Pathogen Inactivation of Whole Blood

Raymond P. Goodrich, PhD
Vice President, Scientific and Clinical Affairs
Chief Science Officer, Blood Bank Technologies
CaridianBCT
Teetering And Tottering On The
The precariousness of blood safety
- WNV
- XMRV
- Chikungunya Virus
- Dengue Virus
- Japanese Encephalitis Virus
- Yellow Fever
- Babesia
- Malaria
- Chagas
- SARS
- Influenza
- Q Fever
- GVHD
- Alloimmunization
- Donor Deferrals and supply
- Agent X, Y and Z
- Other people’s DNA
Teetering And Tottering On The

- The precariousness of blood safety
  - WNV
  - XMRV
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  - Q Fever
  - GVHD
  - Alloimmunization
  - Donor Deferrals and supply
  - Agent X, Y and Z
  - Other people’s DNA

- The precariousness of decision making
  - Potential to decrease product efficacy
  - Potential to increase adverse events
  - Potential to decrease product availability
  - Potential to introduce new risks due to blood product alteration, toxicities due to new chemical entities in the blood supply, or new sources of processing errors
  - Potential that any decision will be criticized as being at fault and wrong, regardless of the facts
Basis of the Mirasol PRT System 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 unable to replicate
  - Not chemistry based on covalent modification

- 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. Piper et al.,2001
5. Kale et al. 1992
6. Hardwick et al. 2004
Mirasol PRT Process for Platelets and Plasma

Connect & transfer product to Illumination bag

Add riboflavin solution

Illuminate (4–10 min)

Transfuse or store for up to 5 days

Total process time ~ 12 min

Connect & transfer product to illumination bag

Add riboflavin

Illuminate 6–10 min

Transfer to plasma storage bag

Transfuse or store for up to 2 years

Total process time ~ 12.8 min

lørdag 11. august 2012
Mirasol Development Program –

Prototype Design – in vitro studies

Human In vivo recovery & survival trial (US, 2 sites)

Patient clinical trial (France)

Exploratory Human trial – in vivo recovery & survival (South Africa)

RBC / Whole Blood work (ongoing)
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Patient clinical trial (France) Oct, 2007

Platelets CE Mark

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Patient clinical trial (France) Oct, 2007

Platelets CE Mark Oct, 2008

CE Mark Platelets in PAS Aug, 2008

Plasma CE Mark

RBC / Whole Blood work (ongoing)

... 2001 2002 2003 2004 2005 2006 2007 2008
Development Program Overview:

Mirasol PRT for Platelets
- Design Exploratory
  - Recovery and Survival
  - Sample Radiolabelled Platelets
  - Healthy Volunteers (N = 18)
  - Cross-Over Design

Core Toxicology Studies
- Ames (17.3 J/ml)
- CHO (17.3 J/ml)
- Acute Toxicity (Rat) (17.3 J/ml)
- Neoantigenicity (17.3 J/ml)
- Ames (6.2 J/ml, ± γ)
- Acute Toxicology (Rat) (6.2 J/ml, ± γ)
- Acute Toxicology (Dog) (6.2 J/mL, ± γ)
- Neoantigenicity (6.2 J/ml, ± γ)
- Acute Toxicology (Saline Bolus Study, Riboflavin and Lumichrome)
- Antigen Profiling (Capture P Assay, 6.2J/ml)
- Embryo – Fetal Development (6.2 J/ml)
- MMN (6.2J/ml)
- CHO (6.2J/ml)
- $^{14}$C-riboflavin PK (6.2 J/ml)
- $^{14}$C-riboflavin Protein Binding (6.2 J/ml)
- Photochemistry (6.2J/ml, ± γ)
- Subchronic Toxicology with Acute Toxicology Arm including Histopathology (Dog) (6.2 J/ml)
- Hemocompatibility (6.2 J/ml)
- Cytotoxicity (6.2 J/ml)

Studies of Platelet and Plasma Protein Function
- Platelet In Vitro Function Tests (17.3 J/ml)
- Platelet Functionality (Escolar) (17.3 J/ml)
- Platelet In Vitro Function Tests (6.2 J/ml, ± γ)
- Complement Protein Activation (6.2 J/ml, ± γ)
- Plasma Protein Profile (6.2 J/ml, ± γ)

No single study, but rather the culmination of results from these studies over 8 years of evaluation led to the CE Mark

CE Mark
What the Testing Results

**In-Vivo Toxicology**

- There is a strong in-vivo history\(^1\) and additional (in vitro & in vivo) CaridianBCT

- No adverse events were attributed to the use of Mirasol-treated platelets in a controlled clinical trial\(^2\)

**In-Vitro\(^†\) & In-Vivo\(^*\) Toxicology\(^3\)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Toxicity(^*)</td>
<td>Negative</td>
</tr>
<tr>
<td>Neoantigenicity(^*)</td>
<td>Negative</td>
</tr>
<tr>
<td>Ames Mutagenicity(^†)</td>
<td>Negative</td>
</tr>
<tr>
<td>CHO Clastogenticity(^†)</td>
<td>Negative</td>
</tr>
<tr>
<td>Cytotoxicity(^†)</td>
<td>Negative</td>
</tr>
<tr>
<td>Reproductive Toxicity(^*)</td>
<td>Negative</td>
</tr>
<tr>
<td>Subchronic Toxicity(^*)</td>
<td>Negative</td>
</tr>
<tr>
<td>MMN Genotoxicity(^*)</td>
<td>Negative</td>
</tr>
<tr>
<td>Blood Compatibility(^†)</td>
<td>Passed</td>
</tr>
<tr>
<td>Leachables and Extractables(^†)</td>
<td>Passed</td>
</tr>
</tbody>
</table>

\(^2\) MIRACLE Clinical Study (2010) Transfusion.
\(^3\) Reddy et al., Transfusion Medicine Reviews 2008; 22: 133–153
## Viral Reduction Results (Infectivity Studies)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Model used</th>
<th>Log Reduction</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV, active</td>
<td>Intracellular human HIV</td>
<td>5.9</td>
<td>Enveloped</td>
</tr>
<tr>
<td>HIV, latent</td>
<td>Cell-associated human HIV</td>
<td>4.5</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Hepatitis C Virus</td>
<td>West Nile Virus</td>
<td>≥5.1</td>
<td>Enveloped</td>
</tr>
<tr>
<td></td>
<td>Sindbis Virus</td>
<td>3.2</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Hepatitis B Virus</td>
<td>Human Hepatitis B</td>
<td>≤4.5*</td>
<td>Enveloped</td>
</tr>
<tr>
<td></td>
<td>Pseudorabies Virus</td>
<td>2.5</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Rabies Virus</td>
<td>Vesicular Stomatitis Virus</td>
<td>≥6.3</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Influenza Virus</td>
<td>Influenza A Virus</td>
<td>≥5.3</td>
<td>Enveloped</td>
</tr>
<tr>
<td></td>
<td>Inf. Bov. Rhinotracheitis Virus</td>
<td>2.1</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Human CMV</td>
<td>≥6.0**</td>
<td>Enveloped</td>
</tr>
<tr>
<td></td>
<td>Inf. Bov. Rhinotracheitis Virus</td>
<td>2.1</td>
<td>Enveloped</td>
</tr>
<tr>
<td>Human B-19 Virus</td>
<td>Porcine Parvovirus</td>
<td>≥5.0</td>
<td>Non-Enveloped</td>
</tr>
<tr>
<td>Hepatitis A Virus</td>
<td>Human Hepatitis A</td>
<td>1.6</td>
<td>Non-Enveloped</td>
</tr>
<tr>
<td></td>
<td>Encephalomyocarditis virus</td>
<td>3.2</td>
<td>Non-Enveloped</td>
</tr>
<tr>
<td>Chikungunya Virus</td>
<td>La Reunion Clinical Isolate</td>
<td>2.1 (Plasma)</td>
<td>Enveloped</td>
</tr>
</tbody>
</table>

*Based on PCR Method. The system has been shown to inactivate up to 29,400 gEq/mL; LOD 30 gEq/mL
**Based on animal infectivity model using infected WBC and cell free CMV
All validated under standard use conditions as per IFU
### Parasite Study Data (Infectivity Studies)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>$\geq$ 3.2</td>
</tr>
<tr>
<td><em>Trypanosoma cruzi</em></td>
<td>$\geq$ 5.0</td>
</tr>
<tr>
<td><em>Leishmania major</em></td>
<td>$\geq$ 4.0</td>
</tr>
<tr>
<td><em>Babesia microti</em></td>
<td>$\geq$4.0 to $\geq$5.0(^1)</td>
</tr>
<tr>
<td><em>Orientia tsutsugamushi</em></td>
<td>$\geq$5.0(^1)</td>
</tr>
</tbody>
</table>

The $>$ symbol is used to indicate inactivation to the limits of detection. Levels of inactivation could be higher but the ability to quantify the full extent of pathogen reduction is limited by the assay sensitivity limits.

\(^1\) Tested in an animal infectivity model. No disease transmission observed with treated products

All validated under standard use conditions as per IFU
Bacteria Studies – Summary

Significantly outperforms bacterial screening

Performed under standard use conditions as per IFU

Foley et al. (IBTS)
Benjamin et al. (ARC)
de Korte et al. (Sanquin)
Dumont et al. (PASSPORT)

Goodrich et al. Transfusion (2009)
## Inactivation of WBC in Blood Products

<table>
<thead>
<tr>
<th>Assay System</th>
<th>Extent of Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong></td>
<td><strong>Mirasol PRT Results</strong></td>
</tr>
<tr>
<td>Limiting dilution assay (LDA)</td>
<td>&gt;6 log&lt;sub&gt;10&lt;/sub&gt; reduction of viable T-cells</td>
</tr>
<tr>
<td>DNA modification</td>
<td>Approximately one event per 245-1850 base pairs</td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td>Amplification inhibited of fragments larger than 245-1850 base pairs</td>
</tr>
<tr>
<td>Cytokine synthesis</td>
<td>Elimination of IL-8, IL-1β synthesis during storage</td>
</tr>
<tr>
<td></td>
<td>AND</td>
</tr>
<tr>
<td></td>
<td>Elimination of cytokine (inflammatory and TH1/TH2) synthesis upon WBC activation</td>
</tr>
<tr>
<td></td>
<td>(IL-2, IL-4, IL-5, IL-10, TNF-α, IFN-γ, IL-8, IL-1β, IL-6, IL-12p70)</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td>Prevention of TA-GVHD in a murine model</td>
</tr>
<tr>
<td>Murine transfusion model</td>
<td>Prevention of antigen presentation in vitro and allo-antibody formation in animal models</td>
</tr>
<tr>
<td><strong>In vitro and in murine allo-immunization model</strong></td>
<td>Prevention of antigen presentation in vitro and allo-antibody formation in animal models</td>
</tr>
</tbody>
</table>

Outperforms gamma irradiation
Mirasol in the World – Status Update (April 2011)

- United States – DOD funded Mirasol Whole Blood studies (multiple in vitro & in vivo studies)
- EMEA – Routine use in 17 countries. Multiple validations underway. (Platelets and Plasma)
- 2 Post-market studies ongoing, 1 completed 3 Clinical trials started in 2010
- Korea – in vitro Platelet studies ongoing
- Malaysia, Singapore – in vitro Platelet studies
- Russia – 41 illuminators placed
- Japan – in vitro Platelet studies ongoing
- Canada – Planning and Regulatory Submissions for Clinical Trial
- United States –
- Canada – Planning and Regulatory Submissions for Clinical Trial
- Russia – 41 illuminators placed
- Japan – in vitro Platelet studies ongoing

- Total of 104 Mirasol illuminators sold /placed in 18 countries worldwide
- Over 120,000 Mirasol treatment sets distributed for platelets and plasma
- Post-market surveillance on over 10,000 platelets and 20,000 FFP transfusions with no Mirasol–related adverse events reported
Clinical Trial and Surveillance Data

- Surveillance data on over 30,000 transfusions (Platelets and FFP)
  - No reports of Adverse Events related to use of Mirasol PRT treated products
  - No reports of TRALI or ALI reported
  - No reports of bacteria contamination in products
  - No reports of increased bleeding or increased platelet product utilization after introduction
  - Clinical measures on platelet products within historical ranges
  - Correction of PT and APTT within expected ranges following FFP use (no difference from historical and real-time untreated products)
## PRT Clinical Trials in Process

<table>
<thead>
<tr>
<th>Trial Name and Location</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>“PREPARES” Trial (platelets in plasma), sponsored by Sanquin in the Netherlands.</td>
<td>Second site in The Netherlands initiated in early March. Third site ready to start. Potential to add international sites.</td>
</tr>
<tr>
<td>“IPTAS” (platelets in PAS), 6 Sites in Italy, sponsored by Italian government.</td>
<td>All three Mirasol sites are up and running</td>
</tr>
<tr>
<td>“PRESS” (7-day storage of platelets in PAS), Denmark, sponsored by CaridianBCT.</td>
<td>Patient enrollment proceeding. Interim analysis on schedule.</td>
</tr>
</tbody>
</table>
White blood cell inactivation after treatment with riboflavin and ultraviolet light

Susanne Marschner, Loren D. Fast, William M. Baldwin III, Sherrill J. Slichter, and Raymond P. Goodrich

Direct Antigen presentation

Donor-derived APC
Donor MHC
Donor peptide

Host-derived T cell

Impaired after Mirasol treatment

Indirect Antigen presentation

Host-derived APC
Host MHC
Donor peptide

Host-derived T cell

Possibly not affected by Mirasol treatment?

Fig. 5. Model of direct and indirect presentation of donor antigen to host immune cells (adapted from http://biomed.brown.edu/courses). MHC = major histocompatibility complex; TCR = T-cell receptor.
Historical Data (Slichter et al. Blood (2005) 105; 4106–4114)
Evaluation of the Safety and Performance of Platelet Transfusion Products Treated with Mirasol® Pathogen Reduction Technology

Plot of Average Slope of the 1-Hour and 24-Hour CCI Values by Number of Transfusions

Probability values are based on testing the null hypothesis that the slope is zero.
Transfusion groups

1. Treated Platelets with leukocytes \(10^6\)
2. Untreated platelets with leukocytes
3. Saline

---

CsA 5mg/kg
3 times/week
Untreated Platelet Products;

IgM and IgG levels over time with markers indicating graft rejection day 1 and day 1 week 1.

lørdag 11. august 2012
Mirasol Treated Products:

Graft >26 days
Graft Survival Post-

Asano et al. (2007)
Transplantation 84: 1174–1182
The TRAP Trial evaluated filtration leukocyte-reduction (F-LR) and UV-B irradiation (UV-BI) to prevent platelet alloimmunization in AML patients receiving induction chemotherapy. Inclusion of UV-BI was based on a 45% rate of preventing platelet alloimmunization in our dog platelet transfusion model. UV-BI was 79% successful in the TRAP Trial. The lower success rate in the dog was probably because we use normal immunocompetent recipient dogs versus chemotherapy-induced immunosuppressed patients. The residual rate of alloimmunization in the TRAP Trial using either F-LR or UV-BI was still 17% to 21%, suggesting that better prevention methods are needed.
**FILTER LEUKOREDUCTION PLUS \( \gamma \)-IRRADIATION OR MIRASOL TREATMENT**

<table>
<thead>
<tr>
<th>Filtration</th>
<th>( \gamma )-IRRADIATION</th>
<th>MIRASOL TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># Donors Accepted*/# Recipients (%)</td>
<td># Donors Accepted*/# Recipients (%)</td>
</tr>
<tr>
<td>Pall PL1B**</td>
<td>0 / 5 (0%)</td>
<td>7 / 7 (100%)</td>
</tr>
<tr>
<td>Fenwal PLS-5A***</td>
<td>2 / 6 (33%)</td>
<td>7 / 8 (88%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2 / 11 (18%)</td>
<td>14 / 15 (93%)</td>
</tr>
</tbody>
</table>
PREVENTION OF ALLOIMMUNE PLATELET REFRACTORINESS IN A DOG MODEL REQUIRES BOTH REMOVAL AND INACTIVATION OF CONTAMINATING DONOR WHITE BLOOD CELLS

S.J. Slichter, MD; E. Pellham, BS, MBA; S.L. Bailey, BS; T. Christoffel, MT(ASCP); L. Gaur, PhD; Y. Latchman, PhD; K. Nelson, PhD; D. Bolgiano, MS

Puget Sound Blood Center and University of Washington; Seattle, Washington USA

- Combining $\gamma$-irradiation with F-LR does not improve the results achieved with F-LR alone.
- Combining Mirasol treatment with F-LR almost completely prevents alloimmune platelet refractoriness.
- If Mirasol-treated/F-LR platelets were given to immunosuppressed patients, it is anticipated that alloimmunization would be completely prevented.

Presented at ASH Meeting, 2010
Mirasol System for Whole Blood
Factors Influencing Virus Inactivation and Retention of Platelet Properties Following Treatment with Aminomethyltrimethylpsoralen and Ultraviolet A Light

G. MOROFF,¹ S. WAGNER,¹ L. BENADE,¹ and R.Y. DODD¹

Fig. 1. The influence of red cell hematocrit level on the inactivation of φ6 by UVA light (4.4 J/cm²) and AMT (40 μg/ml).

Action Spectra and Absorbance Spectra

- DNA Absorption
- 200 Micromolar Riboflavin Absorption
- Action Spectrum-UV Light Effect

UV-Vis of RB in Organic Solvents

<table>
<thead>
<tr>
<th>Type</th>
<th>Legend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Blank</td>
<td>Blue line</td>
</tr>
<tr>
<td>RB in Water</td>
<td>Pink line</td>
</tr>
<tr>
<td>RB in Methanol</td>
<td>Green line</td>
</tr>
<tr>
<td>RB in DMSO</td>
<td>Red line</td>
</tr>
</tbody>
</table>

Absorbance vs Wavelength

Absorbance: 0.0 to 0.9
Wavelength: 300 to 500
Treatment of Whole Blood with the Mirasol System

Objective is to provide a Cost-Effective, Logistically Viable Pathogen Reduction and White Cell Inactivation Process for All Blood Products
Red Cell Quality; Baboon In Vivo Study

No evidence of neoantigen formation; Goodrich et al. Transfusion (2009)
IMPROVE Study

- Treated Whole Blood; Separated into components
- Non–Leukoreduced; White Cell inactivation monitored
- In vitro Cell Quality Evaluation
  - Red Cells; 42 Days storage
  - Platelets (PRP); 5 Days storage
  - Plasma (FFP); 28 Days storage
- Human Clinical Evaluation
  - Radiolabel re–infusion of red cells; volunteer subjects
  - Correlation of in vitro parameters with in vivo

Cancelas et al. Transfusion (2011)
## Predicted 24 Hour Recovery of Red Cells From Whole Blood Products

<table>
<thead>
<tr>
<th>Day of Storage</th>
<th>Untreated Products</th>
<th>Treated Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>ATP 5.27 µMole/gHb</td>
<td>Predicted 24 Hour Recovery 100%</td>
</tr>
<tr>
<td>Day 1</td>
<td>6.66</td>
<td>100%</td>
</tr>
<tr>
<td>Day 5</td>
<td>4.43</td>
<td>91%</td>
</tr>
<tr>
<td>Day 7</td>
<td>2.82</td>
<td>74%</td>
</tr>
</tbody>
</table>
Hemostatic Function of Fresh Whole Blood

- Units of whole blood were treated with the Mirasol PRT Process
  - Collected and stored in CPDA
  - Energy delivered = 80 J/mL_{RBC}

- Units were then stored in standard blood bags at room temperature (No agitation)
- Samples were analyzed after treatment
Analysis of Whole Blood Stored at RT by Impact–R Analysis

Treated Sample

SC: 13 %
AS: 58 μm²
Ob: 1290
Test Image

Untreated Sample

SC: 11 %
AS: 42 μm²
Ob: 1495
Test Image
Lørdag 11. august 2012
## CDMRP Funded Research Consortium

**Mirasol System for Whole Blood**

<table>
<thead>
<tr>
<th>CaridianBCT Biotechnologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhode Island Hospital Rhode Island</td>
</tr>
<tr>
<td>Blood Systems Research Institute California</td>
</tr>
<tr>
<td>Colorado State University Colorado</td>
</tr>
<tr>
<td>University of Colorado Colorado</td>
</tr>
<tr>
<td>American Red Cross Maryland</td>
</tr>
<tr>
<td>New York Blood Center New York</td>
</tr>
<tr>
<td>Hoxworth Blood Center Ohio</td>
</tr>
<tr>
<td>Puget Sound Blood Center Washington</td>
</tr>
<tr>
<td>University of Southern California California</td>
</tr>
<tr>
<td>Army Institute of Surgical Research Texas</td>
</tr>
</tbody>
</table>

**lordag 11. august 2012**
Status of Whole Blood Treatment

- **Pathogen Reduction**
  - Virus reduction evaluations over a range of energies
    - Enveloped and Non-Enveloped viruses (HIV, HBV, HCV, HHV–8, Parvovirus)
  - Bacteria reduction over a range of energies
    - Gram positive and negative bacteria
  - Parasite reduction studies over a range of energies (Chagas, Babesia, Malaria, Leishmania, Anaplasma)

- **WBC inactivation**
  - in vitro data obtained over range of energies, in vivo studies at single dose

- **Blood component quality**
  - RBC quality – room temperature storage of whole blood as well as storage of refrigerated RBCs
  - Platelet quality (PRP) – evaluated post–treatment and on Day 5
  - Plasma quality (FFP) – evaluated post–treatment and on Day 28

- **Toxicology**
  - Baboon immunization and R&S model; rabbit immunization model
  - Neutrophil activation studies

- **Functional Studies**
  - Hemorrhage/Resuscitation Studies in animal model
  - TEG Functionality during storage

- **Initial Human Study Successfully Completed in USA**

Goodrich et al. *Biologicals* (2009)
Cancelas et al. *Transfusion* (2011)
Delivering On The Promise
Has It Been Worth It?

- Window Period Transmissions of HIV, HBV, HCV
- Transmission of CMV and WNV
- Transfusion Associated Bacterial Infections
- Transmission of Chagas, Malaria, Babesia
- Transfusion Associated GVHD
- Reduction in Alloimmunization Rates
- A new way of perceiving and addressing emerging agents
- A way to bring increased blood safety in a practical and affordable way to parts of the
2009 Annual Report
TATRC

Then: World War II Battle of Tarawa first aid station's blood bank.

Now: Mirasol™ Pathogen Reduction Technology (inset) procedure allows for safer blood to be provided on the battlefield.