New Cell for New Vaccines II

Topics:

- Baculovirus Expression Vector System (BEVS) technology
- Development and qualification of PSC’s proprietary insect cell line
  Regulatory issues surrounding insect cells
- FluBIOk® Clinical study results
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 insect cells in a fermenter
Infect cells with engineered virus
Incubate infection for 48 - 72 hrs

Highly purified protein
rHA forms rosettes
Formulate with PBS into vaccine
Technology
“Enabling products where speed, cost, and safety matter”

Key Advantages of BEVS Technology

Authenticity of the antigen
- Antigen in vaccine is an exact match to natural virus

Speed
- Cloning to expression in weeks vs. months

Safety
- No live virus, no need for biocontainment
- >50,000 doses tested in humans with outstanding safety record

Versatility
- Cloned and expressed > 1,000 proteins

Reliable scale-up
- Current scale 500L; scale-up in progress
Inherent Safety Associated with BEVS-based Production Technology

**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
  - PSC has delivered > 50,000 doses in >5,000 subjects

Proprietary Cell Line - Serum-free *expresSF+®*

**Evolved from Sf9 Cells**
- Selective pressure in serum-free media with added insulin (0.4 mM)
- Unique phenotypic and genotypic properties
- Patented
- Qualified as per ICH Q5A and 1993

**Points to Consider**
- Ideal for Manufacturing
  - Serum-free – low cost media
  - Stable for > 50 passages
  - Infected with low MOI \(< 1\)
  - Produces high titer AcNPV
  - cGMP at 500L scale
  - Excellent safety record in clinical trials

- Uninfected
- Infected
Evaluation of New Cell Substrates

Safety and Purity Considerations for Novel Cell Substrates

- Adventitious agents (infectivity)
- Nucleic acids
  - retrotransposons
- Host cell proteins
  - allergies
  - glycosylation impact
- Other possible contaminants induced by raw materials, e.g. FBS
Testing and Qualification of the expresSF+ MCB

expresSF+ Master Cell Bank
generated 1993; new bank in 2004

Identity Testing
- Karyotyping analysis
- Isoenzyme analysis
- Cell morphology/Growth Characteristics (Growth, Infectivity and Protein Production)

Microbial Contaminants
- Sterility (21 CFR 610.12)
- Mycoplasma and Spiroplasma (Direct and Indirect, Agar and Broth – 1993 PTC)

Tumorigenicity
- Tumorigenicity – 16 weeks, nude mice
Testing and Qualification of the expresSF+ MCB

Adventitious Virus Testing

- In Vitro - Vero, MRC-5, BHK-21, and Sf9 cells
  (28 days CPE/hemadsorption)
- In Vivo - Suckling mice, adult mice, and embryonated chicken eggs (1993 PTC, 21 CFR 630.35)
- Developing assays for arboviruses

Retrovirus Testing

- EM of > 200 cells
- Co-cultivation/PERT\(^1\)

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[1] Sf9 cells are known to contain retrotransposons and thus are generally positive for RT.
Testing and Qualification of the expresSF+ WCB

Microbial Contaminants
- Sterility (21 CFR 610.12)
- Mycoplasma and Spiroplasma
  Direct and Indirect – 1993 PTC

Identity
- Cell morphology/Growth Characteristics
  Growth
  Infectivity
  Protein Production
Testing of End of Production (>50p) expresSF+ cells

Identity
- Isozyme analysis
- Cell morphology/Growth Characteristics

Sterility

Adventitious Virus Testing
- In Vitro - Vero, MRC-5, BHK-21, and Sf9 cells (28 days CPE/hemadsorption)
- In Vivo - Suckling mice, adult mice, and embryonated chicken eggs (1993 PTC, 21 CFR 630.35)

Retrovirus Testing
- EM of ≥ 200 cells
- Co-cultivation/PERT

Tumorigenicity
Technology
Cell Line: Serum-free expressSF+

Regulatory Advantages

- FDA finds acceptable for vaccine production
- Non-tumorigenic
- Serum-free medium
- Stable for > 50 passages
- cGMP at 500L scale
- Excellent safety record in clinical trials
- Most mammalian viruses cannot replicate in insect cells and vice versa
  (exception – Arboviruses)
**Technology**

**PSC Virus Background**

*Autographa californica* (Alfalfa Looper)

**Nuclear Polyhedrosis Virus (AcNPV), E2 isolate**

- AcNPV polyhedra were isolated from a single-field-collected alfalfa looper larva
- Propagated and plaque-purified/cloned
- Introduced restriction sites to facilitate generation and cloning of recombinant viruses
- SF+ cells were infected with parental, modified AcNPV
- Harvested supernatant was frozen and designated Master Virus Bank (MVB)
- DNA is isolated from MVB, linearized and recombined with transfer plasmid to form Working Virus Banks
Technology
MVB Qualification Testing

Identity
- Southern Blot
- Comparability to 1994 MVB

Microbial Contaminants
- Sterility (21CFR610.12)
- Mycoplasma/spiroplasma (Direct and Indirect, 1993 PTC)

Adventitious Agents
- In Vitro Assay (VERO, MRC-5, BHK21, S2; 28 days; CPE/hemadsorption)
- In Vivo Assay - Suckling mice, adult mice, and embryonated chicken eggs (1993 PTC, 21 CFR 630.35)
FluBIOk® - Next Generation Vaccine for Influenza

"Making products where speed, cost, and safety matter"

Trivalent recombinant hemagglutinin (rHA) vaccine

- Produced *in vitro* via insect cell culture technology
- Cloned from WHO/CDC recommended strains
- Easier to produce, no eggs, no live viruses, no bio-containment required, no preservatives
- Contains 3x45ug rHA

FluBIOk rHA Antigens

- Highly purified (95%)
- Correct 3-D structure
- Biologically active
  - Hemagglutination activity
- Induces protective immune responses
  - HAI antibodies
  - Neutralizing antibodies
FluBIOk 2004 Phase II/III Field Study
Summary of Results

Clinical dose (45ug/strain)
- Efficacy: 100% (even against drifted H3 strain)
- Effectiveness: 54% reduction ($p \leq 0.05$) in CDC-ILI vs. placebo

Efficacious and effective without neuraminidase

Highly immunogenic
- H3 component - high and long lasting titers
- Protective levels for all antigens for at least 6 months

FluBIOk protects against “drifted” strains
Randomized double-blinded Study

Subjects: Age $\geq$ 65 yr (medically stable)

Subjects randomized to received FluZone, or trivalent rHA containing 45 µg of each rHA

Total enrollment: 869 subjects
PSC03 GMT Results
FluBIOk (135µg) vs. TIV (45µg)

GMT All Subjects

TIV/FluBIOk GMT ratio D28 not to exceed 1.5 →
Endpoint met for all three antigens
PSC03 Serology Results
Flublok (135µg) vs. TIV (45µg)

All Subjects

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Subjects > 75 Years

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Difference in sero-conversion observed in population as a whole versus 279 subjects aged 75 or older

Note: TIV contained B/Malaysia and Flublok contained B/Ohio antigen
FluBIOk® - Next Generation Vaccine for Influenza
"Making products where speed, cost, and safety matter"

The BEVS technology provides:
- Speed, Cost and Safety
- Rapid response to emerging strains
- No need to handle live viruses
- Authentic antigen (no changes due to adaptation of the virus to egg or cell culture)

Next steps for PSC
- Preparing our launch facility
- Two clinical studies in progress for 2007-2008 to support accelerated and traditional approval
- BLA filing starting Q4 2007 (Accelerated Approval)
- FluBIOk product approval expected in 2008
- Development of prophylactic pandemic vaccine

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