DEVELOPMENT OF A DEVICE FOR PRION REDUCTION BASED ON AFFINITY LIGAND TECHNOLOGY

Patrick Gurgel
235th ACS Meeting
April 2008
Pathogen Removal and Diagnostic Technologies - PRDT

- Joint venture of ProMetic and the American Red Cross
- Ruben Carbonell and Robert Rohwer - co-founders
- MacoPharma is a commercial partner
Transfusion Transmission of vCJD

- vCJD is transmissible through blood transfusion
- Four cases have been reported, thus far
- Short incubation times suggest high titer, efficient route
- Study has estimated about 380 possible blood donors as infected with vCJD in the UK
PRDT Solution

- Develop an affinity technology-based device that can reduce endogenous infectivity from red blood cell concentrates (RBCs) while maintaining the integrity of the product
  - Development of ligand selection methodology
  - Screening involving different spikes and ligand sources
  - Infectivity bioassays
  - Hemocompatibility
  - Development of filter device
Ligand selection

Ligand Selection

- Peptide ligands
  - 1-6 amino acid residues investigated
  - Solid-phase libraries
  - Millions of possible sequences

- Polymers
  - Commercially available

- Mimetic ligands
  - Triazine-based ligands
  - Library design based on peptide library results
Ligand Selection

• Primary Screening - Bead Blot

[Diagram showing the process of ligand selection through primary screening using bead blot method.]

Lathrop et al., Anal Biochem (2007), 7, 217,507
Ligand Selection

- Secondary Screening - Western Blots and SDS-PAGE Gels
  - Different spikes
  - Small chromatographic columns

- Human Brain PrPsc
- Hamster Brain PrPsc
- Total protein staining
- 1% spCJD
- PK

Hamster Brain PrPc

© 2008, ProMetic Life Sciences Inc.
Ligand Selection

- Tertiary Screening - Infectivity Study
  - Removal of hamster brain derived infectivity spiked into human leukoreduced red blood cell concentrate
    - Gregori et al. (2006) Transfusion 46:1152-1161
Ligand Selection

- Quaternary Screening - Infectivity Study
  - Removal of endogenous infectivity from scrapie-infected hamster leukoreduced whole blood
    - Gregori et al. (2006) Lancet 368:2226-2230

<table>
<thead>
<tr>
<th></th>
<th>Whole blood</th>
<th>LR WB Challenge</th>
<th>Flow through</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected/Total animals</td>
<td>21/47</td>
<td>15/99</td>
<td>0/100</td>
</tr>
<tr>
<td>Poisson Titer ID/ml</td>
<td>11.8 ± 2.2</td>
<td>3.3 ± 0.8</td>
<td>&lt; 0.2 ± 0.2</td>
</tr>
<tr>
<td>Reduction</td>
<td></td>
<td></td>
<td>&gt; 1.2 log₁₀</td>
</tr>
<tr>
<td>%Leukoreduction</td>
<td></td>
<td>72%</td>
<td></td>
</tr>
</tbody>
</table>

Device removed all detectable infectivity from challenge
Device Development

- Particle-impregnated membrane (PIM) produced as below
- Multiple layers of PIM are stacked, fused together and encased, forming the final device
PIM Characterization

- SEM of particle-impregnated membrane
PIM Characterization

- Binding Isotherms
  - PIM has the same binding behavior as a packed bed column

<table>
<thead>
<tr>
<th>Material</th>
<th>$Q_{\text{max}}$ [mg/g]</th>
<th>$K_d$ [M]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIM (dynamic)</td>
<td>68.1</td>
<td></td>
</tr>
<tr>
<td>Resin (dynamic)</td>
<td>72.9</td>
<td>$\sim 10^{-5}$</td>
</tr>
<tr>
<td>Resin (static)</td>
<td>71.8</td>
<td></td>
</tr>
</tbody>
</table>
PIM Characterization

- Higher permeability than packed beds
  - Allows for the passage of particulate material, such as red blood cells
Binding of PrP to device

- Binding of spiked PrP in RBC by resin columns and P-CAPT device
  - Packed columns and device bind PrPsc similarly

Resin in columns

P-CAPT
Hemocompatibility

- Hemocompatibility of resin with whole blood showed no negative effects
  - No hemolysis
  - No platelet activation
  - No complement activation
  - No factor VII activation
- RBC yields are within the acceptable limits
Device Development

- Approved for commercialization in Europe (CE mark)
- Efficacy of Removal
  - >99.9% (> 3 log 10) reduction of exogenous brain spike infectivity in red blood cell concentrates
  - >1.2 log 10 reduction of endogenous whole blood infectivity
- No impact on red blood cells or activation of coagulation factors, platelets or complement
- Neoantigenicity and Red Cell Recovery and Survival studies have been completed
- No adverse effects detected in Human Safety trials
Prion Removal from Blood Products

I
IgG

II
buffer
- - + +

III
HSA
- - + +

PK

(a)

PK

(b)
Acknowledgements

- **NCSU**
  - Ruben Carbonell, Omon Herigstad

- **VAMC/UM**
  - Robert Rohwer, Luisa Gregori, Brian Lambert

- **American Red Cross**
  - David Hammond, Julia Lathrop, Melanie Poncheri, Liliana Gheorghiu

- **ProMetic**
  - Peter Edwardson, Steve Burton, Yong Zheng