Recombinant protein vaccines produced in insect cells
BREAKING NEWS 16JAN2013

- FDA approves Flublok for the prevention of influenza in adults 18 - 49 years old

“The Evolution, and Revolution, of Flu Vaccines”
http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm336267.htm

- First recombinant influenza vaccine
- The pandemic solution

Only pandemic vaccine that can be quickly manufactured and/or transferred to and manufactured in other countries
Fault was not with the execution of the response, but in inherent shortcomings of current technologies for development & production of influenza vaccines”

All US influenza vaccines are currently produced in embryoed chicken eggs:
- Delayed response time
- Limited capacity
- Limited flexibility

PCAST recommended short-term improvements in surveillance, strain development, testing & fill/finish.

“The greatest potential for substantially shortening the time and increasing the reliability of influenza vaccine production lies in the use of recombinant DNA technologies” - PCAST
Topics:

- Baculovirus Technology Platform (BEVS)
- Flublok and Pandemic Influenza
- Other vaccines opportunities
- Conclusions
Baculovirus Technology Platform (BEVS)
BEVS Technology
“Enabling products where speed, cost and safety matter”

Baculovirus Expression Vector System (BEVS)

- Engineer baculovirus with the gene of interest (e.g. Hemagglutinin)
- Baculoviruses highly specific to insect cells
- Powerful promoter generates high yield of protein of interest
- Culture expression of insect cells in a fermenter
- Infect cells with engineered virus
- Incubate infection for ~48 - 72 hours
- Protein forms rosettes
- Purify protein to > 90% into final product
- Formulate with PBS into vaccine

Flublok® Approval → Validation
Virus to Transfer Vector – 3 Days

Influenza Virus

RNA Extraction

RT-PCR of Full Length HA

Ligation into Transfer Plasmid

Transformation into *E. coli*

Colony PCR

Plasmid Preps

Restriction Analysis

Sequencing

Recombinant Baculovirus Construction

**Influenza Virus**

10 Plasmids per rHA

Full-Length cDNA

Transfer plasmid

Polyhedrin promoter

Processing Signals

PCR-direct cloning

Full Length HA gene

**J Invertebr Pathol 2011 S31-41.**
<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfer Vector Preparation</td>
<td>From influenza virus to rHAs gene in transfer plasmid.</td>
<td>1 days</td>
</tr>
<tr>
<td>Recombinant baculovirus construction.</td>
<td>From transfer plasmid to 300 ml of P3 recombinant baculovirus.</td>
<td>19 days</td>
</tr>
<tr>
<td>Clone selection. Virus bank generation.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus bank freeze down.</td>
<td>From P3 recombinant baculovirus to frozen vials.</td>
<td>1 day</td>
</tr>
<tr>
<td></td>
<td><strong>Material from PD to GMP manufacturing</strong></td>
<td></td>
</tr>
</tbody>
</table>
Universal Purification Process

1. seed
   → Fermentation

2. infect
   → Harvest

   Disk-stack centrifugation

   DNA removal
   → Purify

   Capture

   Purification

   TFF/Formulation

Depth Filtration

Clarification

Extraction
Flublok and Pandemic Influenza
Influenza Vaccine:  
HA = Major Surface Protein

- HA (*Hemagglutinin*):  
  Coat of the influenza virus  
  Antibodies against HA protect against influenza  
  Changes in HA require annual update of vaccine
## Summary Panblok development: Pandemic Flu Vaccine based on rHA

<table>
<thead>
<tr>
<th>Year</th>
<th>Details</th>
</tr>
</thead>
</table>
| 1998-2000| - NIAID conducted two clinical studies with rHA  
- 2 x90 mcg rHA induced reasonable immune response       |
| 2005     | - Revaccination study  
- Broad immunogenicity after a single dose in subject who previously received rHA |
- Significant dose sparing                                |
| 2012/2013| - Study PSC25: rHA in combination with SE only  
- Significant dose sparing                                 |

Note: UNM is also performing studies in Japan non-adjuvanted rHA; Alum did not provide benefit
Other Vaccine Opportunities
## Approved Vaccines for Human or Veterinary Use

<table>
<thead>
<tr>
<th>Disease</th>
<th>Brand name(s)</th>
<th>Originator</th>
<th>Protective Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VACCINES FOR HUMAN USE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>CERVARIX®</td>
<td>GSK</td>
<td>L1 protein</td>
</tr>
<tr>
<td>Influenza</td>
<td>FLUBLOK</td>
<td>Protein Sciences</td>
<td>HA</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>PROVENGE®</td>
<td>Dendreon</td>
<td>PSA</td>
</tr>
<tr>
<td><strong>VACCINES FOR VETERINARY USE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV2</td>
<td>Porcilis® PCV</td>
<td>Merck</td>
<td>PCV2 ORF2 protein</td>
</tr>
<tr>
<td>PCV2</td>
<td>CircoFLEX®</td>
<td>B. Ingelheim</td>
<td>PCV2 ORF2 protein</td>
</tr>
<tr>
<td>Classical swine fever</td>
<td>Porcilis Pesti®</td>
<td>Merck</td>
<td>E2 protein</td>
</tr>
</tbody>
</table>

_Adjusted from M.M.J. Cox/ Vaccine 30 (2012): 1759 -1766_
# Vaccines Candidates for Human Use in Clinical Development

<table>
<thead>
<tr>
<th>Disease</th>
<th>Protective Antigen</th>
<th>Originator</th>
<th>Development Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>GAD</td>
<td>Diamyd</td>
<td>Phase III</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>ORF 2</td>
<td>GSK</td>
<td>Phase II</td>
</tr>
<tr>
<td>Influenza</td>
<td>NA</td>
<td>Protein Sciences</td>
<td>Phase II</td>
</tr>
<tr>
<td>Influenza</td>
<td>HA/NA/M1</td>
<td>Novavax</td>
<td>Phase II</td>
</tr>
<tr>
<td>ParvovirusB-19</td>
<td>Parvovirus VLP</td>
<td>Meridian Life Sciences</td>
<td>Phase II</td>
</tr>
<tr>
<td>Influenza H5</td>
<td>HA</td>
<td>Protein Sciences</td>
<td>Phase I</td>
</tr>
<tr>
<td>Norwalk</td>
<td>Norwalk capsid VLP</td>
<td>Ligocyte</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

*Adjusted from M.M.J. Cox/ Vaccine 30 (2012): 1759 -1766*
## Antigen targets for recommended viral vaccines

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>ETIOLOGICAL AGENT</th>
<th>PROTECTIVE ANTIGEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>Hepatitis B Virus (HBV)</td>
<td>HbSAg</td>
</tr>
<tr>
<td>Polio</td>
<td>Polio Virus serotypes (types 1, 2 or 3).</td>
<td>VP1 and VP4</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Rotavirus</td>
<td>VP6, VP7 and major outer capsid protein</td>
</tr>
<tr>
<td>Measles</td>
<td>Measles virus (genus <em>Morbillivirus</em>, family <em>Paramyxoviridae</em>)</td>
<td>H and F proteins</td>
</tr>
<tr>
<td>Rubella</td>
<td>Rubella virus (= togavirus of the genus Rubivirus)</td>
<td>H, F, and N viral proteins</td>
</tr>
<tr>
<td>HPV</td>
<td>Human papilloma virus (&gt;100 subtypes)</td>
<td>E1</td>
</tr>
<tr>
<td>Japanese</td>
<td>Japanese Encephalitis (JE) virus</td>
<td>L1 structural protein</td>
</tr>
<tr>
<td>Encephalitis</td>
<td><em>Flaviviridae</em></td>
<td>Glycoprotein E</td>
</tr>
<tr>
<td>Yellow Fever</td>
<td>Yellow fever virus (<em>Flaviviridae</em>)</td>
<td>E, and E/NS1</td>
</tr>
<tr>
<td>Tick-Borne</td>
<td>Tick-borne encephalitis virus (<em>Flaviviridae</em>)</td>
<td>E and C</td>
</tr>
<tr>
<td>Encephalitis</td>
<td><em>(Flaviviridae)</em></td>
<td>Polyproteins</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Hepatitis A virus (HAV)</td>
<td>Polyproteins</td>
</tr>
<tr>
<td>Rabies</td>
<td>Rabies virus (RABV) (<em>Rhabdoviridae</em>)</td>
<td>Protein G</td>
</tr>
<tr>
<td>Mumps</td>
<td>Mumps virus (<em>Paramyxoviridae</em>)</td>
<td>Protein H and N</td>
</tr>
<tr>
<td>Influenza</td>
<td>Influenza virus <em>(Orthomyxoviridae)</em></td>
<td>HA; NA; HA, NA, M1 VLP</td>
</tr>
</tbody>
</table>

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Conclusions
Universal production process

**Working Virus Bank (WVB)**

**Single Cell Line SF+**

**“Universal Process”**
- Insect Cell culture
  - WVB is added
- Centrifugation
  - Pellet is solubilized
- Depth filtration
- Capture (IEX)
- Purification (HIC)
- Membrane Filtration
- Ultrafiltration
- Sterile Filtration

**“Plug & Play”**

**Recombinant baculovirus**

**Identify Protective Antigen**
Concluding Remarks

- Key advantage = universal “plug and play” process. The BEVS technology is a versatile rapid production technology to “tackle” emerging diseases of viral or parasitic origin (SARS, H5, Ebola).
- Safe vaccines can be produced fast & for low cost.
- Production facility can be multi-use to manufacture a broad range of products (disposable technology offers flexibility).
- Establishment of large scale manufacturing is high priority! (disposable technology may speed up this process).
- No need to handle live dangerous viruses.
And finally….

- FDA approval of Flublok validates this technology!
- We are working on the scale-up of our robust manufacturing process to ensure availability of this product in sufficient quantity!

This recombinant influenza vaccine became a reality thanks to support of contract HHSO100200900106C and perseverance of the Protein Sciences team!

…. and as Benjamin Franklin Stated: "Energy and persistence conquer all things."