Advantages & Challenges of Vaccine Development in Insect Cells

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Novavax, Inc.
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Renaissance in the Development of New and Safer Vaccines

- Expanding US and World markets
  - Increased need for adult vaccines
- New unmet medical needs
  - HIV, bioterrorism, pandemic influenza
- Advances in Immunology
  - Innate immunity
  - Cellular immunity
  - New adjuvants
- Advances in expression systems
  - More complex vaccines: Virus-like Particles (VLPs)
- Advances in biopharmaceutical manufacturing
Baculovirus – Insect Cell Expression System

Brief Overview
Baculoviruses

- dsDNA, circular genome 133,894 bp
- Limited host range - Lepidoptera (butterflies and moths)
- Biological pesticides
  - 8 baculoviruses registered by the EPA
  - No reported human disease, hypersensitive, or allergies
- *Autographa californica* Nuclear Polyhedrosis Virus (AcMNPV)

AcMNPV OB

AcMNPV infected larvae
Insect Cell Lines

- **Sf9 and Hi 5**
  - Acceptable for the manufacture of human biologicals
  - Commercial License
    - Texas A&M – Sf9
    - Boyce Thompson Institute – Hi 5

![Spodoptera frugiperda](image1)

![Trichoplusia ni](image2)

![Ovarian Cells](image3)

![Egg Cells](image4)

![BV infected Sf9 Cells](image5)
# Genetic Engineering of Baculovirus

<table>
<thead>
<tr>
<th>Cloning methods</th>
<th>AcMNPV</th>
<th>Kits</th>
<th>rBV (%)</th>
<th>Speed (days)</th>
<th>GMP</th>
</tr>
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<tbody>
<tr>
<td>Recombination (original)</td>
<td>wt</td>
<td>No</td>
<td>~ 1%</td>
<td>10 – 15</td>
<td>Yes</td>
</tr>
<tr>
<td>Direct Insertion (linear DNA)</td>
<td>modified</td>
<td>Yes</td>
<td>&gt;90%</td>
<td>10 – 15</td>
<td>Yes</td>
</tr>
<tr>
<td>Transposition in E.coli or Sf9</td>
<td>modified</td>
<td>Yes</td>
<td>100%</td>
<td>5 - 10</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Recombinant AcMNPV**
- Enveloped nucleocapids
- 60 x 330 nm

**Master Seed**
- Sf9 Cells
- Serum – free media
- 10⁸ – 10⁹ pfu/ml
- Stored: 5°C or -80°C
Baculovirus – Insect Cell Expression System

➢ Summary
  – Eukaryotic expression system used to express 1,000s of genes
  – Multiple genes can be expressed
  – Competitive yields
  – Correct protein folding
  – Does not replicate in human or mammalian cells
  – No identified allergens
  – Sf9 and Hi 5 cells are non-tumorgenic
  – Only a few insect-vectored viruses (arborviruses) replicate

➢ Major limitation
  – N-glycosylation pathway is different in insects compared to higher eukaryotes
**N-glycosylation in Sf9 Insect Cells**

- Lack of sialylo and glucosyl transferases
- Lack of the transferases to produce glycoalergens
  - 1,2-xylose and 1,3-fucose in plants and some invertebrates
  - 1,6-fucose in mammalian and Sf9 cells

Mammalian: Complex N-Linked

Insect: Paucimannose
Production of Influenza VLPs in Insect Cells
Why Recombinant Influenza VLP Vaccine

- No eggs
- No pathogenic virus in manufacturing
- Controlled cell culture process
- Safety
  - No Serum
  - No Protein
- Exact genetic match
- SRID potency assay validated for recombinant VLPs
- Improved immunogenicity of flu VLPs without adjuvants
- Speed from strain selection to manufacturing is weeks
Influenza Virus

- Enveloped; segmented, negative stranded RNA virus
- Surface hemagglutinin (HA) and neuraminidase (NA) spike glycoproteins
- Matrix (M1) is the major capsid protein
- Influenza Virions:
  - M1 helical capsid
  - HA and NA spikes in lipid bilayer envelope
  - Pleomorphic
Cloning Strategy to Produce an Influenza Virus-like Particle (VLP) Vaccine

- HA, NA, and M1 genes cloned into Tandem rBV
  - Infection with single cloned rBV – Poisson distribution
  - Mixed infection with multiple rBV – Cells unevenly infected
- Each gene within its own expression cassette
  - Polyhedrin promoter
  - Poly(A) termination signal
Cloning and Expression of Influenza VLPs

**RNA Sequence**
- HA, NA, M1

**DNA Synthesis**
- Codon Optimized

**Cloned Genes**
- HA
- NA
- M1

**Influenza Virus**
- Plaque Isolate

**rBaculovirus**

**Bacmid DNA**

**Tandem Vector**

**Master Virus Seed**

**VLP Manufacturing**

**100 nm**
Kinetics of Secretion of Influenza (H5N1) VLPs in Sf9 Cells

<table>
<thead>
<tr>
<th>Std</th>
<th>Sf9</th>
<th>20</th>
<th>26</th>
<th>30</th>
<th>44</th>
<th>48</th>
<th>72</th>
<th>hours</th>
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<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>24</td>
<td>36</td>
<td>48</td>
<td>60</td>
<td>72</td>
<td>96</td>
<td></td>
</tr>
</tbody>
</table>

Flu BV

HA gp64

p39

M1

p10

Baculovirus VLPs

Hours post infection

NOVAVAX
Sucrose Gradient Purification of Enveloped VLPs

- Buoyant density in sucrose
  - BV = 1.16 – 1.17 g/ml
  - VLPs = 1.14 – 1.15 g/ml

- Sucrose gradient purified VLPs ~ 50% pure

- Novavax has developed chromatographic procedures for the purification of influenza and other enveloped VLPs to >90% pure
Chromatography Purified Influenza A/Indonesia/5/05 (H5N1) VLPs

<table>
<thead>
<tr>
<th>Reference rHA</th>
<th>100L Batch</th>
<th>100 L Batch</th>
</tr>
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<tbody>
<tr>
<td>HA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Batch Consistency of A/Indonesia/5/05 (H5N1) VLPs

### Table of Band MW, IntOD, and % Purity

<table>
<thead>
<tr>
<th>Band</th>
<th>MW</th>
<th>IntOD</th>
<th>% Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 HA</td>
<td>68.4</td>
<td>3.79365</td>
<td>40.64%</td>
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<tr>
<td>2 NA</td>
<td>54.4</td>
<td>1.83645</td>
<td>19.67%</td>
</tr>
<tr>
<td>3 M1</td>
<td>26.7</td>
<td>3.38801</td>
<td>36.29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>96.61%</strong></td>
</tr>
<tr>
<td>1 HA</td>
<td>69.2</td>
<td>3.71364</td>
<td>41.52%</td>
</tr>
<tr>
<td>2 NA</td>
<td>54.4</td>
<td>1.74567</td>
<td>19.52%</td>
</tr>
<tr>
<td>3 M1</td>
<td>27</td>
<td>3.37161</td>
<td>37.69%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>98.72%</strong></td>
</tr>
<tr>
<td>1 HA</td>
<td>68.7</td>
<td>5.25887</td>
<td>42.92%</td>
</tr>
<tr>
<td>2 NA</td>
<td>54.4</td>
<td>1.88479</td>
<td>15.38%</td>
</tr>
<tr>
<td>3 M1</td>
<td>27</td>
<td>4.49662</td>
<td>36.70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>95.00%</strong></td>
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</table>
Cryoelectron Microscopy of Pleomorphic VLPs

A/Indo H5N1 VLPs

Influenza virus pleiomorphy characterized by cryoelectron tomography

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Pre-clinical Immunogenicity H5N1 and H3N2 Influenza VLPs
Preclinical Influenza VLP Immunogenicity

- Mice and Ferret Challenge Models
  - 6 to 10 animals per group
  - IM or IN
  - Doses 15, 3.0, 0.6, and 0.12 µg HA (SRID)
  - Immunogens:
    - H5N1 and H3N2 VLPs
    - HA subunit
    - Whole inactivated virus (WIV)
  - Immunizations at weeks 0 and 3
HAI Antibody H5N1 A/Indo/5/05 VLP Vaccine IM (Mice)

HAI GMT (Log2)

<table>
<thead>
<tr>
<th>VLP Dose</th>
<th>Week 0</th>
<th>Week 3</th>
<th>Week 5</th>
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<tbody>
<tr>
<td>3ug</td>
<td>0/8</td>
<td>2/8</td>
<td>8/8</td>
</tr>
<tr>
<td>0.6ug</td>
<td>0/8</td>
<td>0/8</td>
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<tr>
<td>0.12ug</td>
<td>0/8</td>
<td>0/8</td>
<td>8/8</td>
</tr>
</tbody>
</table>

Protective threshold: 5

NOVAVAX
Anti-HA IgG Isotypes*
H3N2 A/Fujian/411/2002 VLP Vaccine IM (Mice)

*Week 5
H5N1 A/Indo VLP Vaccine Cross-strain Protection in Mice

- Cross-strain protection against virus challenge
  - Immunized 0 and 3 weeks
  - Low dose (0.6µg) VLPs
  - No adjuvant
  - Induced protective antibody and T-cell response
Cross-protection of Flu VLP Vaccine in Ferrets

A/Viet Nam/1203/2004 Challenge (10LD_{50})
Phase I/IIa Trial of Influenza H5N1 A/Indo/5/05 VLP Vaccine

- Blinded, dose – ranging
- Safety and Immunogenicity
- No adjuvant
- 230 young adults
- Initiated July 2007
Regulatory Considerations

- No specific FDA guidelines for products derived from the baculovirus – insect cells

- Quality assessment based on most recent recommendations with specific considerations for:
  
  - Cell banks and Virus seeds: Spiroplasma, adventitious agents (arboviruses)
  
  - Harvest and purification process: removal of insect cell and baculovirus proteins and cell DNA and baculovirus clearance or inactivation

- Drug Substance and Drug Product: standard physical, biological, and safety tests
## Human Vaccines made in Insect Cells

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Type</th>
<th>Product</th>
<th>Company</th>
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</thead>
<tbody>
<tr>
<td>Cervical Cancer - Human Papillomavirus Virus (HPV)</td>
<td>VLP non-enveloped</td>
<td>Cervarix™</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>Influenza Seasonal Vaccine</td>
<td>rHA subunit</td>
<td>Phase III</td>
<td>Protein Sciences</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Subunit ex vivo</td>
<td>Phase III</td>
<td>Dendrion</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>Subunit ex vivo</td>
<td>Phase I/II</td>
<td>Favrille</td>
</tr>
<tr>
<td>Influenza pre-pandemic H5N1</td>
<td>HA-NA-M1 VLP</td>
<td>Phase I/II</td>
<td>Novavax</td>
</tr>
</tbody>
</table>
SUMMARY

Baculovirus – Insect Cell Expression System

- Mature Technology
  - 25 years in development
  - 2007 licensed human product

- Safe
  - No human pathogens
  - Not tumorigenic
  - No reported allergens

- Rapid (weeks)

- Efficient production enveloped VLPs

- Trials of Influenza pre-pandemic influenza VLP vaccine – 2007

- Trials of Influenza trivalent seasonal flu VLP vaccine – 2008