free growth
- Histopathology at passage 98 is in agreement with the literature for MDCK cells
1c. Cell characterization – Tumorigenicity summary

- Tumorigenic potential not observed in immune-competent animals
- Lysates of $10^7$ MDCK cells not tumorigenic
- 0.1 mg MDCK-DNA not considered oncogenic; confirmative studies initiated
MDCK safety assessment

1. Cell line characterization

2. Downstream processing (DSP)
   - Elimination of intact cells
   - Elimination of cellular DNA
MDCK-based vaccine production
2a. DSP - Elimination of intact cells

- MDCK from WCB
- Cell Production
- Virus Production
- DNA digestion
- Homogenization
- Centrifugation
- Inactivation
- DNA digestion

- Chromatography
- Detergent treatment
- Ultracentrifugation
- 0.22μ filtration
- Monovalent
- 0.22μ filtration
- Trivalent
- 0.22μ filtration
- Filling
## 2a. DSP - Elimination of intact cells

### Intact cells

10 log clearance factor

<table>
<thead>
<tr>
<th></th>
<th>Pilot scale</th>
<th>Production scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenization</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Centrifugation</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Detergent treatment</td>
<td>≥3.6</td>
<td>Validation ongoing</td>
</tr>
<tr>
<td>Ultracentrifugation</td>
<td>≥3.6</td>
<td></td>
</tr>
<tr>
<td>0.22μ filtration x3</td>
<td>≥3.6</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>≥ 21.4</td>
<td></td>
</tr>
</tbody>
</table>
2b. DSP - Elimination of DNA
## 2b. DSP - Elimination of DNA

### DNA content clearance factor

<table>
<thead>
<tr>
<th>Step</th>
<th>Pilot scale</th>
<th>Production scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA digestion 1</td>
<td>51</td>
<td>Validation ongoing</td>
</tr>
<tr>
<td>DNA digestion 2</td>
<td>88</td>
<td>(including content and size)</td>
</tr>
<tr>
<td>Detergent treatment/ Ultracentrifugation</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td><strong>Total clearance</strong></td>
<td><strong>760,000</strong></td>
<td></td>
</tr>
</tbody>
</table>

**specification: < 10 nanograms per dose**
2. DSP - Summary

- Adequate purification and testing warrants vaccine safety
- Solvay is committed to follow the latest scientific insights as well as regulatory guidance
MDCK safety assessment

1. Cell line characterization
2. Downstream processing
3. Final product safety
   - Pre-clinical experience
   - Clinical experience
### MDCK-based vaccine – Pre-clinical experience

<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Route</th>
<th>Dose (µg HA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local tolerance</td>
<td>rat, rabbit</td>
<td>s.c. / i.m. / i.v.</td>
<td>45</td>
</tr>
<tr>
<td>Systemic toxicity</td>
<td>rat, rabbit</td>
<td>s.c.</td>
<td>45, 75, 450</td>
</tr>
<tr>
<td>Pyrogenicity</td>
<td>rabbit</td>
<td>i.v.</td>
<td>45</td>
</tr>
<tr>
<td>Mutagenic potential</td>
<td>mouse</td>
<td>s.c.</td>
<td>45</td>
</tr>
<tr>
<td>Active and passive anaphylaxis</td>
<td>guinea pig</td>
<td>s.c. for induction i.v. for challenge</td>
<td>45</td>
</tr>
</tbody>
</table>
MDCK-based vaccine – Pre-clinical experience

- Local tolerance
  - No local irritation
- Systemic toxicity
  - No adverse effects
- Pyrogenicity
  - No distinct increase in body temp

- Mutagenicity
  - No increase in number of micronuclei
- Anaphylaxis
  - No active anaphylaxis
  - Passive anaphylaxis favorable to egg-based
MDCK-based vaccine – Clinical experience

• 14 randomized, double-blind studies
• 1,023 subjects on MDCK-based subunit vaccine
• Population: 18-60, over 60, patients at-risk for complications for influenza, atopic patients and subjects with an egg-allergy
• Major objectives: Show immunogenic non-inferiority and comparable safety to existing egg-based subunit influenza vaccine
MDCK-based vaccine – Clinical experience

- Local and systemic reactogenicity profile is comparable to egg-based vaccine
- Reactions are minor and short-lived
- No unexpected safety findings
- Non-inferior in immunogenicity

MDCK-based vaccine has comparable safety and immunogenicity profile as egg-based vaccine
Conclusions

- Solvay is confident MDCK is a safe substrate for inactivated influenza vaccines.
- The use of MDCK will improve reliability of influenza vaccine supply, and enhance pandemic preparedness.
- Solvay will pursue licensing worldwide, including US.
Participants

- Iris de Bruijn, Ph.D.
  - Global Clinical Director, Influenza Vaccines
- Ed Geuns, Pharm.D.
  - Director, Regulatory Affairs
- Michael Hare, B.S.
  - Manager, Regulatory Affairs
- Alex Kersten, D.V.M.
  - Senior Scientist, Pre-Clinical Development
- Jeroen Medema, MSc.
  - Senior Scientist, Vaccines
- Peter Finn, MRCVS., FRCPath.
  - Consultant
- Michael Williams, B.S.
  - Vice President, Viral Products
  The Biologics Consulting Group
- Ruth Wolff, Ph.D.
  - Director, Therapeutics
  The Biologics Consulting Group
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