GMP Purification of Recombinant Proteins from Plants at Commercial Scale

New Cells for New Vaccines

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Presentation Outline

• GMP Contract Manufacturing from (Transgenic) Biomass
• Product Range and Platform Range
• GMP Process Considerations and New Facility Design
• Economical Targets
• Summary
Plants as Production Vehicles

- Transgenic plants as protein production vehicle since ~1990
- Proof of concept provided for:
  - Functionality and activity
  - Safety
  - Scalability – Speed to production - Surge capacity
- How about Low Cost of Goods Potential?
  - Accumulation level (>100-fold variations)
    - Platform dependent
    - Product dependent
  - Speed of processing / purification
  - Scale of processing / purification
  - Capital investment (marginal)
• Four SAFC Divisions:
  – SAFC Pharma: GMP Contract Manufacturer
  – SAFC Biosciences: Cell Culture Media Development
  – SAFC Supply Solutions: Custom Reagents
  – SAFC Hi-Tech: Servicing Electronic Industry
SAFC Pharma: GMP Contract Manufacturer

• **Bio-Molecule APIs**
  - Aqueous extraction and purification from biomass (bulk)
    • Facilities in St. Louis, MO
  - Virus production (vaccines & viral therapy) & fill-finish capability:
    • Facility in Carlsbad, CA (formerly Molecular Medicines BioServices)
  - Conjugation of small molecule API to bio-molecules
    • Facility under construction (ready Q1 2008)
  - Microbial fermentation
    • Facility under construction (ready Q1 2009)

• **Small Molecule APIs**
  - Including highly potent and toxic compounds
  - Facilities in St. Louis, Madison, Arklow, Gillingham, Manchester
SAFC Pharma: GMP Product Examples

- **Product Examples**
  - **APIs**
    - Plant and animal allergens
    - Vaccines
    - Therapeutic proteins and enzymes
  - **Blood products**
    - Thrombin
    - Recombinant Human Serum Albumin (under development)
  - **Excipients**
    - Keyhole limpet hemocyanin (KLH)
  - **GMP Process reagents (enzymes and inhibitors)**
    - Trypsins (recombinant from plants and from animal sources)
SAFC Pharma: New GMP Facility Drivers

- Accelerated Trend to Minimize Risk of Transmittable Diseases to Humans
- Increasing Demand of Biologics from Natural & Transgenic Sources
- Transgenic Potential of Cost-Efficient Production at Large Scale
- Accelerated Trend to Improve Production Economics
- Good Fit with Our Core Competency

Consequently: Design for Two Modern, Multi-Product Facilities
- Animal-free biomass (plant, bacteria, yeast)
- Animal-origin biomass
SAFC Pharma: Plant Facility Design Considerations

• **Recombinant bovine APROTININ expressed in tobacco leaves**
  – Collaboration with LSBC, now Kentucky BioProcessing, LLC
  – Commercialized: Used as GMP process aid,
  – Indistinguishable from native molecule
  – Ability to produce multi-kg quantities

• **Recombinant bovine TRYPSIN expressed in corn seed**
  – Collaboration with Prodigene, now Stine Seeds company
  – Commercialized: Used as GMP process aid
  – Indistinguishable from native molecule
  – Produced under GMP conditions from flour; Annual target in multi-kg range

• **Recombinant human SERUM ALBUMIN (rHSA) expressed in rice seeds**
  – Collaboration with Ventria company
  – In late-stage development: Marketed for use as an excipient
  – Annual target volumes app. 500 kg
Plant Facility Design, Upstream 1

Goal: Process Maintained Continuous As Long As Possible

- Tobacco Leaf Biomass
  - Physiologically active material: Immediate processing required
  - Cell disruption by knife-based equipment
  - Up to 2 tons/hr input
  - Buffer from concentrate to arrest unwanted reactions
  - No prolonged extraction time required (dilution of cell liquid)

- Installations:
  - Three extraction/hold tanks of 2,500 L capacity
  - One 7,000 L buffer tank
  - On 2,100 sq ft floor space
  - Delta V control system (DCS) w/ transfer panel integration
  - Controlled, yet not classified
Plant Facility Design, Upstream 2

**Goal:** Process Maintained Continuous As Long As Possible

- **Grain Seeds**
  - Seeds provide long-term stability for recombinant protein
  - Cell disruption by milling (off site)
  - Up to 0.4 tons/hr input
  - Buffer from concentrate to arrest unwanted reactions
  - Prolonged extraction times required (~0.5 hrs to ~2 hrs)
  - Continuous process options, extraction:
    - Plug-flow (no mixing): Too long pipes required, infeasible for required extraction times
    - Alternating tank fill-drain cycles: Appropriate for short extraction times
    - Sequential tank flow-through: Appropriate for longer extraction times
Plants Facility Design, Upstream 3

- Solid-Liquid Separation
  - Screw press (tobacco)
  - Decanter centrifuge (grain flour)
- pH Treatment
  - In-line injector w/ in-line mixer
  - Plug-flow concept for short adjustment times
- Clarification (Controlled, yet not classified on 2,200 sq ft floor space)
  - Disk-stack centrifuge
- Filtration
  - Banks of dead-end filters in alternating operations
  - (UF/DF systems, NOT continuous operations, however)
  - 1 x 3,200 L and 2 x 2,000 L tanks
Plant Facility Design, Downstream 1

- Initial, Capturing Chromatography (Classified 100,000, ISO-8):
  - Packed bed
    - Highly clarified feed stream required
    - Does not tolerate continued precipitation
    - Relative slow flow rate (<30 dm/hr)
    - Up to 1 m chromatography columns w/ broad selection of resins
      - IEX, HIC, Affinity, IMAC, Proprietary
  - Suspended bed
    - No fully clarified feed stream required
    - Does tolerate continued precipitation
    - Relative fast flow rate (60 dm/hr)
    - Restricted selection of media
Plant Facility Design, Downstream 2

• Feed stream preparation by
  – UF/DF (not continuous operations)
  – In-line pH or conductivity adjustment

• Second Chromatography in classified area (100,000, ISO-8):

• Packed bed
  – To match elution rate of first column requires large second column
  – High-capacity pre-filtration required

• Suspended bed
  – Easier to match elution rate of first column with small second column
  – No pre-filtration required
Plant Facility Design, Downstream 3

• Following Process Steps Similar to Any Biologicals Production:
  • 660 sq ft Classified area (10,000, ISO-7)
  • Polishing chromatography (packed bed)
  • UF/DF
  • Formulation (DF)
  • Stabilization
    – Aseptic/sterile fill (in classified 100 bio-hood)
    – Freeze-drying (with classified 100 area)
    – Spray drying (PD scale, future plans for commercial scale)
SAFC Pharma: Plant Facility Features

- Over 17,000 square feet of manufacturing space features fully validated equipment
  - 7 large, fixed tanks w/ space for non-fixed equipment
- Enclosed processing
- USP purified water with bagged WFI capability on campus
- Delta-V process control
- Controlled, not classified environment for upstream operations
- Classified environment (ISO 8 [1,100 sq ft] and ISO 7 [650 sq ft])
- Validated Cleaning-In-Place (CIP) system
- 200L Lyophilization capability
- Controlled environment packaging capability
TrypZean™ (recombinant bovine trypsin expressed in corn)
- Collaboration with Stine Seeds company
- Current expression level 0.05g/kg flour
- Approx. 50% recovery
- Low expression resulting in high costs
- Progress made towards increased expression level

Cost reduction through:
- Increased expression levels
- Increase batch sizes
- Shortened process times
• Recombinant human serum albumin (r-HSA) in rice
  – Collaboration w/ Ventria company
  – In late stages of Process Development
  – Very high expression levels (6g/kg)
  – Targeting multi – kg batch sizes
  – Annual output >500 kg
  – Pricing expected to be significantly lower than current recombinant alternatives
Contract GMP Bio-Molecule Manufacturing

- **Key process parameters to aid economics**
  - High expression level
  - High input capability
  - Design of continuous upstream operations
  - Short processing times
  - Large scale operation and large batch size

- **Supported by:**
  - Process engineering for manufacturing design utilizing process modeling software
  - Experienced GMP production staff (currently 24/5 operations)
  - Process Development group to develop or optimize process design
  - Quality control group w/ analytical assay development, product release
  - Project management for internal coordination and interface with customer
SAFC Pharma GMP Bio-Molecule Contract Manufacturing: SUMMARY

- Two spacious, multi-product GMP contract manufacturing facilities for biologics from plant and animal biomass, including transgenic biomass
- Build for less then $20 million with output capacity of up to 1,000 kg/year
- Gradual expansion capability adjacent; alternatively build dedicated facility for product commercialization
- Acknowledgement of corporate partners:
  - Stine Seed Company
  - Kentucky BioProcessing, LLC
  - Ventria Bioscience