Preparing for the Next Pandemic: Rapid “Novel” Influenza Vaccine Product

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FluBlok®

- First recombinant influenza vaccine
- First cell-based influenza vaccine in U.S.
- FDA licensure in 2010?
  - No additional safety or efficacy studies required – FDA letter 01/11/10
- The pandemic solution
  - Only pandemic vaccine that can be quickly manufactured and/or transferred to and manufactured in other countries
Topics

- Influenza Viruses and Vaccines
- BEVS Technology
- Insect Cells as Substrate for Vaccine Manufacturing
- Flublok: a Recombinant Influenza Vaccine
- Pandemic Influenza Vaccine: PanBlok
- Summary

The Influenza Viruses

- Orthomyxoviruses (Greek, myxa=mucus)
- Three types of influenza virus (A, B, and C)
  - A viruses
    - Divided into subtypes based on genetic and antigenic differences among surface proteins (HA & NA)
    - Current subtypes found in people are A(H1N1) and A(H3N2)
  - B viruses
    - No subtypes
  - C viruses
    - Cause mild respiratory illness
- Antigenic “Drift” of A and B viruses leads to epidemics every winter
- Antigenic “Shift” of A viruses leads to pandemics
  - 3x in the past 100 years
Production of influenza vaccine

Characteristics
- Trivalent vaccine: 2 A strains and 1 B strain
- Protection correlates with hemagglutinin (HA) antibodies

Production process:
- Chicken Embryo's
- Isolation of Virus
- Kill Virus
- Isolate virus proteins

Long production cycle
One egg = one dose
Production affected by Avian influenza outbreaks
Adaptation required
Adverse reactions
Less effective in the elderly

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
BEVS Technology
“Enabling products where speed, cost and safety matter”

Key Advantages of BEVS Technology

- Versatility
  - Produced > 1,000 proteins

- Speed
  - Single serum-free cell line for all products
  - Cloning in weeks vs. months

- Low cost
  - High yields in a low-cost proprietary media
  - High-density fermentation

- Safety
  - Reliable scale-up
  - Current scale 500L; others up to 5,000L

Insect Cell Substrate

Inherent Safety?

- Baculovirus
  - Daily exposure - typical serving of coleslaw contains 112 million polyhedra (each polyhedron contains multiple baculoviruses)\(^1\)
  - Limited Host Range (Lepidopteran Species of Insects)
  - Do NOT Replicate in Mammalian Cells

- Insect Cells
  - Virtually No Known Adventitious Agents Can Replicate in both Insect Cells and Mammalian Cells
  - Arboviruses are Rare Exceptions (West Nile Encephalitis)
  - Derived from Non-biting Insects – Low Adverse Events

Insect Cell-Produced Products & Regulatory Approval Status

Cervarix – First insect cell product licensed by FDA
- Papillomavirus vaccine
- Oct. 19, 2009 - Approved in U.S
- 2007 - Approved in EU & Australia

Provenge® - Prostate cancer treatment
- First cancer immunotherapy to be approved by the Agency
- Approved May 2010

Impact
- Removes a “barrier” for insect cell-based production platform from regulatory viewpoint

Insect Cell-Produced Products Approaching FDA Approval with PSC’s Assistance

- Glybera® - Lipoprotein Lipase Deficiency
  - Recombinant Adeno-Associated Virus (rAAV) -based gene therapy
  - Orphan disease indication
  - BLA filed January 11, 2010

- Diamyd® – Type I Diabetes Vaccine
  - Phase III studies ongoing in U.S. and Europe
  - Preservation of insulin secretion
  - Major partnership deal with J&J

- FluBlok - Influenza Vaccine
  - First non egg-based flu vaccine in U.S.
  - Under final review at FDA
Examples of Vaccines that are being Produced in Insect Cells

- **Human therapeutic & prophylactic vaccines**
  - SARS – Spike – entering Phase I
  - HIV
  - Norovirus – Phase I
  - Hepatitis B, C and E
  - West Nile
  - Malaria
  - Dengue
  - Marburg, Ebola

- **Veterinary vaccines**
  - PCV
  - Influenza (avian; porcine; horse)

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**SARS Vaccine**

S = Major Surface Antigen

Coronaviruses are large enveloped RNA viruses that infect mammals and birds

Target for vaccine: S - Spike glycoprotein (surface protein) = major antigen

Rationale:
- Veterinary vaccine development
- Key to Infection - ACE2 receptor binding
- Antibodies to S-Protein identified from SARS survivors neutralized the virus
**Major Influenza Surface Protein**

- **HA (Hemagglutinin):**
  - Coat of the influenza virus
  - Antibodies against HA protect against influenza
  - Changes in HA require annual update of vaccine

**Hemagglutinin properties**

- Trimeric integral membrane protein
- Cleavage of HA with host protease into HA1 and HA2 needed for fusion activity
- HA1 and HA2 linked by disulfide bonds
- Contains four antigenic sites (A, B, C, and D)
- Contains many glycosylation sites
- Hydrophobic transmembrane domain
Cloning of influenza HA gene

Downstream Process

Fermentation

Harvest

Extraction

Clarification

Capture

Purify

DNA removal

Purification

TFF/Formulation

Depth Filtration

Disk-stack centrifugation

2. Infect

1. Seed

Production

~ 4 wks
Safety & Immunogenicity of FluBlok
Potential Benefits (3x45µg rHA)

- Influenza rHA antigens are produced in insect cells – protein based vaccine with low endotoxin content
- rHA protein is highly purified and does **not** contain egg protein or other contaminants from eggs
- Selection or adaptation of influenza virus strains that produce at high levels in eggs is not required => the best genetic match
- Cloning, expression and manufacture of FluBlok within 2 months
- FluBlok does not require any embryonated chicken eggs
- Manufacturing of FluBlok does not require biocontainment facilities
- Manufacture of rHA does not include formalin inactivation or organic extraction procedures

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PanBlok:
Pandemic Flu Vaccine based on rHA

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<th>1997 Hong Kong “bird flu”</th>
<th>8 weeks from development to product</th>
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<td>FDA authorized immediate use</td>
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<td>200 healthcare workers &amp; researchers vaccinated</td>
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| Safety During Production | No need to grow or handle a live virus |

<table>
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<tr>
<th>Authenticity of Antigens</th>
<th>Antigen is exact match to natural H5N1 (or any other) virus</th>
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<td>No induced structural changes as occurs with reverse genetics</td>
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<th>Manufacturing</th>
<th>Any monoclonal antibody facility</th>
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<td>More than adequate existing capacity</td>
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NIAID-Sponsored Study by Drs. Topham & Treanor at University of Rochester

Serum Neutralizing (NT) Antibody Responses Following One or Two Doses of H5 Vaccine in Naïve Subjects or Following a Single Dose in H5 Vaccine-Primed Subjects

- Determine the ability of a clade 3 H5 Protein Sciences recombinant vaccine administered in 1998 to prime for immune responses to a subsequent clade 1 H5 subvirion vaccine in healthy adults
- Comparison of responses in H5 primed subjects to those of H5 naïve subjects

### Time-line for Vaccine Development

**Novel rH1 Vaccine**

- Receipt of virus
- Plaque isolation
- Freeze virus bank
- Start manufacturing
- Product release
- Start Australia study

**Development time of novel rH1 vaccine**

- Product release testing, i.e.:
  - Mycoplasma/spiroplasma: 30 days
  - Sterility: 14 days
  - General safety testing: 7 days

**Start commercial production**
Pandemic Vaccine Production

Insect Cell culture
Baculovirus is added
↓
Centrifugation
Pellet is solubilized
↓
Depth filtration
↓
Capture (IEX)
↓
Membrane Filtration
↓
Purification (HiC)
↓
Ultrafiltration
↓
Sterile Filtration

Mammalian Cell culture
Centrifugation
Supernatant is processed
↓
Depth filtration
↓
Capture
↓
(Membrane Filtration)
↓
Purification
↓
Ultrafiltration
↓
Sterile Filtration

Shortage of vaccine is unnecessary as there is adequate cell culture capacity available worldwide.

PanBlok: Clinical Studies (H5)

- Studies in Japan by UMN (Japanese Partner)
  - Phase 1/2 study: Completed
    - A/Vietnam w/o Alum
    - Conclusion: Alum offered no benefit
  - Phase 2 study: Ongoing
    - Two doses of A/Vietnam, followed by booster dose with A/Indo/H5
    - Two doses of unadjuvanted 135mcg meet EMEA license criteria
**PanBlok: Clinical Studies H5 (2)**

- Clinical study ongoing in the U.S. by PSC with BARDA support (Contract # HHS0100200900106C)
- Phase 1/2 study: Test H5 in combination with adjuvant (GLA/SE)
- Data expected: by end 2010

**Summary**

- FluBlok was tested in >3000 Subjects
- FluBlok can be produced much faster than the egg-based vaccine
- FluBlok may provide better protection against influenza for adults ≥ 65 yr (specifically ≥ 75yr)
- FluBlok may be approved in the U.S. in 2010
- PanBlok would address the need for large quantities of vaccine within short time.

**Next Steps**

- Scale-up manufacturing
- Test vaccine in children
- Alternative formulations (patch?)
Cell Substrate Issues

- Develop screens for (un)known viruses
  CODEHOP PCRs developed for the following virus families:
  - Ascoviridae
  - Iridoviridae
  - Densoviridae A, B, C and *Penaeus merguiensis* densovirus
  - Nodaviridae (*TNCL Virus*)
  - Tetraviridae
  Master, Working and End of Production cells were screened.

- RT Activity of insect cell substrate
  Co-cultivation assays, EM screening of (un) stressed cells, viral clearance studies, multiple PERT assays with variable results

FluBlok Outlook – 2010 and beyond

- FluBlok to market
  - Large scale manufacturing deal
  - Address remaining FDA cell substrate questions
  - FDA approval under traditional approval regulations expected in 2010
  - Filing for market authorization in EU and Australia in 2010
  - Licensing of FluBlok/PanBlok