DEVELOPMENT OF A DEVICE FOR REDUCTION OF PRION INFECTIVITY FROM RED BLOOD CELL CONCENTRATE

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Pathogen Removal and Diagnostic Technologies - PRDT

- Joint venture of ProMetic and the American Red Cross
- R. Carbonell and R. Rohwer are co-founders
- MacoPharma is a 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
- Leukofiltration removes only 50-70% of infectivity
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
Ligand Selection

- Secondary Screening - Western Blots and SDS-PAGE Gels
  - Different spikes
  - Small chromatographic columns
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>Infected/Total animals</th>
<th>Whole blood Challenge</th>
<th>LR WB Challenge</th>
<th>Flow through</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poisson Titer ID/ml</td>
<td>21/47</td>
<td>15/99</td>
<td>0/100</td>
</tr>
<tr>
<td></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 log_{10}</td>
</tr>
<tr>
<td>%Leukoreduction</td>
<td></td>
<td></td>
<td>72%</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
Device Development
PIM Characterization

- SEM of particle-impregnated membrane

~200 µm
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>
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</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
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
P-Capt Device

- Approved for commercialization in Europe (CE mark)
- Efficacy of Removal
  - >3 log_{10} reduction of exogenous brain spike infectivity in RBC containing 2,000,000 times the level of infectivity expected in RBC
  - Removal of all detectable endogenous infectivity from whole blood
- 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 Plasma Products

- One presumed plasma-derived transmission case to date
  - Patient was a hemophiliac
- Low level of contamination (approx. 3 ID$_{50}$/ml based on 263K hamster studies)
- Large plasma pools (many 1000’s of units)
- Dilution of infectivity does not eliminate the risk
- Precautionary measures for a potential risk
Prion Removal from Plasma Products

PRDT resins challenged with 25% HSA

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Resin 1</th>
<th>Resin 2</th>
<th>Resin 3</th>
<th>Challenge</th>
<th>Resin 4</th>
<th>Resin 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>PK</td>
<td>-</td>
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PRDT Resin 3 challenged with 3% IgG

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In Conclusion

- P-Capt, a device for removal of prion infectivity from Red Blood Cells
  - Demonstrated infectivity removal at endogenous levels
  - Device is safe and effective
  - Currently available for adoption by Blood Services
Acknowledgements

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

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- **MacoPharma**
  - Iwona Walicka, Chryslain Sumian