Vaccine Production Strategies: Ensuring Alignment and Sustainability

Second WHO Consultation on Global Action Plan for Influenza Vaccines (GAP-II)

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“Bench to Bedside”
Considerations when investing in Influenza Vaccine Manufacturing Infrastructure

- Multiple new influenza vaccine products and manufacturing technologies are becoming available
  - This creates an opportunity to bypass outdated technology, as has been done with cellular telephone technology
- Investments should result in sustainable capabilities
  - Preparedness requires that facilities and personnel are kept busy making products
  - New “flexible manufacturing facility” technology, equipment and designs can be used to manufacture many different biologicals in the same facility
  - Use of disposables rather than traditional “stainless steel” fermenters reduces cleaning requirements, turn around time, and risk of contamination
Considerations when investing in Influenza Vaccine Manufacturing Infrastructure

• Standardization can facilitate technology transfer
  – Facilities built and equipped to standardized specifications may benefit from standardized manufacturing parameters and operating procedures

• Fill, finish and distribution are important manufacturing chokepoints
  – 10% – 30% of influenza vaccine cost per dose is associated with the fill/finish process
  – New fill and finish technologies such as “Blow-Fill-Seal” (BFS) can reduce cost, reduce overall manufacturing time, increase flexibility, and facilitate tracking/distribution
Egg-based Manufacturing Current “Gold Standard”

Time to First Trivalent Vaccine Lot after Strain Change

Week
0  2  4  6  8  10 12 14 16 18 20 22 24+

REFERENCE VIRUS
SEED VIRUS PREP
MONOVALENT PRODUCTION
TRIVALENT PRODUCTION
FILLING
VACCINE RELEASE
VACCINE DISTRIBUTION

less critical when using “gene to vaccine” technologies

From a presentation by Norman Baylor, US FDA/CBER
Examples of Influenza Vaccine products and manufacturing technologies

- **Products and Technologies requiring development of seed virus**
  - Egg-based manufacturing
    - Inactivated, split, purified subunit products
    - Live attenuated vaccines
  - Mammalian Cell-based manufacturing
    - Inactivated, split, purified subunit products
    - Live attenuated vaccines

- **Products which are manufactured from “gene to vaccine”**
  - Baculovirus
    - insect cell culture, whole larvae manufacturing
    - subunit versus VLP
  - Prokaryotic manufacturing (*E. coli*)
  - Recombinant Adenoviral-vectored influenza vaccines
  - Plant-based manufacturing (subunit versus VLP)
  - Peptide epitope products (computational vaccinology)
Baculovirus-based influenza antigen manufacturing processes (cell culture)
Protein Sciences, subunit rHA

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
Baculovirus-based influenza antigen manufacturing processes (cell culture)

Protein Sciences, subunit rHA

**FluBlok®**

- First recombinant influenza vaccine
- Nine clinical studies completed
- First cell-based influenza vaccine to be developed in U.S.
- FDA licensure in 2011
  - *No additional safety or efficacy studies required – FDA letter 01/11/10*

- One pandemic solution
  - A pandemic vaccine that can be quickly manufactured and/or transferred to and manufactured in other countries

**Not currently adapted to use flexible, disposable manufacturing technologies**
Baculovirus-based influenza antigen manufacturing processes (whole insect)
C-PERL, subunit rHA, rNA, rNP, rM2

High expression in vivo baculovirus system
- High expression levels
- Pre-occluded baculovirus technology
- Eukaryotic
- Automation
- Rigorous process control
- Linearly scalable
Baculovirus-based influenza antigen manufacturing processes
C-PERL, subunit rHA, rNA, rNP, rM2

- Eukaryotic
- Leverages powerful BEVS platform
- 11 years of operational experience
- Scalability with minimal optimization
- No process re-engineering during scale-up
- Current capacity to 200 kg biomass/ week
- Protein expression in 1 – 10 g/ kg range typical
- Often outperforms cell culture 10 – 100 X
- Difficult proteins more readily expressed (e.g., lipophilic)
- H1N1 A/California hemagglutinin
  - Baculovirus receipt to protein delivery: 314 hours
  - 150mg of H1N1 hemagglutinin protein (>99% pure)
- A/Perth hemagglutinin (His-tagged, untagged)
  - Gene sequence receipt to protein delivery: 52 days
  - >100 mg (>99% pure) tagged and untagged
- Over 40 influenza protein lots shipped to government customer
- High purity (>95%)
- Hemagglutinin, neuraminidase, matrix, nucleoprotein
E. coli-based influenza antigen manufacturing processes

VaxInnate’s Vaccine Technology

- Most viral vaccines are attenuated, inactivated or split viruses grown in eggs or cultured mammalian cells
- VaxInnate’s vaccines are highly purified recombinant proteins consisting of vaccine antigens genetically fused to a potent immune stimulator: the bacterial protein flagellin
- These constructs mimic the antigen presentation of a natural infection and elicit strong innate and adaptive immune responses at low doses.
- These vaccines can be produced by simple fermentation processes in bacteria with high capacity at low cost and in a very short time.
E. coli -based influenza antigen manufacturing processes

VAX125: A Seasonal Vaccine Based on A/SI/3/2006
Geometric mean HAI antibody titers to HA Solomon Islands by dose in 128 healthy adult subjects 18-49 years old

Efficiency of the Major Influenza Vaccine Manufacturing Processes

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Average Yield/L (net mg)</th>
<th>Est. dose (µg)</th>
<th>Required bioreactor runs*</th>
<th>Time in Months to Last Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>7</td>
<td>15</td>
<td>128</td>
<td>6-12</td>
</tr>
<tr>
<td>Cell culture</td>
<td>3</td>
<td>15</td>
<td>300</td>
<td>6</td>
</tr>
<tr>
<td>Baculovirus</td>
<td>45</td>
<td>45</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>Bacterial (Vaxinnate)</td>
<td>400</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*Assumes a 5,000L bioreactor to make 300 million doses

High-throughput, efficient manufacturing system, low-cost product

Optimal antibody response is obtained with 1.2 µg of vaccine

From Treanor et al. Vaccine 2010;28:2068-74
Recombinant Adenoviral-vectored Influenza Vaccines
Vaxin (intranasal)

**Cheaper**
- High yield (1000L produces 75M doses)
- Reliable manufacturing process
- Dramatically lower capital requirements ($25M v $1B)
- COGS 25-50% less than other recombinant vaccines
- Reduces biohazard waste

**Faster**
- 4 months vs. 8 months to manufacture
- Scalable for pandemic outbreaks
- Consistent manufacturing process

**Safer**
- No needles
- No live virus (FluMist®)
- No viral shed
- No influenza virus required
- No potential reassortment
- No eggs
- No egg allergies
- No need for medical personnel to administer
Recombinant Adenoviral-vectored Influenza Vaccines
Vaxin (intranasal)

-Two Phase I studies completed

| Vaxin's Intranasal Vaccines Versus Currently Marketed Vaccines and Newer Vaccines in Development | Killed (shots) | Live Attenuated (FluMist®) | Killed Produced in MDCK Cells¹ | Live Attenuated Produced in MDCKCells¹ | Recombinant | DNA Based | Virus Vectored | Vaxin |
|---|---|---|---|---|---|---|---|---|---|
| Propogation of Flu Virus | Yes | Yes | Yes | Yes | No | No | No | No |
| Egg Dependence | Yes, Low Yield | Yes, Low Yield | No | No | No | No | No | No |
| Speed | Slow | Slow | Fast | Fast | Fast | Fast | Fast | Fast |
| Virus Replication | No | Yes | No | Yes | No | No | Yes | Yes |
| Mode of Administration | Injection | Nasal Spray | Injection | Nasal Spray | Injection | Injection | Injection | Nasal Spray |
| Reassortment | No | Yes | No | Yes | No | No | No | No |
| Sterility | + | - | + | + | Baculovirus Contaminant | + | + | + |
| Self Adjuvanted | No | Yes | No | Yes | No | No | Yes | Yes |

(1) MDCK: Madin-Darby Canine Kidney
Recombinant Adenoviral-vectored Influenza Vaccines
PaxVax (oral)

PaxVax Vaccine Concept
Replication Competent Ad4 Vector, Single Dose, Oral Vaccine
Example: H5 Flu Completed Phase 1a/1b trials

Potential Advantages
- Ad4 vector allows rapid strain switching and possible universal antigen use if/when available
- A549 cell culture with disposable components allows more rapid, scale-able, less expensive and more consistent manufacturing yields than traditional egg based
- Room temperature formulations eliminates need for cold chain and allows wider distribution
- Oral formulations, pills and powders, allow timely distribution and delivery
Recombinant Adenoviral-vectored Influenza Vaccines
PaxVax (oral)

Clinical Status:
- finished 160 subject ph1
- proceeding to ph2 challenge study
PaxVax conclusions:
- vaccine is safe
- one dose elicits sufficient vaccine take
- strong cellular responses
- primes well for subsequent H5
- higher doses appear to overcome pre-existing immunity to the vector.
Recombinant Plant-manufactured Influenza Vaccines
Fraunhofer USA

Schematic representation of “launch vector”-based production of target antigens in plants

Step 1. Cloning of a target gene into “Launch Vector” System

Step 2. Inoculation of “Launch Vectors” containing target genes into plants

Step 3. Accumulation of target antigen in plants

Step 4. Purification of recombinant antigen from plant biomass
Recombinant Plant-manufactured Influenza Vaccines
Fraunhofer USA

**FhCMB’s Goal:** Development of technology that will address unmet needs

- Rapid Response
- Surge Capacity
- Scale
- Cost
- Platform Technology
- Simple Manufacturing
- Safety
- Regulatory

-GMP facility in Delaware, USA
-Clinical Phase I Influenza vaccine

Center for Molecular Biotechnology
Recombinant Plant-manufactured Influenza Vaccines
Medicago

Plant-based manufacturing

1. Synthesis
   Gene synthesized from sequence of pandemic virus

2. Vacuum Infiltration
   Genetic material introduced into plants through vacuum infiltration

3. Incubation
   Plants are incubated four to six days in greenhouse for protein expression and VLP formation

4. Harvest
   Plants are harvested to extract VLPs

5. Purification
   VLPs are purified to obtain clinical grade material

6. Medicago vaccine
   • Potent immune stimulation
   • Immunological memory
   • Lower dosage
   • No genetic material (non-infectious)

Medicago's solution: first responder

- April 24: Identification of genetic sequence of A (H1N1)
- May 8: Genetic material introduced into plants
- May 8-12: Plants incubated in greenhouse for vaccine production
- May 15: First purified vaccine lot
- June 30: Positive preclinical results (mice)

- Start production of any new vaccine in < 4 weeks
- Substrate easy to supply (plants in greenhouses)
- Can produce high yields of complex products (VLPs, antibodies)
Recombinant Plant-manufactured Influenza Vaccines
Medicago

Clinical results

Pandemic – H5

<table>
<thead>
<tr>
<th></th>
<th>Dosage (ug)</th>
<th>Seroconversion</th>
<th>Seroprotection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicago - PI</td>
<td>2x20+alum</td>
<td>70%</td>
<td>60%</td>
</tr>
<tr>
<td>18-49 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medicago - PII A</td>
<td>2x20+alum</td>
<td>65%</td>
<td>65%</td>
</tr>
<tr>
<td>18-49 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Split in eggs</td>
<td>2x45+alum</td>
<td>Not reported</td>
<td>58%</td>
</tr>
<tr>
<td>18-45 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recombinant</td>
<td>2x45+alum</td>
<td>8%</td>
<td>Not reported</td>
</tr>
<tr>
<td>20-40 years</td>
<td></td>
<td>(22% without alum)</td>
<td></td>
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</tbody>
</table>

- PI: 48 subjects
- PII: 235 subjects

- Good safety profile
- One of the best immunogenicity results obtained with H5

Clinical results

Seasonal – H1

<table>
<thead>
<tr>
<th></th>
<th>Dosage (ug)</th>
<th>Seroconversion</th>
<th>Seroprotection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicago - PI</td>
<td>1x5</td>
<td>61%</td>
<td>63%</td>
</tr>
<tr>
<td>18-49 years</td>
<td></td>
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</tbody>
</table>

- PI: 100 subjects
- Good safety profile
- All 3 CHMP criteria met with one single 5ug dose
Peptide Epitope-based Influenza Vaccines
Immune Targeting Systems

- Solid phase Fmoc chemistry
- Lyophilised nanoparticulate formulation
- APIs stress tested in lead optimisation
- Commercially scalable process
- Utilizing industry standard methods & analytics
- Competitive COGs

Densigen™ (35aa conserved immunoprevalent antigen)

Immune Targeting Systems in Summary

London based biotech developing vaccines for mutating viruses

Lead Program: Synthetic thermostable universal Flu vaccine (Flunisyn™)
- Phase I positive data validates approach Phase-II expected to initiate in 2011
- Safe, well tolerated and highlights its best-in-class immunological potential

Technology Platforms
- Next generation broadly applicable T-cell vaccine platform (mutating viruses)
- DepoVaccine™ technology (synthetic, antigen depot)
- Densigens™ (conserved immunoprevalent antigens)
### Flunisyn™ - Universal Influenza Vaccine Profile

<table>
<thead>
<tr>
<th>Category</th>
<th>Target product profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal flu vaccine</td>
<td>- One vaccine for all seasonal &amp; pandemic flu strains</td>
</tr>
<tr>
<td></td>
<td>- Synthetic and thermostable</td>
</tr>
<tr>
<td>Mode of action</td>
<td>- Boosts residual T-cell memory to kinetically responsive levels</td>
</tr>
<tr>
<td></td>
<td>- Reduces viral load &amp; symptom severity</td>
</tr>
<tr>
<td>Clinical indication(s)</td>
<td>- Elderly: weak T-cell immunity needs boosting (not possible with commercial flu vaccines)</td>
</tr>
<tr>
<td></td>
<td>- Combination with conventional flu vaccine (Abs + T-cells)</td>
</tr>
<tr>
<td></td>
<td>- All population: Stand alone potential (pre-pandemic)</td>
</tr>
</tbody>
</table>

**Targeting improved vaccine efficacy for the elderly**

**Would provide Governments a ubiquitous long term vaccine stockpile**
Blow-Fill-Seal Fill and Finish Systems

Nephron

BFS Advantages

• Plastic bottles and vials deliver tremendous pricing advantages over the traditional glass ampoule
• High capacity BFS machines yield well over 15M units per month
• BFS delivers a high fill accuracy; within 0.25 ML
• Reduced over fill versus glass can significantly lower price per unit where costly formulations are used
• All plastic vials are individually tested for leaks and fill volume
  – BFS machines yield better than 95% efficiency
  – Less than 2% rejects during leak detecting
• BFS delivers savings due to the aseptic fill model
  – Prep and post sanitation of plastic bottles in not required
  – Rubber stoppers and caps can be inserted intro the plastic vials in the Class 100 chamber of the BFS machine
• BFS can readily deliver a single dose preservative free bottle of injectable drug
• Disposal of plastic over glass yields additional costs savings
Blow-Fill-Seal
Fill and Finish Systems
Nephron
Nephron Pharmaceuticals Corp. is a US based, generic drug manufacturer with over 25 years experience making BFS packaged drugs. The company ships well over 1 billion plastic vials of sterile drugs per year. All of Nephron’s blow fill seal machines and high speed secondary packaging lines operate out of GMP and EU compliant facilities located in Orlando, FL. Nephron’s BFS fill and finish capabilities include respiratory and ophthalmic solutions, suspensions and topical gels. All of Nephron’s BFS fill lines are aseptic sterile fill lines with inline leak detecting technology. With a vaccine development partner and committed capacity agreement in place, Nephron could fill a 50 million vial order in less than three months from the day an order is placed. In addition to fill and finish capabilities, Nephron maintains a global distribution network, distributing low priced drugs to large retail pharmacy and grocery chains, all major and specialty wholesalers and most major hospital systems. Nephron maintains its own trucking company to replenish stock at its 3 private distribution centers around the country and distributes directly to any licensed location in the US.
Thank you for your interest, patience, attention and participation!

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