Continuous and Semi-continuous Cell Culture for Production of Blood Clotting Factors

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**Prevnar**
Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM197 Protein)

**Prevenar 13**
Pneumococcal/Saccharide Conjugate Vaccine Assorted, 13-valent

**Benefix**
Coagulation Factor IX (Recombinant)

**ReFacto**
Antihemophilic Factor (Recombinant)

**elelyso**
(taliglucerase alfa) for injection

**Fragmin**
(dalteparin sodium injection)

**Genotropin**

**Rebif**
22 mcg/0.5 mL (interferon beta-1a)

**SOMAVERT**
(pegvisomant for injection)

**SOMAVERT**

**BioMolecules**

**Vaccines**

**Biosimilars**

Robust pipeline
BeneFIX or rFIX

- Recombinant therapy for Factor IX deficiency (hemophilia B)
- 55 kDa zymogen with 12 γ-carboxylation sites
- Several other complex post-translational modifications
- Extensive structure and functionality assays
- First approved recombinant factor IX product (1997)
- CHO-based batch re-feed process
- ADRM free culture medium from the beginning
ReFacto AF/ Xyntha or BDD-rFVIII

- Recombinant therapy for Factor VIII deficiency (hemophilia A)
- FVIII gene sequenced in 1984
- Engineered version of FVIII (B domain deleted), maintains procoagulant function
- Increased transcription efficiency
- ReFacto approved in U.S., EU (1st licensed in 1999)
- ReFacto AF approved in 2008
- CHO-based perfusion process
- DS manufactured at an external partner site for Pfizer

Key Process Challenges

- Low level expression (transcription/ translation level)
- Growth associated expression
- Labile product
  - Degradation during processing
  - Activation during processing
- Complex post-translational modifications
  - Disulfide bond formation (11 for rFIX)
  - N-linked and O-linked glycosylation
  - Tyrosine sulfation (rFVIII, rFIX)
  - Metal ion binding
  - Heavy and light chain cleavage
  - $\gamma$-carboxylation sites
- Process changes while maintaining quality/ comparability
  - Removal of ADRMs (Albumin) from culture medium (BDD-rFVIII)
  - Changes in production sites/ scales
  - Use of disposables
  - Ongoing process updates (PAT, vendor changes, ICH guidelines)
- Supply sensitive customer base
Cell culture process - BeneFIX

Re-feed Process

Seed

Low cell density

After n days

High cell density

Retained culture

Low cell density

Re-feed

Harvest

Recovery

Nutrient media

Bioreactor
BeneFIX production data

BeneFIX (1997 – present)

Number of batches

Bioreactor

2.5kL
6kL
12kL

Non-validated database of GMP data
Benefits/ challenges of continuous process

- **Productivity vs Titer**
  - Perfusion/ re-feed processes reduce BRX turn-around time
  - Lower product quality risk than increasing specific productivity of cells
  - Easy to model volumetric productivity vs titer benefit analysis

- Data from multiple bioreactors
- Range of variation in productivity yet consistent product quality

Non-validated database of GMP data
Benefits/ challenges of continuous process

- **Campaign lengths**
  - Several weeks to months
  - Multiple bioreactors allow back up inoculation sources
  - Savings in reduced SIP/ CIP cycles of bioreactors

- **Number of cell generations**
  - Extensive LIVCA studies required
    - At scale EOP samples tested for genetic stability
    - Routine testing for culture purity (mycoplasma and virus) at EOP only
    - Additional product characterization to account for cell age (early-mid-late stage campaign effects)
  - CHO, in general, stable for extended number of generations
Specific growth rate vs generations

- Growth rate is independent of cell generation number
- Range in growth rate giving consistent product quality

Non-validated database of GMP data
Benefits/ challenges of continuous process

- Maintenance of aseptic BRX conditions for extended time
  - A major concern with extended period cell culture
  - SIP / CIP procedures have vastly improved
    - Years of experience
    - Multiproduct facilities
    - Rigorous PM and engineering controls (o-rings!)
    - Disposable use with minimum open operations

1.3% contaminated
Two of eleven bioreactors were never contaminated
Cell culture process – ReFacto AF

Perfusion Process

Culture medium → Bioreactor → Return of CHO cells to the bioreactor → Cell separator → Harvest of product containing broth → Harvest tank → To clarification, primary capture
ReFacto AF

- New MCB for HSA-free ReFacto
  - Same cell lineage, BDD-rFVIII genotype unchanged
  - Albumin-free media for production

- Replace hybridoma-derived MAb column

- Analytical Comparability
  - Very similar biochemical profile
  - Relationship to historical ranges well understood

- Non-clinical/Clinical Comparability

Kelley et al., 2009, Haemophilia.
ReFacto AF production data

Consistent product quality data over the years

Non-validated database of GMP data
**Benefits/ challenges of continuous process**

**Batch definition**
- Critical for biologics from a compliance perspective
- Each DS lot traces back to a single cell bank thaw event
- Pooling of multiple batch streams during purification
- Batch stream could originate from multiple scales

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**BeneFIX**

Bioreactor 1 → Bioreactor 2 → Bioreactor 3 → Harvest and Cell Separation → Concentration/ Diafiltration by Ultrafiltration 1 → Chromatography 1 → Chromatography 2 → Purification

**ReFacto AF**

Bioreactor → Harvest and Cell Separation → Chromatography 1 Frozen eluate → Eluate pool → Viral inactivation & Purification
Conclusions

- Semi-continuous (re-feed) and continuous (perfusion) processes of complex recombinant proteins commercially successful for over 15 yrs
- Product/ process life-cycle changes successfully implemented
- Large data sets demonstrate process robustness and cell line stability
- Increased plant productivity with shorter bioreactor turn-around times
- Manageable contamination rates over extended periods of operation add substantial operational savings
Acknowledgements

Almost two decades of contributions towards continuous development and manufacturing of BeneFIX and ReFacto AF/ Xyntha

- Pfizer Global Supply (Tanya Alcorn)
  - Site Operations (Erin Beal, Erik Roos)
  - Quality
  - Manufacturing Sciences and Technology (Dan Lasko, Enda Moran, Kesav Reddy, Jim Booth)
- Pharmaceutical Sciences (Tim Charlebois, Mike Jankowski, Shamik Sharma)
- External supply partners