Pathogen Reduction: Where Are We?

Blood Matters
Halifax
November 8, 2013

Dr. Dana Devine, Chief Medical & Scientific Officer
Reducing the Risk of Transfusion-Transmitted Infection

• What do we do now?
  – Donor history
  – Donor examination
  – **Testing**
  – Diversion of initial blood flow
  – Leukoreduction
  – Post donation information
  – Limit exposures
A Chronology of Donor Testing for Infectious Disease

1938: Syphilis
1970: HBsAg
1975: Anti-HIV
1980: Anti-CMV
1985: Anti-HTLV
1990: Anti-HCV
1995: HIV & HCV NAT
2000: HIV p24 Ag
2005: Anti-HBc
2010: WNV NAT

Other Pathogens Confirmed to Be Transmitted by Transfusion: Dengue, HHV-8, Simian Foamy virus, Chikungunya, HEV, Babesia, Erhlichia, Malaria

Will it ever stop? Certainly not by taking this 'reactive' approach to threats.
Pathogen Inactivation of Blood

An alternative strategy to additional testing.
What’s Pathogen Inactivation?

• A process for killing living cells including
  – Viruses
  – Bacteria
  – Parasites

• In the blood industry it is an established collection of methodologies to treat fractionated products during manufacture. It is a highly effective way to increase fractionated product safety as evidenced by over 20 years with no known transmissions of HIV, HCV or HBV.

• Progress is being made to apply it to increasing the safety of fresh products (RBCs, platelets, plasma).
• Treatment of plasma or platelet products post-production to improve safety profile

• Products in the global marketplace
  – Cerus: psoralen additive + UV light; unreacted psoralen must be removed by filtration after treatment. Works for either platelets or plasma.
  – Caridian: riboflavin additive + UV light; no removal after treatment is required. Treat either platelets or plasma.

• PRT plasma can also be purchased as a pooled product; Octaplasma is currently available in Canada for limited use.
Landscape – PRT Platelets

• Non-North America (becoming standard of care); overall 50% of European countries have implemented or are implementing some form of PRT
  – Implemented to a significant degree in many European countries: Belgium (by law), France, Poland
  – Implementation underway or under evaluation in Germany, Italy, Netherlands, Australia
  – Recommended but no funding yet in UK

• US
  – Terumo and Cerus both received FDA agreement on path to licensure in spring of 2013
## Pathogen reduction treatment

### System name

<table>
<thead>
<tr>
<th>System name</th>
<th>Mirasol</th>
<th>Intercept</th>
<th>Theraflex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Company</strong></td>
<td>TerumoBCT, Lakewood, CO</td>
<td>MacoPharma, Mouvexaux, France</td>
<td>Cerus Cooperation, Concord, CA</td>
</tr>
<tr>
<td><strong>Photosensitizer</strong></td>
<td>Riboflavin (50µM)</td>
<td>Amotosalen (150µM)</td>
<td>-</td>
</tr>
<tr>
<td><strong>UV type</strong></td>
<td>UVA + UVB (280 – 400 nm; max at 313 nm)</td>
<td>UVA (320 – 400 nm)</td>
<td>UVC (254 nm)</td>
</tr>
<tr>
<td><strong>Energy</strong></td>
<td>6.24 J/ml for 10 min</td>
<td>3 J/cm² for 3 min</td>
<td>0.2 J/cm² for &lt; 1 min</td>
</tr>
<tr>
<td><strong>Mechanism</strong></td>
<td>Oxidization of guanosine bases causing single strands breaks</td>
<td>Covalent crosslinking between nucleic acids</td>
<td>Cyclobutane pyrimidine dimers causing blockage of nucleic acid transcripts</td>
</tr>
<tr>
<td><strong>Platelet storage medium</strong></td>
<td>Plasma, SSP+</td>
<td>Intersol and SSP+</td>
<td>SSP+</td>
</tr>
</tbody>
</table>

What effect does PRT have on the product?

– PRT is known to cause some platelet injury
  • Aubuchon, Transfusion 2005: Radiolabelled studies: % Recovery 24.8% less; Survival 26.8% less
  • Zone of non-inferiority (Mirasol) was set at 20% of reference.

– Expect this reduction to be reflected in CCI

– Injury is not specific to a particular PRT
  • Eurosprite: 12% decrease in CCI_{1\text{hour}}; 30.2% decrease in CCI_{24\text{hour}}
  • SPRINT: 30.6% decrease in CCI_{1\text{hour}}; 33.7% decrease in CCI_{24\text{hour}}

– This is the cost of increased safety.
Basis of the Mirasol technology

• **Riboflavin + UV Light (UVA and UVB):**
  - Riboflavin modifies nucleic acids upon exposure to light\(^1,2,3\)
  - When applied to blood, this mechanism renders pathogens and leukocytes unable to replicate
  - Chemistry is not based on covalent modification. Electron transfer chemistry
  - Riboflavin and its photo-products are non-toxic\(^4\) and non-mutagenic\(^4,5\) and are naturally present in normal blood\(^6\)

1. Kuratomi & Kobayashi 1977
2. Speck et al. 1975
4. Reddy et al., 2008
5. Kale et al. 1992
6. Hardwick et al. 2004
Platelets Treated and Stored in Plasma

Transfer product to illumination bag
Add riboflavin solution
Illuminate 6 to 10 minutes
Split as needed‡
Transfuse or store for up to 5 days

Product Requirements

<table>
<thead>
<tr>
<th>Source, Apheresis Collection</th>
<th>Apheresis platelets in ACD-A</th>
<th>Whole blood (WB)-derived platelets in CPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>170 mL to 360 mL</td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>0.8 to 2.1 x 10^6/µl</td>
<td></td>
</tr>
<tr>
<td>Yield*</td>
<td>Up to 7.5 x 10^{11}</td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td>0.7 to 2.1 x 10^6 platelets/µL (max 5.1 x 10^{11}/bag)</td>
<td></td>
</tr>
</tbody>
</table>

Process Requirements

<table>
<thead>
<tr>
<th>Apheresis platelets: treat within 2 to 22 hrs of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB-derived platelets: treat within 8 hrs of pooling, but no more than 32 hrs after WB collection</td>
</tr>
</tbody>
</table>

‡ DOUBLE+ kit with second storage bag available.

*As determined by the upper limits for platelet concentration and volume; different limits may apply in a given blood center.
Effort to Bring PRT to Canada

• CBS has opted to engage in a strategic partnership with Terumo BCT to assist them with licensure of the Mirasol® Pathogen Reduction Technology for the following reasons:
  – Requires minimal change to our current process
  – Riboflavin (Vitamin B2) has fewer safety concerns
  – Process time is very rapid (approx. 12 minutes/unit)
  – Technology looks promising for RBC/WB
  – We have an established working relationship with Terumo BCT
  – This joint effort does not bind CBS to this specific manufacturer’s technology.
Pathogen Reduction Evaluation & Predictive Analytical Rating Score (PREPAReS)

- Initiated in the Netherlands in November 2010
- Sponsored by the Sanquin Blood Supply Foundation, the national blood operator in the Netherlands and financially supported by Terumo BCT.
- Canadian Blood Services’ role in this study is to produce, at its Ottawa manufacturing site only, the Mirasol-treated pooled platelets strictly for use in PREPAReS by the participating hospitals.
Acquiring clinical data for Mirasol PRT

• Clinical Trial Overview
  – Terumo BCT has added CBS to the PREPAREs clinical trial underway in the Netherlands (Sanquin);
    • Terumo holds the Canadian CTA.
    • Nancy Heddle (McMaster) is the principal investigator
    • CBS produces the Mirasol-treated platelet concentrates for the trial sites
  – Clinical trial primary objective: Determination of clinical non-inferiority of Mirasol-treated Pooled Platelets compared to “regular” Pooled Platelets
  – Study design: Randomized, single blinded, multicenter, non-inferiority trial
  – Patient population: Hospitalized hemato-oncology patients
PREPAReS

• **Patient population:**
  - Over the course of the study, approximately 618 patients will be recruited from participating sites in Canada, the Netherlands and Norway.
  - The inclusion criteria for patients are as follows:
    - Hospitalized hemato-oncological patients,
    - aged $\geq 18$ years,
    - at the time of consideration, expected to require $\geq 2$ platelet transfusion,
    - have signed an informed consent form.
PREPAReS Data Collection

• Study Data:
  – Daily bleeding assessments
  – Daily platelet counts.
  – Coagulation testing and antibody screens will be performed weekly.

• The study period will last for 6 weeks from the first platelet transfusion (post-randomization) unless one of the following occurs first: hospital discharge, greater than 7 days without requirement for platelet transfusion, patient death, or patient request for withdrawal.

• The efficacy of the two products will be evaluated as the percentage of patients with a WHO standardized grading scale of bleeding severity score equal or greater than 2. (i.e.: from mild blood loss to clinically significant bleeding).
  – 1-hour CCI and 24-hour CCI will be calculated, as well as the percentage of days that a patient has ≥ grade 2 bleeding score.
Canadian Arm of PREPARES

– Terumo holds the Canadian Clinical Trial Authorization

– Prof. Nancy Heddle (McMaster Univ) is the principal investigator of the Canadian study

– CBS produces the Mirasol-treated platelet concentrates for the trial sites. We have now been producing Mirasol platelets for 12 months.
**Current Buffy Coat Process (4 BC)**

1 LP

Add all volume of the largest compatible male plasma

Pool

4 BC

- Centrifuge
- Extract using on Compomat (settings differ between 4BC and 5BC)

Plt. Pool

Ready for transfusion

**Clinical Trial Process (5 BC)**

1 LP

Add a portion of a compatible male plasma

Pool

5 BC

5BC Plt. Pool

Ready for Mirasol treatment
Product Overview

- Standard of care platelet pool (4BC)
- Mirasol treated platelet pool (5BC)
- Mirasol bag (UV Illumination/Storage bag)
- Apheresis platelet bag
### Product Overview

**What is in the bag?**

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Mirasol-treated pooled platelet (5 BC pool)</th>
<th>Standard of care pooled pooled platelet (4 BC pool)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average residual white blood cell (X 10^6 rWBC /unit)</td>
<td>0.0096 ± 0.0242 (n = 83)</td>
<td>0.011 ± 0.11 (n = 1322)</td>
</tr>
<tr>
<td>Average unit volume (mL/unit)</td>
<td>338 ± 17 (n = 91)</td>
<td>343 ± 31 (n = 1298)</td>
</tr>
<tr>
<td>pH</td>
<td>6.9 ± 0.17 (n = 85)</td>
<td>7.4 ± 0.07 (n = 120)</td>
</tr>
<tr>
<td>Average yield (X 10^9 plt/unit)</td>
<td>399 ± 59 (n = 91)</td>
<td>318 ± 102 (n = 1298)</td>
</tr>
</tbody>
</table>
Participating Study Sites

- Juravinski Cancer Centre (Hamilton) 20
- Sunnybrook Health Sciences Centre (Toronto) 1
- London Health Sciences Centre 6
- Ottawa General Hospital 1
- Kingston General Hospital 0
- Total recruitment to date = 28 patients
Currently supplying Ontario study sites from our Ottawa production lab. Transportation validated for product shipments to Nova Scotia.
The CBS Team at the completion of Mirasol process validation

Back row: Colleen, Danielle, Susan, Raj, Lynne, Heather, Tamiko, Lynne, Chantal
Front row: Karen, Kim, Myrna, Tracy, Terry
Other Progress in PRT

• Ideally, we would like to use PRT to extend platelet shelf-life. Thus it is important to understand the effects of PRT on platelet function in order to develop ways to minimize any negative effects.
The Mirasol study design

Two-arm pool-and-split study

- Pool
- Split
  - Untreated + riboflavin + UV-A/B
  - Sample collection on Day 0, 1, 4 & 6
  - Storage

Examples of *in vitro* data

- CD62 Activation
- pH
Arrays Identify Effects of Mirasol on Platelet Signalling
Treatment with p38 kinase inhibitor ameliorates the effect of Mirasol treatment
Phosphorylation of VASP at Ser239 by Mirasol treatment correlates with platelet activation/degranulation

Apheresis platelet concentrates

Buffy-coat platelet concentrates

Phosphorylation of VASP at Ser239 by Mirasol treatment correlates with platelet activation/degranulation.
Proteomic and biochemical assessment of the Mirasol treatment: Current model of molecular action

UV/Mirasol

? → p38 → Akt

p38 → VASP

? → GSK3β

HSP27 → actin dynamics

Glycogensynthase

Glucose → Glycogen
# Cytokine release upon Mirasol PRT treatment

<table>
<thead>
<tr>
<th></th>
<th>day 1</th>
<th>day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>day 1</th>
<th>day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>1.12 ± 0.25</td>
<td>1.07 ± 0.12</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.18 ± 0.13</td>
<td>1.17 ± 0.08</td>
</tr>
<tr>
<td>MCP-1</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>MCP-2</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>MCP-3</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>MCSF</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>MDC</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>MIP1-β</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>RANTES</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>SCF</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>TARC</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>TFG-β1</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>TNF-β</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>EGF</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>IGF1</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td>ANG</td>
<td>1.16 ± 0.22</td>
<td>1.15 ± 0.12</td>
</tr>
</tbody>
</table>

**Ratio**

- Ratio 0-1.25
- Ratio 1.26-1.50
- Ratio 1.51-2.00
- Ratio >2.01

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**Key Cytokines**

- **PDGF**: 1.06 ± 0.20
- **EGF**: 2.22 ± 0.91

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**Additional Information**

- **PDGF** and **EGF** are highlighted as key cytokines for their significant release in response to Mirasol PRT treatment.

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**References**

- [Canadian Blood Services](www.blood.ca) - "it's in you to give"
Cytokine release upon Mirasol PRT treatment

**EGF**

**PDGF-BB**

**Ena78**

**RANTES**

(normalized concentration)

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**Day 0**

- Con: [Data Point]
- Irr: [Data Point]

**Day 1**

- Con: [Data Point]
- Irr: [Data Point]

**Day 4**

- Con: [Data Point]
- Irr: [Data Point]

**Day 7**

- Con: [Data Point]
- Irr: [Data Point]
Cytokine release upon Mirasol PRT treatment:
Effect on endothelial cells
Those Who Do the Research…

And those who fund the research:
- Canadian Blood Services
- The Burroughs Wellcome Fund
- Canada Foundation for Innovation
- CIHR
- NSERC

Dr. Peter Schubert
Thank you for your attention. Questions are welcome!