Implementation of a novel Plasma Protein Purification Scheme (PPPS™) for high-yield manufacture of therapeutic plasma proteins

Tom Chen, Davida Blackman, Sharon Williams,
Gordon Harris & Steve Burton*

ProMetic Life Sciences Inc.
Company Overview

- Fully integrated biopharmaceutical company (PLI:TSX).
- HQ in Montreal with R&D and operations in the USA, UK and Canada.
- >220 employees.
- Market Cap ~ $1.3 Billion.
- Strategic alliances / licensing agreements with >35 companies world-wide.
- Prometic’s drug candidates in clinical phases:
  - Plasminogen phase I
  - PBI-4050 entering phase II
  - IVIG entering Phase III
  - 3 other Rx expected to enter Phase III 2015/2016.
- Bioseparations business with 17 products used in the production of FDA/EMEA licenced biopharmaceuticals/medical devices.
A New, High Yielding, Affinity Cascade for Sequential Isolation of Plasma Proteins of Therapeutic Value

Christopher Bryant, Dev Baines, Ruben Carbonell, Tom Chen
John Curling, Timothy Hayes, Steve Burton, David Hammond
Plasma Protein Precipitation

Plasma

Cryosupernatant

Cryoprecipitate

100% FVIII
100% Fibrinogen

~50% FVIII
~30% Fibrinogen

~50% FVIII
~70% Fibrinogen
Affinity chromatography offers several major advantages compared to other protein purification techniques:

- Selective binding and elution
- Very pure product in a single unit operation
  - removal of non-target proteins
  - removal of aggregates/truncated/modified isoforms
- Stabilization of bound protein
- High yields of purified product
- Reduced processing time
- Reduced cost of goods
- Applicable to process-scale applications
PPPSTM Process

Filtered plasma

Clotting Factor Capture

Plasminogen Eluate

Flow-through

Flow-through

Fibrinogen Eluate

Immunoglobulin Eluate

C1 INH Eluate

AIPI Eluate

Human Serum Albumin Eluate

Flow-through

Flow-through

Flow-through

Flow-through

Flow-through

Flow-through

Flow-through
Each column step in the PPPS™ backbone may be considered the equivalent of a Cohn precipitation step:

- Plasma Load
- Secondary purification (1 or 2 columns)
- V.I. Step
- Elution [“PPT”]
- Nano Filtration
- UF/DF
- Sterile Filtration
- Bulk Active
- Flow-Through [“S/N”]
<table>
<thead>
<tr>
<th>Cohn (Oncley, Kistler-Nitschmann)</th>
<th>PPPS™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designed for albumin</td>
<td>Designed for most needed proteins</td>
</tr>
<tr>
<td>Ethanol precipitation</td>
<td>Synthetic ligand affinity chromatography</td>
</tr>
<tr>
<td>Liquid-solid separation</td>
<td>Liquid handling only</td>
</tr>
<tr>
<td>Manual precipitate handling</td>
<td>Closed, highly automated systems</td>
</tr>
<tr>
<td>Extensive refrigeration</td>
<td>Ambient temperature (controlled)</td>
</tr>
<tr>
<td>Solvent (ethanol) recycling</td>
<td>Greater requirement for WFI/buffers</td>
</tr>
</tbody>
</table>
PPPS™ Advantages

- Improved yield and purity compared to industry standards.
- Ambient temperature process operation.
- No alcohol used.
- Excellent viral safety; typically 8 logs for non-enveloped viruses and ≥11 logs for enveloped viruses.
- Process can be run in a closed, highly automated system.
- Process amenable to “process analytics” (In-process controls).
- Enables extraction of low abundance/labile proteins in high yield and therefore ideally suited to orphan product purification.
## PPPS™ Capture vs Cohn

### Protein isolation sequences:

<table>
<thead>
<tr>
<th>Cohn</th>
<th>PPPS™</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII (Cryo ppt)</td>
<td>Clotting factors (Column 1)</td>
</tr>
<tr>
<td></td>
<td>Plasminogen (Column 2)</td>
</tr>
<tr>
<td>Fibrinogen (Cryo ppt/Fraction I)</td>
<td>Fibrinogen (Column 3)</td>
</tr>
<tr>
<td>IVIG (Fraction II + III)</td>
<td>IVIG (Column 4)</td>
</tr>
<tr>
<td>A1AT (Fraction IV)</td>
<td>C1INH (Column 5)</td>
</tr>
<tr>
<td>Albumin (Fraction V)</td>
<td>A1AT (Column 6)</td>
</tr>
<tr>
<td></td>
<td>Albumin (Column 7)</td>
</tr>
</tbody>
</table>
Cohn Method 6

PPPS™ Capture vs Cohn

Cohn, E. J. et al., J. Am. Chem. Soc. 68 (1946) 459-475
## PPPSTM Yields vs Cohn

<table>
<thead>
<tr>
<th>Product</th>
<th>Plasma conc./L</th>
<th>PPPSTM bulk-active Yield (g)</th>
<th>Industry Yield* (g)</th>
<th>Increase%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasminogen</td>
<td>0.2 g/L</td>
<td>0.12 g</td>
<td>N/A</td>
<td>_</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>3 g/L</td>
<td>1.9 g</td>
<td>0.9 g</td>
<td>111%</td>
</tr>
<tr>
<td>IVIG</td>
<td>7.5g/L</td>
<td>5.6 g</td>
<td>4 g</td>
<td>40%</td>
</tr>
<tr>
<td>C1INH</td>
<td>0.25 g/L</td>
<td>0.16 g</td>
<td>0.08g/L</td>
<td>100%</td>
</tr>
<tr>
<td>A1PI</td>
<td>1.2 g/L</td>
<td>0.74 g</td>
<td>0.22 g</td>
<td>235%</td>
</tr>
<tr>
<td>HSA</td>
<td>35 g/L</td>
<td>25 g</td>
<td>25 g</td>
<td>0%</td>
</tr>
</tbody>
</table>

ProMetic’s PPPS™ facility in Laval, Canada

- cGMP facility, operational 2013
- Recovery of therapeutic proteins from plasma
- Up to 150,000 L annual plasma capacity
- cGMP clinical trial supplies
- Conformance lots post BLA
- Commercial production of orphan products
- Supply of bulk active proteins to partners
- Provides a validated blue print for partners’ future plants
- Technology showroom and training of partners’ staff
- Technology transfer at scale
ProMetic’s PPPS™ facility in Laval, Canada

- Modern “biotech” facility
- Viral zoning (3 zones with unidirectional materials flow and controlled personnel access).
- Radial flow column technology (Proxcys) for all chromatographic steps.
- Filtration technology (micro, UF, nano, sterile) from Sartorius.
- Extensive use of single-use disposable technology (buffers, column fractions etc.).
Currently 75% of revenues are generated with 4 proteins: Albumin, IgG, Factor VIII and AAT

Plasma Proteins Currently Commercialized

- Factor IX
- IVIG
- Factor VIII
- Fibrin sealant
- Antithrombin III
- Factor VIIa
- Factor XIII
- C1 Esterase Inhibitor
- Protein C
- Protein S
- Factor V
- Plasmin
- Haptoglobin
- Ceruloplasmin

>10
Plasminogen Deficiency

Multi-system disease that affects the prevention and healing of tissue damage.

- Eyes
- Ears
- Sinuses
- Gingiva
- tracheobronchial tree
- genitourinary tract
**Plasminogen Capture**

4 mL column  
Linear flow rate: 50 cm/hour  
Equilibration buffer: 50 mM Sodium Phosphate pH 7.5 (5CV)  
Load: 100 mL filtered human plasma  
Post load wash buffer: 50 mM Sodium Phosphate pH 7.5 (10CV)  
Elution buffer: pH 7.5 (5CV)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic Binding Capacity†</td>
<td>(g/L of adsorbent)</td>
</tr>
<tr>
<td>Recovery</td>
<td>(%)</td>
</tr>
<tr>
<td>Purity</td>
<td>(%)</td>
</tr>
<tr>
<td>Purification Factor</td>
<td></td>
</tr>
</tbody>
</table>

† @ 10% breakthrough

Lane 1: MW Marker  
Lane 2  
Lane 3: Human plasma load  
Lane 4: Flow through fraction  
Lane 5: Elution fraction  
Lane 6:  
Lane 7: MW Marker
## Plasminogen Process Reproducibility

<table>
<thead>
<tr>
<th>Plasminogen</th>
<th>Pilot Scale (100 L)</th>
<th>Scale-up Run (1000 L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Recovery (bulk active)</td>
<td>66%</td>
<td>66%</td>
</tr>
<tr>
<td>Purity</td>
<td>99.5%</td>
<td>99.4%</td>
</tr>
</tbody>
</table>
Plasminogen Clinical Programme

FDA clearance of the IND (October 2014)
FDA acceptance of the Phase II / III surrogate end point for licensure*

Plasminogen IV Clinical program for hypoplasminogenemia

Phase I
~ 6 patients
Safety & PK

Phase II/III
~ 15 - 18 patients
Safety + surrogate end point

H1 2015
H2 2015

BLA
Market

accelerated approval Pathway*

2016

*To secure an accelerated pathway approval, a drug must treat a serious condition, provide a meaningful advantage over available therapies and demonstrate an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit.
Global Plasma Protein Market

(2012; US$15.2bn)

- IVIG: 48%
- HyperImm: 5%
- Albumin: 13%
- Factors pd: 24%
- AAT: 4%
- Fibrin: 3%
- C1: 2%
- Others: 1%
- HyperImm: 5%
## IVIG Process Reproducibility

<table>
<thead>
<tr>
<th></th>
<th>10% IVIG</th>
<th>Pilot Scale (100 L)</th>
<th>Scale-up Run (1000 L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery</td>
<td></td>
<td>70%</td>
<td>74%</td>
</tr>
<tr>
<td>Purity</td>
<td>99.9%</td>
<td>99.9%</td>
<td></td>
</tr>
</tbody>
</table>
ProMetic’s IVIG has demonstrated exceptionally low thrombogenicity/FXIa activity:

<table>
<thead>
<tr>
<th>Sample</th>
<th>TGA Assay 1 FXIa activity (mU/ml vs NIBSC 11/236 std)</th>
<th>Biophen FXIa Assay 1 FXIa activity (mU/ml vs NIBSC 11/236 std)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FXIa Std (NIBSC 11/236)</td>
<td>10,000</td>
<td>-</td>
</tr>
<tr>
<td>ProMetic IVIG; lot 1</td>
<td>&lt;0.05</td>
<td>&lt;1</td>
</tr>
<tr>
<td>ProMetic IVIG; lot 2</td>
<td>&lt;0.05</td>
<td>&lt;1</td>
</tr>
<tr>
<td>High +ve control</td>
<td>134.65</td>
<td>122.95</td>
</tr>
<tr>
<td>Low +ve control</td>
<td>3.56</td>
<td>4.89</td>
</tr>
</tbody>
</table>

CBER Ig-Thrombin Generation Test Protocol 3.0 (manual and automated) Version: May 26 2014
IVIG Clinical Programme

Pre-IND Meeting
FDA – 28 Mar

Engineering runs
GMP runs

IND Submission Q4 2014

Clinical Strategy

Bioequivalence Clinical Trial

End enrollment

End trial

BLA submission
IVIG Consumption Per Capta (2010)

Source: MRB and company reports

(Grams IVIG per thousand population)
Strategic Alliance with Generium Pharmaceuticals

- Construction of a PPPS™ facility in Volginsky, Russia

- Capacity to handle up to 600,000 L of plasma / year
  - Design based on ProMetic PPPS™ Laval facility
  - Facility to be operated by Generium
  - Facility to be built, equipped and operated to meet FDA/EMEA guidelines

- Portion of Generium plant capacity to serve as CMO capacity for Prometic and ProMetic licensees

- Manufacturing of two coagulation factors for Global commercialization

- ProMetic to provide tech transfer services and chromatography resins
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Richard Dodd  
Dev Baines

Our Technology Partners

[Logos for ProMetic and other partners]

PPB, May 2015, Sardinia