versatile Technologies for vaccine Manufacturing

DCVMN 17th Annual General Meeting
24-27 October 2016
Buenos Aires, Argentina

Damon Asher, PhD
Associate Director, Vaccine Initiative
Process Solutions
Biopharma top growth strategies
Top regions / countries of interest for expansion
Types of products in development and which will be most disruptive
ROI expectations on growth strategies
Top of mind risks associated with growth strategies
Approaches to managing risks
Confidence in ability to execute
The Economist Intelligence Unit Survey Demographics

One goal: assess the balance between risk and growth in biopharma

- 31% Europe
- 30% North America
- 29% Asia
- 10% Rest of World

- 9.8% Latin America (Brazil & Mexico)
- 254 biopharma industry leaders
- 25 questions
LATAM Plans to Expand Beyond its Borders...

64% intend to add production and/or development capacity or grow market share in EMEA & Asia

Most important strategies for growth over the next five years:

- 52% Expansion into new products
- 40% Expansion into new geographical markets
while the rest of the world moves to LATAM...

**Most important** strategies for growth over the next five years:

- **40%** Expansion into new products
- **30%** Expansion into new types of therapeutic categories

**52%** intend to add production and/or development capacity or grow market share in LATAM & Middle East/Africa
Vaccine Development & Manufacturing Strategy in LATAM

36% Biotechs are already developing or plan to develop vaccines

40% LATAM Biotechs plan to partner with other pharmaceutical companies to manufacture new drug and therapy products

30% Global Biotechs plan to invest in production facilities in emerging markets to manufacture new drug and therapy products
LATAM: The Changing Biopharma Risk Equation

Manufacturing and Development of New Drug and Therapy Products

**Risks will increase** somewhat or significantly over the next five years:

**LATAM**
- 52% controlling costs in development and production
- 44% maintaining IP protection
- 44% scaling up and supplying market demand

**Vs.**

**GLOBAL**
- 41% controlling costs in development and production
- 40% maintaining IP protection
- 36% maintaining regulatory compliance
Get a Copy of the Report

www.gobeyondbiopharma.com

- Download the EIU report
- Explore the survey results through interactive infographics
- Stay engaged with updated blog content
Case study and testbed for modern vaccine scale-up and optimization

VLPs
Powerful platform for creation of new vaccines
- Processes are currently quite diverse

Standardized platforms needed to accelerate vaccine development!
Production & Purification of VLP-Based Vaccine

Work carried out in collaboration with iBET

iBET: Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Hepatitis C

- 170 million people infected, over 350,000 deaths/year
- Causes cirrhosis and liver cancer
- Current therapies only partially effective, costly and poorly tolerated
- No vaccine currently exists

Production & Purification of VLP-Based Vaccine
Insect Cell Expression System for VLP

VLP produced in Sf9 insect cells co-infected with MLV-GAG and HCV-E1E2 using baculovirus
Production & Purification of VLP-Based Vaccine

Process Challenges

1. Scalable production in bioreactor
2. Efficient purification
3. High recovery of VLP
4. Baculovirus clearance
Production & Purification of VLP-Based Vaccine

Insect Cell / Baculovirus VLP Production Platform

1. Media and Inoculum Preparation
2. Cell growth in Bioreactor and Virus Inoculation
3. Primary Clarification
4. Bioburden Reduction
5. UF/DF
6. Sterile Filtration
7. UF/DF
8. Polishing Chromatography
9. Baculovirus Inactivation
10. Purification Chromatography
Production & Purification of VLP-Based Vaccine
Process Step Optimization

Strategy

Reusable Stirred Tank Bioreactor (STR)

Disposable Mobius® CellReady 3L Bioreactor (CR)

Clarification
- Optiscale® depth filters
- Polygard® CN filters

Concentration
- Pellicon® XL UF cassettes

Purification
- AEX prototypes
- B/E and F-T processes
Production & Purification of VLP-Based Vaccine
Establish Scalable, Single-Use Bioreactor Production

1. Media and Inoculum Preparation
2. Cell growth in Bioreactor and Virus Inoculation
3. Primary Clarification
4. Bioburden Reduction
5. UF/DF
6. Sterile Filtration
7. UF/DF
8. Polishing Chromatography
9. Baculovirus Inactivation
10. Purification Chromatography
Production & Purification of VLP-Based Vaccine

Establish Scalable, Single-Use Bioreactor Production

Reasons for increasing Disposables

(% Indicating Attribute is "Very Important" or "Important")

- Eliminate cleaning requirements
  - 48.5%
  - 41.7%
- Reduce capital investment in facility & equipment
  - 40.0%
  - 45.0%
- Reduce time to get facility up and running
  - 40%
  - 45%
- Faster campaign turnaround time
  - 34.6%
  - 47.1%
- Decrease risk of product cross-contamination
  - 42.4%
  - 39.2%

Source: 8th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production, April 2011
# Production & Purification of VLP-Based Vaccine

## Establish Scalable, Single-Use Bioreactor Production

### Challenge #1
*Leap to mfg scale*

### Challenge #2
*Get on the path!*

<table>
<thead>
<tr>
<th>Ratio</th>
<th>3 L</th>
<th>50 L</th>
<th>200 L</th>
<th>1000 L</th>
<th>2000 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Volume : Total Volume</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Impeller Diameter : Vessel Diameter</td>
<td>0.6</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vessel Height : Vessel Diameter</td>
<td>1.8:1</td>
<td>2.0:1</td>
<td>2.0:1</td>
<td>2.0:1</td>
<td>2.0:1</td>
</tr>
<tr>
<td>Liquid Height : Vessel Diameter</td>
<td>1.4:1</td>
<td>1.7:1</td>
<td>1.6:1</td>
<td>1.6:1</td>
<td>1.6:1</td>
</tr>
<tr>
<td>Internal Baffle</td>
<td>No</td>
<td>Single (Paddle-Type)</td>
<td>Single (X-Type)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual sparger (open pipe/microsparger)</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Min – Max Working Volume (L)</td>
<td>1 – 2.4</td>
<td>10 – 50</td>
<td>40 – 200</td>
<td>200 – 1000</td>
<td>400 – 2000</td>
</tr>
</tbody>
</table>
Transition from Glass to Scalable Single-Use Bioreactor

Initial challenges

**CR Conditions:**
- 27 °C, pO₂ = 30%
- Agitation rate: 70–200 rpm
- Impeller: 1 marine
- Aeration rate: 0.01vvm
- Working volume = 2 L

**STR Conditions:**
- 27 °C, pO₂ = 30%
- Agitation = 70–250 rpm
- Impeller: 2 six-blade Rushton
- Aeration rate: 0.01vvm
- Working volume = 2 L

**Sf9 cells in CR**
- Observation of cell aggregates for unoptimized CR culture

**Sf9 cells in STR**
- Long lag-phase observed for CR culture

STR=Glass stirred tank
CR=Single-use bioreactor
Transition from Glass to Scalable Single-Use Bioreactor

Process Optimization

Table 1. Shear stress in the two bioreactors

<table>
<thead>
<tr>
<th>Agitation Rate (rpm)</th>
<th>Shear stress (N/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STR</td>
</tr>
<tr>
<td>70</td>
<td>0.14</td>
</tr>
<tr>
<td>90</td>
<td>0.20</td>
</tr>
<tr>
<td>110</td>
<td>0.27</td>
</tr>
<tr>
<td>130</td>
<td>0.34</td>
</tr>
<tr>
<td>150</td>
<td>0.43</td>
</tr>
<tr>
<td>170</td>
<td>0.51</td>
</tr>
</tbody>
</table>

New CR Conditions:
Agitation rate: 150 rpm

<table>
<thead>
<tr>
<th></th>
<th>$\mu_{\text{max}}$ (h⁻¹)</th>
<th>$T_d$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>0.20</td>
<td>35</td>
</tr>
<tr>
<td>STR control</td>
<td>0.20</td>
<td>34</td>
</tr>
</tbody>
</table>

CR match STR hydrodynamic parameters for agitation rates above 150 rpm

✓ Enhanced cell growth in CR
✓ CR and STR show similar growth profiles
Transition from Glass to Scalable Single-Use Bioreactor

Optimized Conditions

- Increased agitation rate
- Increased cell density of inoculation
- Replaced micro sparger with an open-pipe sparger
Transition from Glass to Scalable Single-Use Bioreactor

Sf9 Growth Profiles

Images of Sf9 cell during culture in CR or STR

45 h 70 h 95 h

STR

CR

No formation of cell aggregates

Metabolite concentration in culture supernatants

Comparative metabolite profiles in STR and CR

STR=Glass stirred tank
CR=Single-use bioreactor
Transition from Glass to Scalable Single-Use Bioreactor
VLP-HCV Productivity in the Two Bioreactors

WB analysis of VLP-HCV production kinetics

Similar GAG and HCV-E1E2 expression kinetics

Gag-MLV titer (P30) in the bioreactor harvested bulk

Equivalent productivity in STR and CR culture bulks

Baculovirus replication kinetics

Comparable baculovirus replication kinetics

STR=Glass stirred tank
CR=Single-use bioreactor
Growth kinetics of Sf9 cells in the Mobius® 50 L and 3 L bioreactors. The black arrow indicates the infection of the bioreactors and control shake-flask.

GAG-MLV titre in culture bulks of Mobius® 50L and 3L and in glass stirred tank bioreactor (Glass-STR).
Production & Purification of VLP-Based Vaccine
Insect Cell / Baculovirus VLP Production Platform

1. Media and Inoculum Preparation
2. Cell growth in Bioreactor and Virus Inoculation
3. Primary Clarification
4. Bioburden Reduction
5. UF/DF
6. Sterile Filtration
7. UF/DF
8. Polishing Chromatography
9. Baculovirus Inactivation
10. Purification Chromatography
Production & Purification of VLP-Based Vaccine
Clarification Optimization

**Disposable capsule filters**
Eliminates the time and expense associated with assembling, cleaning, and validating stainless steel housings.

**Replacement for centrifugation**

Polygard® CN, nominal pore sizes of 10, 5, 0.6 and 0.3 μm
Pleated, all-polypropylene depth filters
Filter area: 17 cm²; Inlet flux: 988 LMH
Unlike centrifugation (CFG), depth filtration resulted in ~70% DNA clearance.

**Clarification**

- **Filter-only clarification train** can be used without compromising recovery yield of VLPs.
- Filter cascade composed of a Polygard® CN 5 μm filter followed by a 0.3 μm depth filter showed the highest recovery of HCV-VLP, improving on centrifugation/2° depth filtration.
- **Moderate DNA removal** with depth filtration was seen.
Production & Purification of VLP-Based Vaccine

Insect Cell / Baculovirus VLP Production Platform

- Media and Inoculum Preparation
- Cell growth in Bioreactor and Virus Inoculation
- Primary Clarification
- Bioburden Reduction
- UF/DF
- Sterile Filtration
- UF/DF
- Polishing Chromatography
- Baculovirus Inactivation
- Purification Chromatography
## Optimization of TFF for Concentration and Purification

### Membrane Choices

<table>
<thead>
<tr>
<th>Cellulosic Membrane (e.g. Ultracel® membrane)</th>
<th>PES Membrane (e.g. Biomax® membrane)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Membrane</strong></td>
<td>Composite regenerated cellulose (void free structure)</td>
</tr>
<tr>
<td>** Typical Available NMWL**</td>
<td>3, 5, 10, 30, 100, 300, 1000 kD (new 1 kD in PD)</td>
</tr>
<tr>
<td><strong>Relative protein binding</strong></td>
<td>Ultra low (~0.1 g/m2) Use with any protein concentration (good with dilute)</td>
</tr>
<tr>
<td><strong>pH Stability</strong></td>
<td>2-13</td>
</tr>
<tr>
<td><strong>Comments</strong></td>
<td>▪ Organic solvent resistance ▪ High yield ▪ Very hydrophilic – use with solutions containing hydrophobic components (i.e. antifoams or detergents)</td>
</tr>
</tbody>
</table>
Optimization of TFF for Concentration and Purification

Purification Results

**UF/DF**

Pellicon® cassette with **300 kD regenerated cellulose** membrane offered the best combination of recovery and purification.

4-5X concentration achieved.

Better removal of baculovirus, DNA, and host-cell protein!

Both membranes were fully retentive of the VLP
Production & Purification of VLP-Based Vaccine
Insect Cell / Baculovirus VLP Production Platform

Media and Inoculum Preparation → Cell growth in Bioreactor and Virus Inoculation → Primary Clarification → Bioburden Reduction

Sterile Filtration ← UF/DF ← Polishing Chromatography ← Baculovirus Inactivation ← Purification Chromatography
Purification strategy

Resin Selection

Exigencies and constraints regarding impurity removal and product stability are very specific. The approach is thus similar to rec. proteins, but viruses are more sensitive to high salt and pH. This is why AEX media (e.g. DEAE) are often used to reduce the salt concentration needed to elute the virus.

Capture

pH < pI < pH (pH = 7 for binding of sensitive Viruses)

Viruses and derivatives are generally negatively charged → AEX is the preferred route

Selectivity

Throughput (> 250 cm/h)

Fractogel® SE HircSP(COO-)

Fractogel® or Eshmuno® resin family

Chrom Step # 2, 3 and 4

Fractogel® or Eshmuno® resin family

Fractogel® or Eshmuno® resin family
Anion Exchange Chromatography (AEX) for VLP Purification

Resin Selection

Tentacle morphology increases surface area and virus binding capacity
Anion Exchange Chromatography (AEX) for VLP Purification

Separation of VLP from Baculovirus

**Inputs:** [NaCl] (100/200/300 mM) and flow rate (100/200/400 cm/hr)

**Responses:** % VLP recovery and Baculovirus LRV

Fractogel® TMAE
Anion Exchange Resin

![Graph showing recovery and LRV with input variables of NaCl and flow rate](image-url)
VLP-Based Vaccine Process

Optimal Purification Balance

- Successfully purified VLPs using Fractogel® TMAE commercial resins
- Yield of >60% with ~2 LRV baculovirus can be achieved with a flow-through/wash purification strategy
- Options to increase recovery or purification depending on product value by varying process conditions

![Purification and Recovery Balance](image)
VLP-Based Vaccine Process

**Pre-Packed Chromatography Columns Add Flexibility**

- Elimination of labor-intensive packing
- No capital investment
- No hardware to clean
- Fast setup and qualification

**Chromabolt® Prepacked Chromatography Columns 10cm, 20cm, 32cm**

**Time Savings**
Summary

1. Successfully used Mobius® 3L disposable bioreactor for production of VLP-based vaccine in insect cell culture system

2. Optimized downstream processing using Polygard® CN 5.0→0.3 μm depth filters followed by UF/DF using Pellicon® cassette with Ultracel® 300 kD membrane

3. Purified VLP by using Fractogel® commercial AEX resins

4. Integrated all the above components to create a fully scalable process that meets recovery and impurity clearance requirements.
Thank You

Damon Asher, PhD
damon.asher@emdmillipore.com

https://www.linkedin.com/in/damonasher