

THE MIRASOL SYSTEM FOR WHOLE BLOOD

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RDCR CONFERENCE



TERUMOBCT

Agenda

- Introduction and Background
- Leukocyte Inactivation and Pathogen Reduction Results
- Treated Whole Blood Stored at Room Temperature – Blood Quality Measurements
- Components from Treated Whole Blood
- Next Steps and Summary



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INTRODUCTION AND BACKGROUND



Introduction

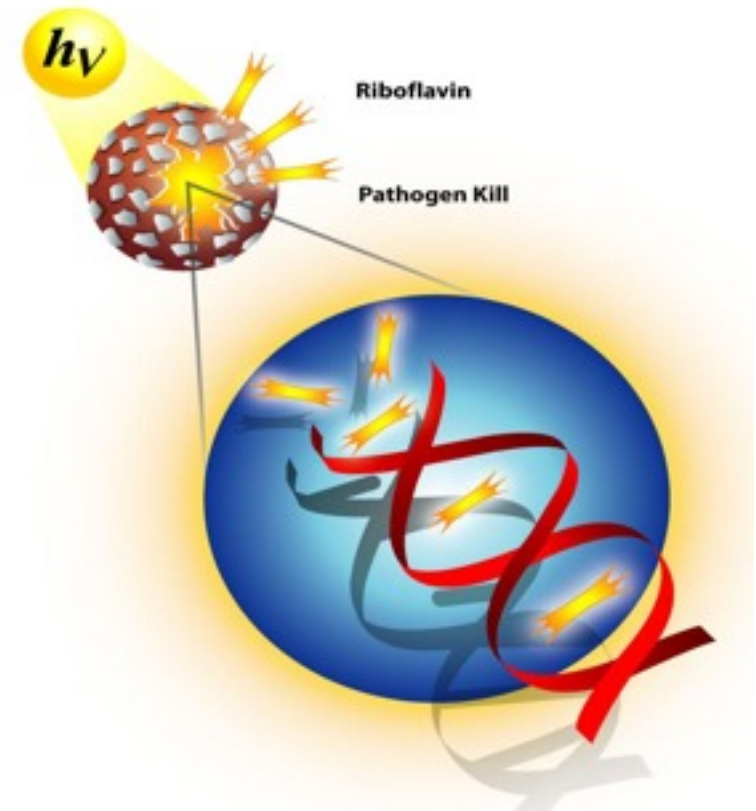
- **Transfusion of fresh whole blood is associated with risks that are similar to those for components**
- **The Mirasol System for Whole Blood, adapted from the Mirasol PRT System for Platelets and Plasma, was developed to mitigate risks associated with pathogens and leukocytes in fresh whole blood transfusions**
 - Transfusion of whole blood to combat casualties occurs within a short time frame, and the blood is not fully screened for known transfusion risks.
 - Fresh whole blood is also not leukoreduced or gamma-irradiated in that situation
- **An added benefit to the treatment of whole blood is the production of treated components from one treated whole blood unit**

Basis of the Mirasol PRT System

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
- Riboflavin and its photo-products are non-toxic⁴ and non-mutagenic^{4,5} and are naturally present in normal blood⁶

1. Kuratomi & Kobayashi 1977
2. Speck et al. 1975
3. Korycka-Dahl & Richardson 1980
4. Piper et al., 2001
5. Kale et al. 1992
6. Hardwick et al. 2004



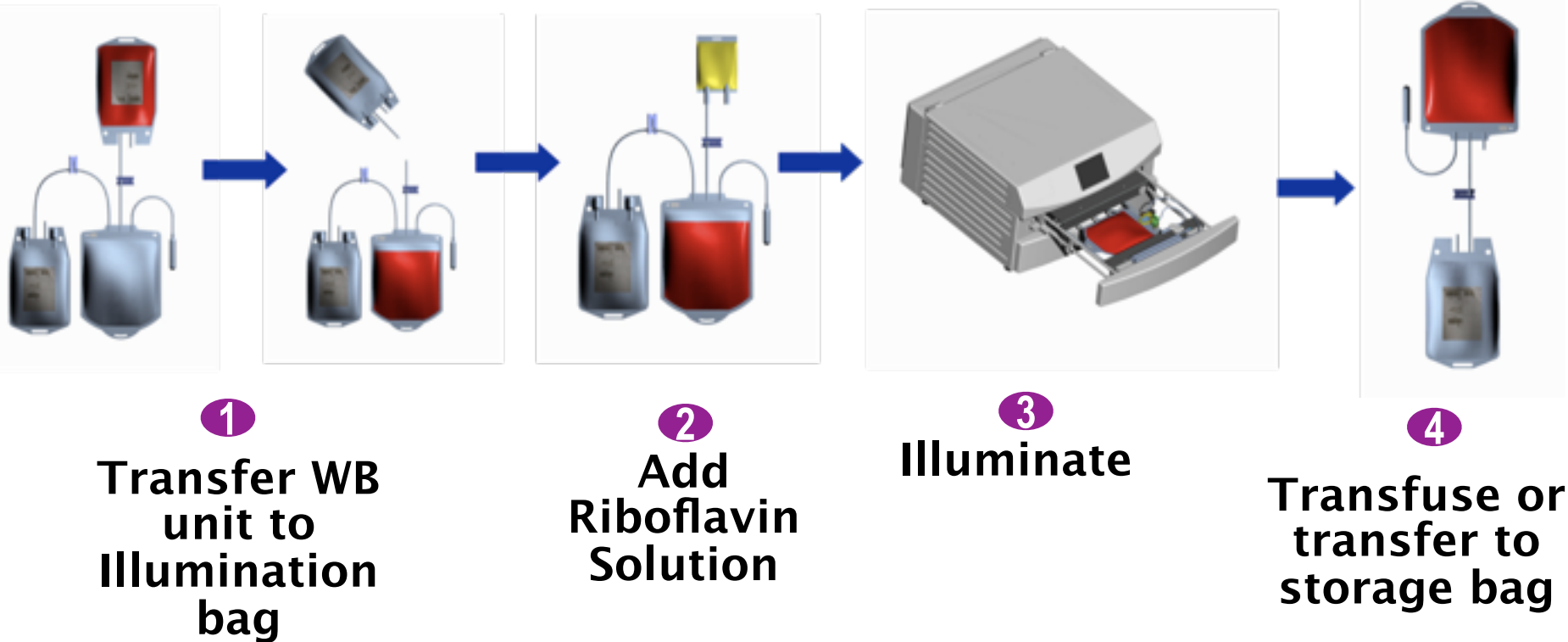
Safety of Riboflavin and Mirasol Treatment

- There is a strong history (*in vivo*) and additional Terumo BCT Biotechnologies safety testing (*in vivo* and *in vitro*, summarized below) supporting the safety of riboflavin and its use in the Mirasol System
 - Reddy et al. Transfus Med Rev. 2008 Apr;22(2):133-53.

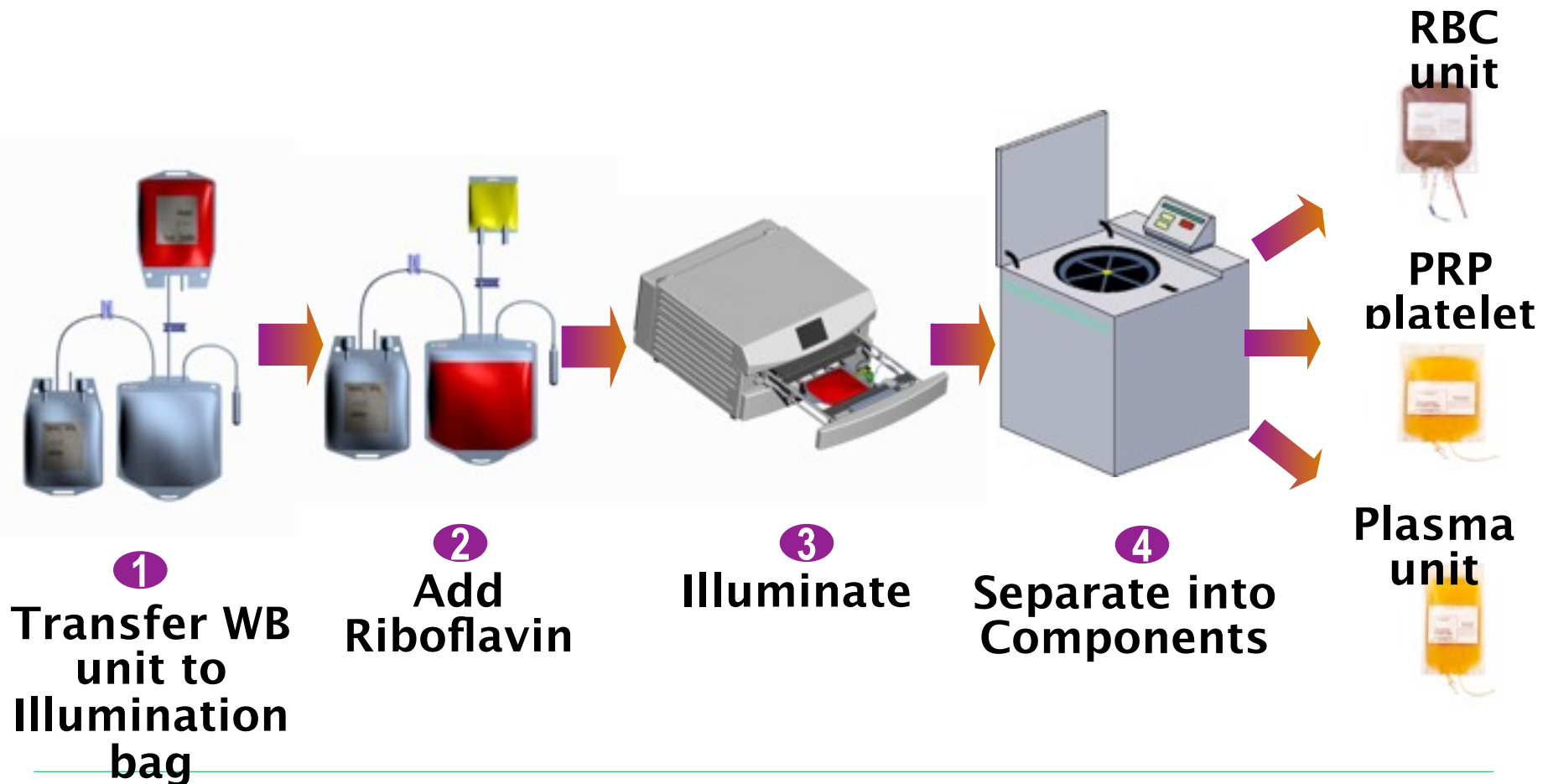
Test	Result
Acute Toxicity	Negative
Subchronic Toxicity	Negative
Neoantigenicity	Negative
Ames	Negative
Chromosomal Aberration	Negative
Mouse Erthrocyte Micronucleus	Negative
Embryo-Fetal Development	Negative
Hemocompatibility	Passed
Leachables and Extractables	Passed

Treatment of Whole Blood with the Mirasol System

UV energy dose is scaled to the volume of RBCs in the whole blood – $80 \text{ J/mL}_{\text{RBC}}$



Treatment of Whole Blood with the Mirasol System



LEUKOCYTE INACTIVATION AND PATHOGEN REDUCTION

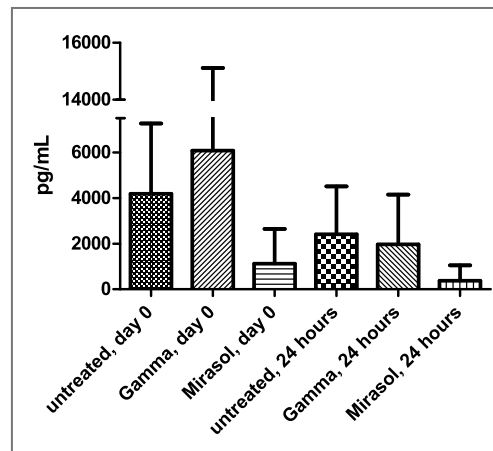


Leukocyte Inactivation

- Studies were performed to compare Mirasol treatment of whole blood with gamma irradiation (25 Gy) – paired comparisons
 - The EU Council recommends a 25 Gy minimum dose, and the FDA recommends 15 Gy minimum dose
- *In vitro* tests assessed inactivation with measurements of:
 - Proliferation (PHA, anti-CD3/CD28, allogeneic stimulators)
 - Antigen presentation
 - Activation (CD69 expression)
 - Viability (Limiting Dilution Assay)
 - Cytokines (LPS stimulation, CD3/28 stimulation)
 - Apoptosis (PI/AnnexinV, TUNEL)
 - Phenotype (Flow cytometry)
- The *in vivo* test was performed in a mouse model of xenogeneic graft-vs-host disease

Leukocyte Inactivation

- *In vitro* results
 - Mirasol treatment decreases viability to the same extent as gamma irradiation
 - Mirasol treatment provides **greater** reduction of cytokines and of antigen presentation

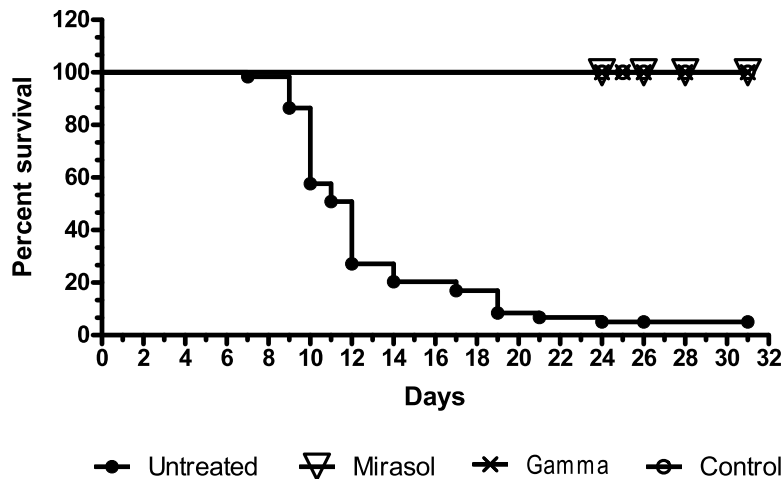


IL-8 secretion in untreated, Mirasol treated and gamma-irradiated cells on Day 0 or 24 hours after treatment (without stimulation). Shown are mean values \pm one standard deviation

Effect of Mirasol treatment and gamma-irradiation on allogeneic stimulator cells in a MLC. Shown are mean values \pm one standard deviation. All values were corrected for the background of cells incubated in PBS, in the absence of any stimulus.

Mirasol System for Whole Blood: Comparison with γ -Irradiation & Leukoreduction

- *In vivo* results
 - Mirasol treatment is as effective as gamma irradiation at preventing xenogeneic graft-vs-host disease (evaluated in a mouse model)
 - Fast et al. Transfusion epub May 2012



Survival of mice injected with untreated, Mirasol-treated or gamma-irradiated donor cells. Control mice were injected with PBS. Data points for the following number of mice were available: untreated n=59, Mirasol n=60, gamma n=59, control n=18

Mirasol System for Whole Blood: Comparison with γ -Irradiation & Leukoreduction

	Leukoreduction	γ -irradiation (25 Gy)	Mirasol System for Whole Blood
WBC viability	Residual amount of viable WBCs	5 log reduction	5 log reduction
Allo-immunization	Reduced but not prevented	Not prevented	No antigen presentation <i>in vitro</i> Animal models in progress
TA-GVHD	Not prevented	Prevented	Prevented in animal model
Cytokine production	Still produced by residual WBCs	Cytokines still produced	Production prevented

Pathogen Reduction

- Intraerthrocytic parasites are of concern in the U.S. (*Babesia* spp.) and in parts of the world where malaria is endemic (*Plasmodium* spp.)
 - Studies with *Babesia microti* yielded reduction values of 5 log (Tonnetti et al. in press)
 - Pilot studies with *B. divergens* indicate 5 log or greater reduction
 - Studies with *Leishmania donovani* and *Plasmodium* spp. are in progress
- Chagas' disease is caused by *T. cruzi*, which is reduced by ≥ 3.5 log with Mirasol treatment (Tonnetti et al. 2012)
- Reduction of HIV was tested with a cell-associated form of the virus; reduction levels of 4.5 ± 0.5 were observed
- Testing of bacterial reduction is ongoing

Pathogen Reduction

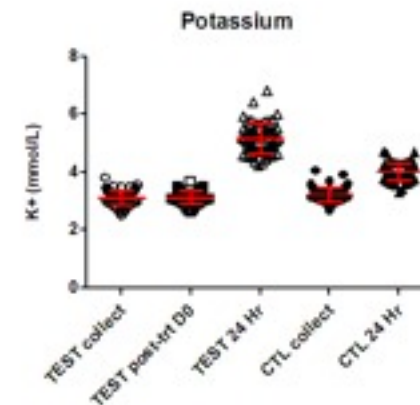
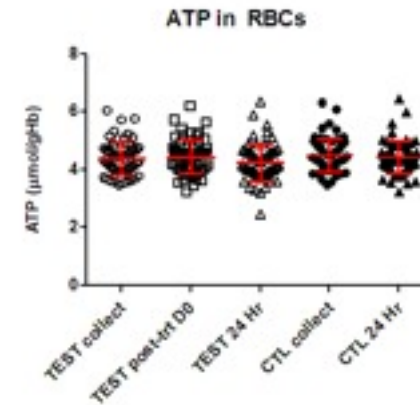
Parasite	Disease	Reduction with Mirasol System	Status
<i>Babesia microti</i>	Babesiosis	$\geq 5.0 \pm 0.2$	Complete, manuscript in press
HIV	AIDS	4.5 ± 0.5	Complete
<i>Trypanosoma cruzi</i>	Chagas' disease	≥ 3.5	Tonnetti et al. Transfusion 2012
<i>B. divergens</i>	Babesiosis	N/A	In progress
<i>Leishmania donovani</i>	Leishmaniasis	N/A	In progress
<i>Plasmodium falciparum</i>	Human malaria	N/A	In progress
<i>P. yoellii</i>	Murine malaria	N/A	In progress
<i>Yersinia enterocolitica</i>	Sepsis	N/A	In progress

TREATED WHOLE BLOOD STORED AT ROOM TEMPERATURE – BLOOD QUALITY MEASUREMENTS



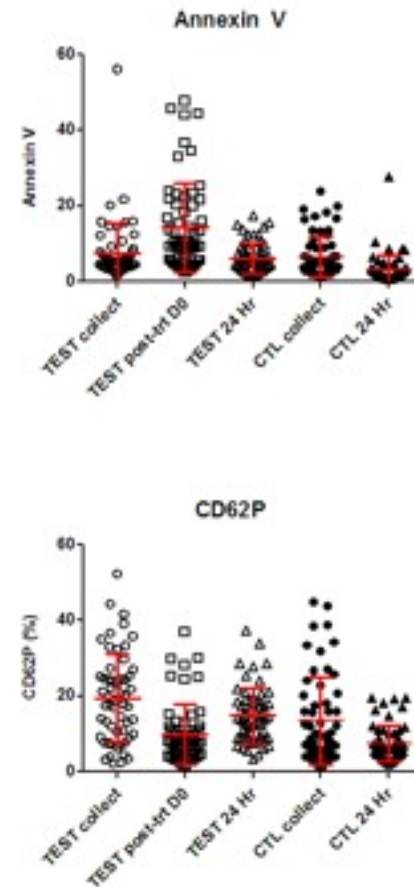
Whole Blood Stored at 22°C: RBC Quality

- Treated whole blood and paired units of control whole blood were stored for 24 hours from the time of collection.
 - Hemolysis values for remained well below 1% throughout storage
 - ATP values indicate that the FDA criterion for recovery will be met by RBCs in treated WB
 - Methemoglobin levels post-illumination range from 1.6 to 8.2%, and are reduced to background levels during storage
 - RBCs in treated whole blood release more potassium during storage; levels remain low at 24 hours



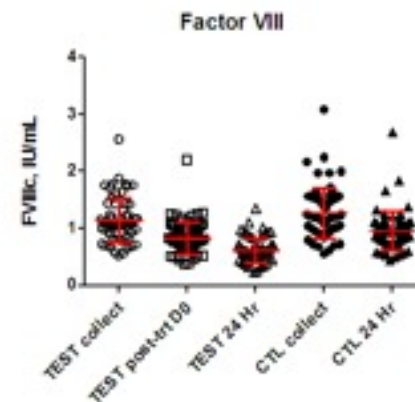
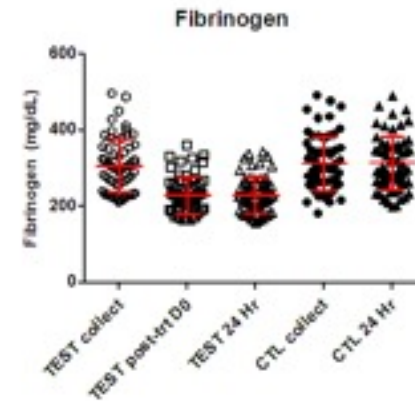
Platelets from Treated Whole Blood

- Platelets from treated whole blood and paired units of control whole blood, were stored for 24 hours from the time of collection, was evaluated for factors and other proteins requested by the FDA.
 - No statistically significant differences were observed in platelet ATP and HSR values for test and control units.
 - CD62P and Annexin V values were significantly higher in the 24-hour samples from test units. However, the values in 24-hour samples for test and controls are lower than those reported for 5-day stored platelets.
 - ESC values were significantly lower in test samples, although the measured values are well within the range where in vivo viability is not affected (Holme and Moroff, 1998).



Plasma from Treated Whole Blood

- Plasma from treated whole blood and paired units of control whole blood, were stored for 24 hours from the time of collection, was evaluated for factors and other proteins requested by the FDA.
 - No statistically significant differences were observed in levels of von Willebrand Factor Activity, anti-thrombin III, Protein S, Protein C, or thrombin-antithrombin complex.
 - Prothrombin time (PT) and activated partial thromboplastin (aPTT) time were greater in treated units than in controls. PT values for treated units remained within the normal range for controls, while aPTT values were slightly above the range.
 - Fibrinogen and Factors V, VIII and XI were significantly lower in treated units than in controls.

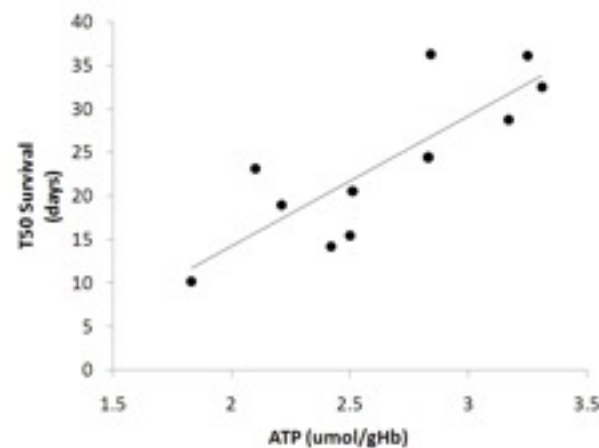
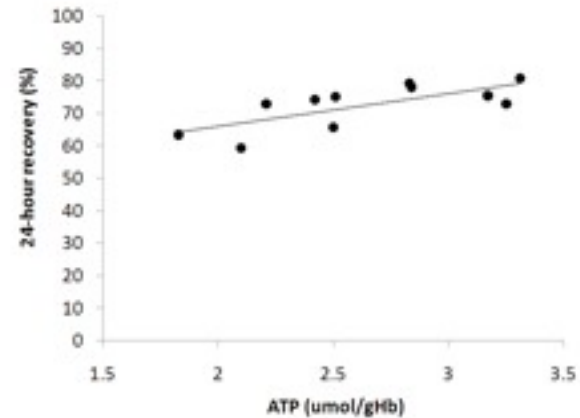


COMPONENTS FROM TREATED WHOLE BLOOD



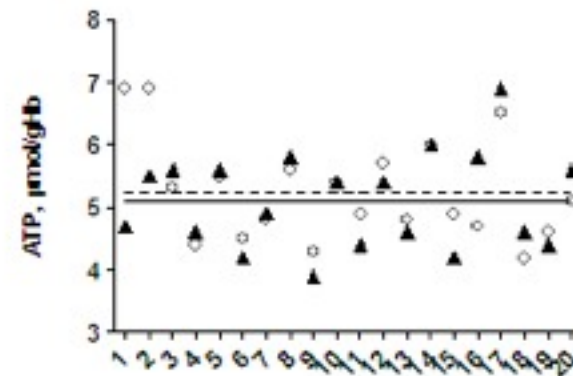
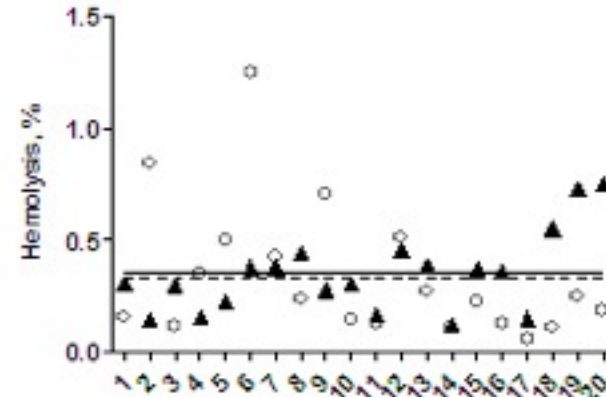
IMPROVE Feasibility Clinical Trial with Treated pRBC stored for 42 days in AS-3

- The FDA requires a 24-hour recovery value of 75% for new blood products containing RBCs.
- Based on the ATP correlation from the clinical data, RBCs with ATP levels of ≥ 3.0 $\mu\text{mol/gHb}$ will meet the FDA criteria for recovery.
 - For the 24-hour recovery data: Spearman Coefficient of Correlation = 0.752; $p = 0.008$
- These correlations, obtained by testing the prototype device, provided a guide for further development
- Figures from Cancelas et al. Transfusion (2011) Jul;51(7):1460-1468



Treated pRBC Quality

- For these data, treated pRBC (derived from Mirasol-treated whole blood) were suspended in AS-3 and leukoreduced prior to storage
 - Hemolysis values for treated unit remained below 1% throughout storage
 - ATP values indicate that the FDA criterion for recovery will be met by treated RBC
 - Release of K⁺ by treated RBC is similar to that observed for gamma-irradiated RBC
 - Methemoglobin levels post-illumination range from 1.6 to 8.2%, and are reduced to background levels during storage
- X-axis in graphs is individual donor number. Symbols in graphs: open circles are values from untreated pRBC, stored in AS-3 at 4°C; closed triangles are values from treated pRBC, stored in AS-3 at 4°C for 21 days



Treated Plasma Quality

- Treatment affects protein factors, with decreases similar to those observed for Mirasol - treated FFP (included for comparison).
 - Values for treated group are corrected for dilution; significant differences from untreated controls are marked with †

Parameter (N=61)	Plasma from Untreated Whole Blood	Plasma from Mirasol-treated Whole Blood	Mirasol-treated FFP (N=90)
Fibrinogen, mg/dL	312±72	229±48†	224±59
FV, IU/mL	0.87±0.14	0.67±0.12†	0.77±0.21
FVIIIc, IU/mL	1.27±0.44	0.83±0.29†	0.83±0.30
FXI, IU/mL	0.93±0.16	0.64±0.14†	0.72±0.17
Protein S, IU/mL	0.88±0.16	0.85±0.13†	0.98±0.19
Protein C, IU/mL	1.15±0.21	1.09±0.21†	0.97±0.22
AT-III, IU/mL	0.98±0.08	0.97±0.09	1.00±0.10
vWf, IU/dL	102±41	103±40	88±30

Platelets from Treated Whole Blood

- Preliminary studies have been performed with platelet concentrates, derived from Mirasol-treated whole blood that was centrifuged to create PRP and stored for 5 days at 22°C
 - When compared to control platelet concentrates, values for pH, lactate and glucose are in the range observed for untreated controls
 - Production of platelet concentrates via the PRP method requires refinement
 - Both PRP platelet products and Buffy Coat platelet products will be evaluated in the coming year

NEXT STEPS AND SUMMARY



Mirasol System for Whole Blood: Next Steps

- ❑ Pathogen reduction evaluations continue
 - Parasite reduction studies continue with *Plasmodium* spp., *Babesia* spp., and *Leishmania donovani*
 - Novel PCR assays are in use to test virus reduction (Dengue virus, Parvovirus B-19, HHV-8)
 - Bacteria reduction studies with Gram positive and negative bacteria continue
- ❑ Leukocyte inactivation: Studies of the potential for alloimmunization and for TRALI are in progress
- ❑ Blood component quality/function
 - Further studies of platelet and plasma components are planned for 2012/13
 - *In vivo* tests of blood function and hemostasis will start this year and next
- ❑ The next phase of clinical work will evaluate R&S of ⁵¹Cr-labelled RBCs in healthy volunteers. FDA approval obtained April 2012
 - ❑ Further clinical work includes tests of the R&S of platelets in whole blood stored for 24 hours.
- ❑ First phase of Operational Testing of the device will occur in mid-July
- ❑ Development of the System for Whole Blood is funded by the U.S. Department of Defense

Summary

- The Mirasol System for Whole Blood uses the same illuminator, disposable kit, and illumination solution as the CE-marked Mirasol PRT System for Platelets and Plasma
- Treatment with the Mirasol System is as effective as gamma-irradiation for the elimination of leukocyte viability and the prevention of TA-GvHD
- The Mirasol System for Whole Blood is effective at the reduction of intraerythrocytic parasites and of *T. cruzi* and HIV; further studies of pathogen reduction are planned.
- Treated whole blood is expected to provide acceptable hemostasis upon transfusion; *in vivo* pre-clinical studies are in progress or planned
- Whole blood treated with the Mirasol System yields platelet and plasma with characteristics similar to components treated with the Mirasol System. Treated pRBC are expected to meet FDA criteria for stored pRBC.

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THANK YOU!

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