Towards making biologic drugs on demand

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Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Defense Advanced Research Projects Agency (DARPA) and SPAWAR Systems Center Pacific (SSC Pacific).
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Making biologic medicines on demand for patients

- Drug manufacturing: 6 - 12 months
- QA/QC testing
- Delivery
- Real-time release (logistics & operations)
- Real-time production, release, and use: 1 - 2 days
Making biologic medicines on demand for patients

**Bio-MOD Capabilities**

- Enables novel, flexible methodologies for genetic engineering/modification of microbial strains and cell-free systems to **synthesize multiple and wide-ranging protein-based therapeutics**
- Develops **flexible, portable device platforms for manufacturing multiple biologics** with high purity, efficacy, and potency, **at the point-of-care**, in short timeframes (**<24 hours**), when specific needs arise
- Includes **end-to-end manufacturing chain** (including downstream processing) in **continuous flow** within a **microfluidics-based platform**
- Focuses on **currently approved therapeutics by FDA** (i.e. no drug discovery)
Interferon-α2b (IFNα2b)

- 165-aa single chain polypeptide (19.2 kD MW)

- Licensed products
  - Intron A (Merck; Schering Corp legacy product)
  - Shanferon (Shantha/Sanofi; India)

- Extensive experience in industry with this drug – first marketed by Schering Corp in 1986

Key interacting residues of IFNα2b are: A19, Q20, L26, F27, M148, E159, K164
Human growth hormone (hGH)

- 191-aa single chain polypeptide (22.1 kD MW)

- Licensed products
  - Pfizer (Genotropin)
  - Novo Nordisk (Norditropin)
  - Genentech (Nutropin)
  - Eli Lilly (Humatrope)
  - Sandoz (Omnitrope)

- Extensive experience in industry with this drug – first marketed by Genentech in 1981
Integrated and Scalable Cyto-Technology (InSCyT) biomanufacturing platform

Upstream
- Sterile Media
- Yeast Inoculum
- Perfusion
- Crude Product Hold Tanks

Downstream
- Column Tanks
- Affinity Chromatography
- Polishing Membrane #1 Tanks
- Polishing Membrane #2 Tanks
- Polishing Membrane #1
- Polishing Membrane #2
- Waste
- UF/DF Membrane

Analytics
- pH, DO, T
- Sterility
- Safety
- Potency
- Purity
- Identity
- Concentration

Real-Time Process Data

On-line Reactor Control

Multivariate Model
- OK for Release
- Not OK

Final Product

Fill

Integrated and Scalable Cyto-Technology (InSCyT) biomanufacturing platform
Upstream

- Sterile Media
- Yeast Inoculum
- Crude Product Hold Tanks
- Real-Time Process Data
- On-line Reactor Control
- Perfusion

Downstream

- Column Tanks
- Polishing Membrane #1 Tanks
- Polishing Membrane #2 Tanks
- Purified Product

Analytics

- pH, DO, T
- Sterility
- Safety
- Potency
- Purity
- Identity
- Concentration

Multivariate Model

- OK for Release
- Not OK

Final Product

- Fill

Micro - bioreactor inoculation

Biomass accumulation

Biologic production

Real-Time Process Data

Micro - bioreactor inoculation

Biomass accumulation

Biologic production

Final Product

Micro - bioreactor inoculation

Biomass accumulation

Biologic production

Final Product
Microbial host: *Pichia pastoris*

**Advantages from regulatory perspective**
- Many products on market or in late-stage development (including IFNα2b, insulin, HepB)
- Reduced risk for viral contamination in InSCyT process
- Human-like post-translational modifications (folding, glycosylation, etc…)

**Technical benefits**
- Rapid and dense biomass accumulation
- High yields of secreted proteins (up to ~15 g/L)
- Limited host cell protein (HCP) profile (eases burden on downstream)
- Amenable to lyophilization
Small-scale perfusion demonstrates extended production

Strains

Base strain: Wildtype *K. phaffii*
Promoter: *AOX1*
Insertion Locus: *AOX1*
Gene Copy #: hGH – 10

Nicholas Mozdzierz, Kartik Shah (Love)
Continuous culture yields stable hGH production and diminishing HCP

M = Marker (Precision Plus Dual Color Standard)
B = Blank
S1 = Reference hGH (2.0 μg load)
S2 = Reference hGH (1.0 μg load)
1 = Pre-induction (vessel)
2 = 9.4 h PI
3 = 24.3 h PI
4 = 49.2 h PI
5 = 76.8 h PI

Strain
Base strain: Wildtype K. phaffii
Promoter: AOX1
Insertion Locus: AOX1
Gene Copy #: multi-copy

Nicholas Mozdzierz (Love)
Design strategies for affinity peptide ligands for purification

- De novo design
- In silico screening

Phage display

Epitope mapping of natural binding partners

Affinity ligands of homologous and structurally similar proteins

Sequences that complement secondary structural elements

Karande and Cramer, RPI
Batch screening to select column operating conditions

hGH Elution (%) From Affinity Peptide Resins

- Design of many peptides allows for selection of the candidate with the most desirable properties

Steve Timmick (Cramer)
Chemistry & chromatography for polishing

Ligands Design, Synthesis, & Coupling

Additional candidates or base matrix

Provide Feedback

Column

Bead

Membrane

Pall Corp.
96-Well plate screening for polishing operating conditions

Bind/Elute polishing step #1

High DNA clearance

MM CEX bead 1
- Binding: Lower pH
- Lower/medium cond.
- Elution: Higher pH
- Full cond. range

Flow-through polishing step #2

High hGH passage

AEX membrane FT:
- Mid to higher pH
- Low to medium cond.
Draft downstream process for hGH purification

Capture Select hGH (B/E) $\xrightarrow{\text{pH 3}}$ MM CEX bead 1 $\xrightarrow{\text{pH 8}}$ AEX membrane (FT)

<table>
<thead>
<tr>
<th>Step</th>
<th>Product Yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture Select</td>
<td>95-98</td>
</tr>
<tr>
<td>MM CEX Bead 1</td>
<td>83-95</td>
</tr>
<tr>
<td>AEX Membrane</td>
<td>95-98</td>
</tr>
</tbody>
</table>

Overall process yield: 82-93%

Characterization of purified hGH from draft process (shake flask CCFs)

- HCP: Undetectable (Cygnus *Pichia* HCP ELISA)
- DNA: Undetectable (PicoGreen)
- N-terminus: no Arg leader sequence detected
- Oxidation: Met125, 15.4% (CV 10.6%) > Reference (6.6%, CV 6.4%)
- Deamidation: Asn149, 2.3% (CV 22%) < Reference (7.8%, CV 32%)
Existing off-line analytical techniques

Size exclusion & ion exchange chromatography

Cell based assays

Plate-based fluorescence assays:
- HCP (ELISA)
- Endotoxin

Identity/Purity

Activity/Potency

Quality

Safety

LC/MS

Biacore
Product **identity** assessment using Raman spectroscopy

Miniaturized NIR Raman system

50 µL 5 min

Unique spectra for protein biologics

- hGH
- G-CSF

Sensitivity improvements

120 mW, 830 nm
Ref. hGH in formulation buffer

**Improved Limit of Detection**

< 0.075 mg/mL
(~0.015% [hGH])

Gajendra Singh (Ram)
Product purity assessment by sizing methods

Size exclusion chromatography (SEC)

- ~100 µL
- ~30 min

Nanofluidic size separator

- 10’s of nL
- 30 min


Sung Hee Ko (Han Group)
Product activity / potency assays

Cell-based assays

~100 µL
~1 week

Biacore

~100 µL
~1 h

Biacore sensogram from: http://www.reproduction-online.org/content/127/2/239/F7.expansion.html

PLoSOne 2013, 8, e56168.

Biacore sensogram from: http://www.reproduction-online.org/content/127/2/239/F7.expansion.html
Product activity assessment with carbon nanotubes

Detection scheme works with any analyte binding pair (e.g.):
- Antibody — antigen
- hGH — hGH receptor
- Protein A – mAb
- Target protein/endotoxin – peptide affinity ligand

Justin Nelson (Strano)
dx.doi.org/10.1021/nl201033d | Nano Lett. 2011, 11, 2743–2752
Novel on-line analytical techniques to support Real-Time Release

Identity/Purity

Activity/Potency

Safety

Quality

Endotoxin Assay (work in progress)

Miniature Raman

Microfluidic electrokinetic device

Nanofluidic size separator

Portable CNT sensor platform

Microfluidic binding assays

Inlet Outlet

Nanofluidic size separator

Inlet Outlet

Portable CNT sensor platform
Production and purification of hGH on InSCyT prototype platform
**Human growth hormone (hGH)**

### Dosages

**Indication:** Adult Growth Hormone Deficiency  
Starting range: 0.15-0.3 mg/day  
Max. Dose (< 35 yo): 0.025 mg/kg/day  
(For 70 kg person, 1.75 mg/day)

### Quality Target Product Profile (QTPP)

<table>
<thead>
<tr>
<th>Intended use in clinical setting</th>
<th>Stoppered vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Container closure system</td>
<td>Liquid formulation (not in Nutropin product information sheet)</td>
</tr>
</tbody>
</table>

**Dosing**

- dosage form
- dosage strength(s)
- route of administration
- delivery systems
  - attributes affecting in vivo pK, pD

- Max. Dose (< 35 yo): 0.025 mg/kg/day  
(For 70 kg person, 1.75 mg/day)

**Drug product quality criteria**

- Product purity:  
  - >90% at early phase  
  - >95% at project end

- Product related impurities:  
  - HCP < 1000ppm/dose ([HCP]/[drug])  
  - DNA < 100 pg/dose

**Process-related impurities:** (same for all products)
Automated three-stage chromatography unit

Alan Stockdale, Amos Lu, Aleksander Cvetkovic, Steve Timmick
Prototype breadboard chromatography unit

Buffer Tanks  Affinity Capture  Polishing
Automation GUI for chromatography unit

- **Pressure Sensor at Dev1 ai5: 5.8174**
- **pH Sensor at COM18: 11.1359**
- **Conductivity Sensor at COM17: 0.3919**
- **Photodiode at Dev1 ai2: 0.4071**
- **Pressure Sensor at Dev1 ai6: 0.4384**
- **Conductivity Sensor at COM19: 17.8997**
- **Photodiode at Dev1 ai3: 0.1717**
- **Pressure Sensor at Dev1 ai7: 10.7741**
- **Conductivity Sensor at COM21: 31.3373**
- **pH Sensor at COM22: 8.5499**
- **Photodiode at Dev1 ai4: 0.4327**
Fully-integrated production and purification of hGH

M = Marker (Standard)
S1 = Reference hGH (2 μg load)
B = Blank

1 = Capture FT
2 = Capture FT
3 = Capture Elution
4 = Polish FT
6 = Polish 1 & 2 Elution
7 = Polish Strip

Alan Stockdale, Amos Lu,
Nicholas Mozdzierz, Aleksander Cvetkovic
Cumulative timeline for integrated production

Outgrowth and Production

Affinity capture

Polish 1

Polish 2

Titer: ~100-150 μg/mL

Timing: 40-44 h

Load: 3 h

Wash: 0.17 h

Elute: 0.17 h

Recovery: 0.17 h, 0.12 h

2+ mg (65-80% yield)

Single purified maximum adult dose of hGH achieved in ~ 48 h

Nicholas Mozdzierz (Love)
InSCyT rhGH comparable to reference standard

<table>
<thead>
<tr>
<th>Met14</th>
<th>Oxidation (%)</th>
<th>Reference standard</th>
<th>InSCyT purified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.5%</td>
<td></td>
<td>8.8%</td>
</tr>
<tr>
<td>Met125</td>
<td>9.5%</td>
<td></td>
<td>5.6%</td>
</tr>
<tr>
<td>Met170</td>
<td>9.0%</td>
<td></td>
<td>7.5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Asn149</th>
<th>Deamidation (%)</th>
<th>Reference standard</th>
<th>InSCyT purified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.2%</td>
<td></td>
<td>7.1%</td>
</tr>
<tr>
<td>Asn152</td>
<td>N.D.</td>
<td></td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Bioactivity comparable to WHO standard in cell-based assays

Steve Timmick (RPI), Annie Wang (Hancock), Amanda del Rosario (KI Swanson Biotechnology Center)
Future Vision:
Small, portable & flexible manufacturing facilities
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2015 Vaccines Bioprocess Development & Commercialization Workshop

**When:** June 23-25, 2015  
**Where:** MIT, Cambridge, MA

- Explore critical issues of vaccine development through:
  - Expert lectures (Keynote: David Kaslow, PATH)
  - Manufacturing at-risk case study
  - Networking

- Who should attend:
  - Scientists new to vaccines
  - Scientists and technical managers
  - Project managers, funders and policy-makers


Register by March 1 for Early Bird rate of $1988