13th Annual Canadian Blood Services
International Symposium

Blood-Borne Pathogens: Defend, Detect, and Destroy

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The Biological Impact of Pathogen Inactivation on Blood Product Quality

CBS Annual International Symposium – September 16, 2015
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Clinical Associate Professor, Pathology, UBC

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Conflict of Interest Disclosures
Peter Schubert

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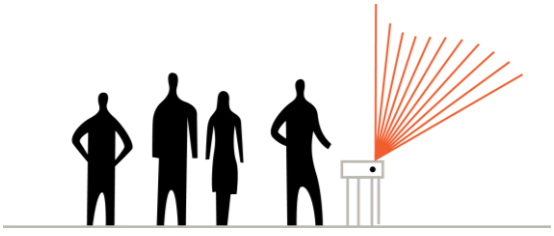
OVERVIEW

- Compare the currently available pathogen inactivation technologies and their mechanisms of action
- Discuss the impact of pathogen inactivation technology on product quality



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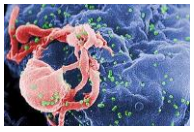
QUALITY and SAFETY

- Shelf life restriction of platelet concentrates
  - Quality reduction during storage
  - Risk of pathogen contamination
- Blood safety has historically been achieved by mitigating known risks
  - Donor screening
  - Testing of every donation for specific pathogens
- Risk still is present
  - Detection limit (concentration)
  - Unknown or unexpected pathogens



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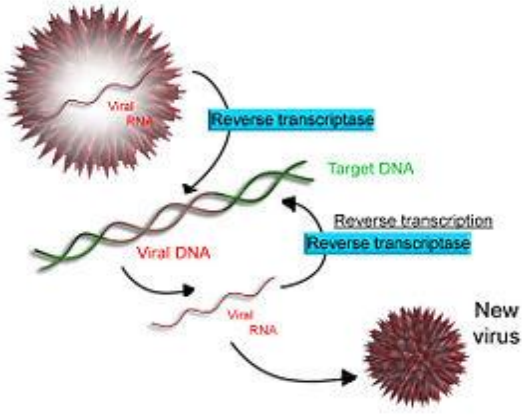
To be infectious, agents must be able to reproduce



- Pathogens with nucleic acids reproduce by replicating their nucleic acid 'roadmap'.
- This is a mandatory step for all pathogenic agents except prions.

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Pathogen inactivation systems for platelet concentrates

System	Manufacturer	UV (nM)	Photo-sensitizer	Solution	Mechanism of action
Intercept	Cerus	320-400	Psoralens	Intersol/ SPP+	Irreversible cross linking of nucleic acids.
Mirasol	TerumoBCT	280-360	Riboflavin	Plasma/ SSP+	- Irreversible photo oxidative damage to nucleic acids. - Photolysis of the complex induces guanine oxidation, single strand breaks and the formation of covalent bonds.
Theraflex	MacoPharma	254	none	SSP+	Nucleic acid presumably occurs due to cyclobutyl ring formation.

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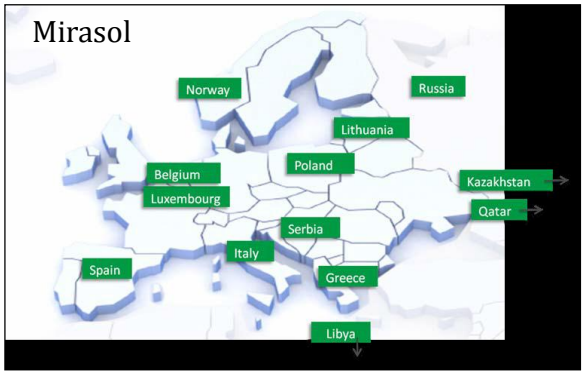
Implementation of PI systems world-wide



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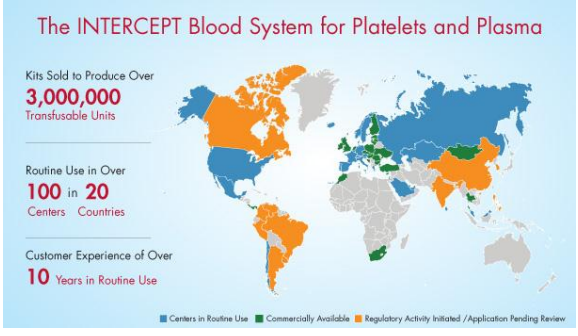

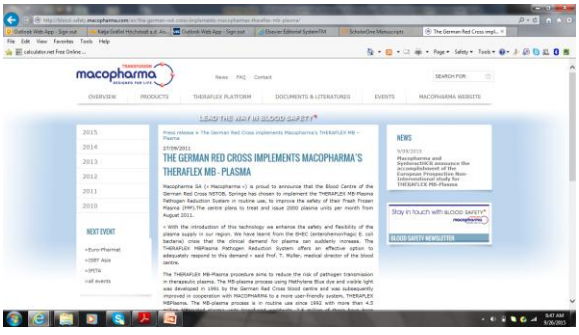

Clinical trials with PRTs

Study	Technology	Design	N (Test/Ref) Mean Storage	Primary endpoint	Secondary endpoint
EUROSPRITE	Intercept BC/PC	56d or 8Tx	52/51 3.4d / 3.5d	CCI & CI at 1h	CCI & CI at 24h, Bleeding score
SPRINT	Intercept AP/PC	28d or 8Tx 12.5% non-inf.	318/327 3.4d / 3.6d	Grade 2 bleeding	Grade 3&4 bleeding, CCI & CI 1&24h
MIRACLE	Mirasol AP/PC	RCT 20% non-inf.	58/64 2.8d / 2.6d	CCI 1h	CCI 24h
PREPARES (CBS, Sanquin, & Norway)	Mirasol BC/PC	42d or 8Tx <15% non-inf.	618	Grade ≥2	CCI & CI 1&24h

Observation: ~25% of platelet are damaged

→ What are the features for the damage?

→ Why does this happen? Mechanisms….

→ How can we fix it?

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Pathogen Reduction Evaluation & Predictive Analytical Rating Score (PREPAReS)

- A prospective, randomized, single-blinded, multicenter non-inferiority trial for the side by side evaluation of Mirasol-treated and standard of care Pooled Platelet products in hemato-oncological patients.
- 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.

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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.

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Participating Study Sites: All Canadian Study Sites Now Entering Patients

Juravinski Cancer Centre (Hamilton)	49
Sunnybrook Health Sciences Centre (Toronto)	11
London Health Sciences Centre	12
Ottawa Hospital	26
Kingston General Hospital	2
Total recruitment to 2015-08-27 =	100 patients

→ now eagerly awaiting results.....

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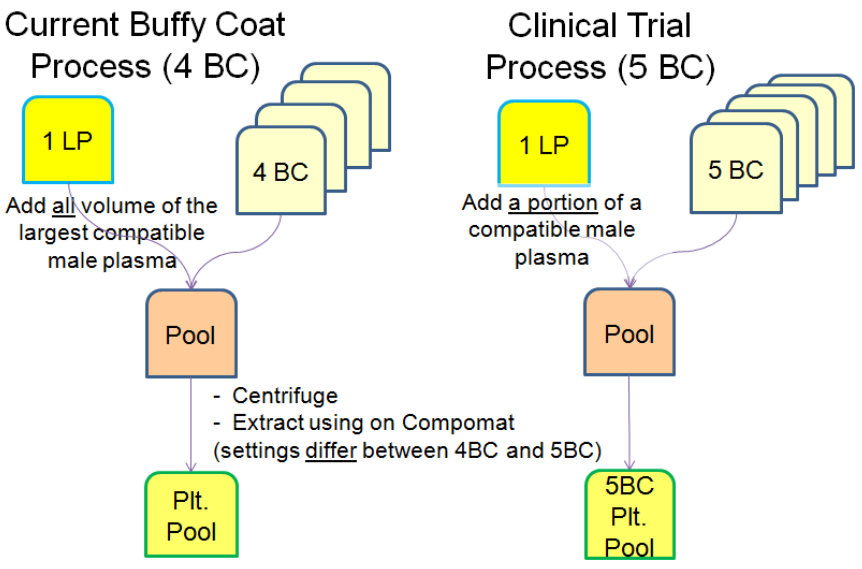

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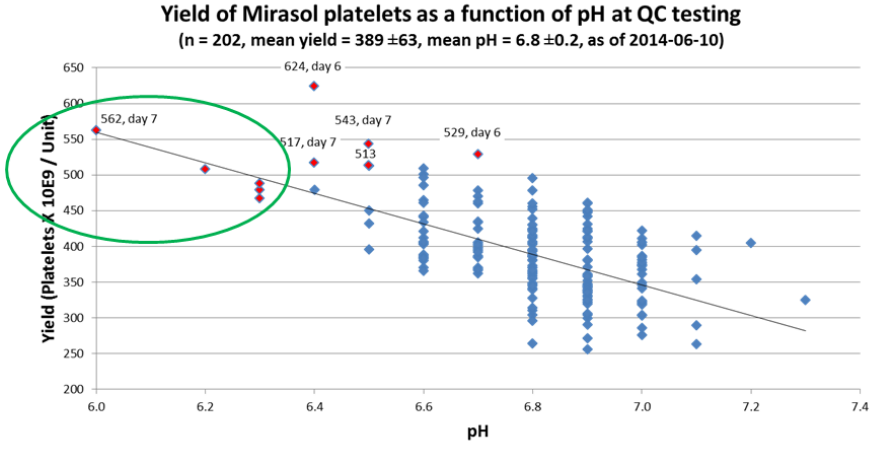
Connection between yield and pH



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The CBS Team at the completion of Mirasol process validation

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PI effect on routine platelet quality parameters

Assay	Read-out	Equipment	Intercept	Mirasol	Theraflex
Platelet concentration	Count => yield, MPV	Hemology analyzer	±	±	±
Morphology	Shape	Microscope	↓	↓	n.d.
Metabolism	Glucose, lactate, pH	Blood gas analyzer	± / ↑	↑↑	↑ (ox)
Apoptosis	Phosphatidylserine exposure	Flow cytometer	↑	↑↑ (d7)	↑
Activation	• P-selectin (CD62P) surface expression • Glycoprotein II/III activity	Flow cytometer	↑ n.d.	↑↑ ↑	↑ n.d.
Responsiveness	• agonist-triggered activation • agonist-triggered ESC	Flow cytometer Aggregometer	↓↓ ✔	↓ ↓	↓ ↓
Clotting	Thrombus formation	microfluidic flow chambers	↓ coag time	↓	↓

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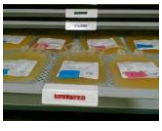

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PI effect on 'novel' platelet features

PI treatment

PI system	Study objective	Citation
Mirasol	Cytokine release	Transfus Apher Sci (2012) 46(1):33-7
	mtDNA release	Vox sang (2014) 107(4):351-9
	ROS formation	Transfus Med Hemother (2015) 42(3):167-73
	NFκB signaling	Transfus Med Hemother (2015) 42(3):167-7
	TRALI in SCID mice	Transfusion (2014) 54(1):74-85
	Mitochondrial function	Transfus Apher Sci (2009) 40(2):79-85
Mirasol Intercept	mRNA, miRNA levels	Platelets (2015) 26(2):154-63
Mirasol Intercept Theraflex	mRNA profile	PLoS One (2015) 10(7):e0133070
Theraflex	Microvesicles	Transfusion (2013) 53(5):990-1000

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Proteomic approaches towards elucidating molecular mechanisms triggered by PI systems

Contents lists available at ScienceDirect

Transfusion Medicine Reviews

journal homepage: www.tmreviews.com

Proteome Changes in Platelets After Pathogen Inactivation—An Interlaboratory Consensus

Michel Prudent [a,*,1], Angelo D'Alessandro [b,1,2], Jean-Pierre Cazenave [c], Dana V. Devine [d,1], Christian Gachet [c,1], Andreas Greinacher [e], Niels Lion [a], Peter Schubert [d,1], Leif Steil [f], Thomas Thiele [e,1], Jean-Daniel Tissot [a], Uwe Völker [f], Lello Zolla [b]

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[f] Interfakultäres Institut für Genetik und Funktionelle Genomforschung, Universitätsmedizin Greifswald, Greifswald, Germany

- weak impact of PI on the overall proteome profile

Impact found:

- Intercept: intracellular signaling leading to platelet activation
- Mirasol: adhesion and shape change
- Theraflex: aggregation and shape change

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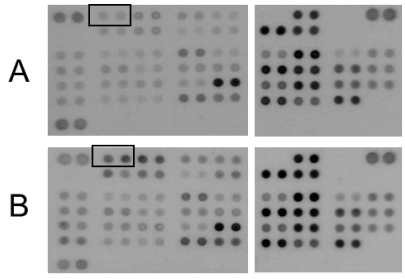
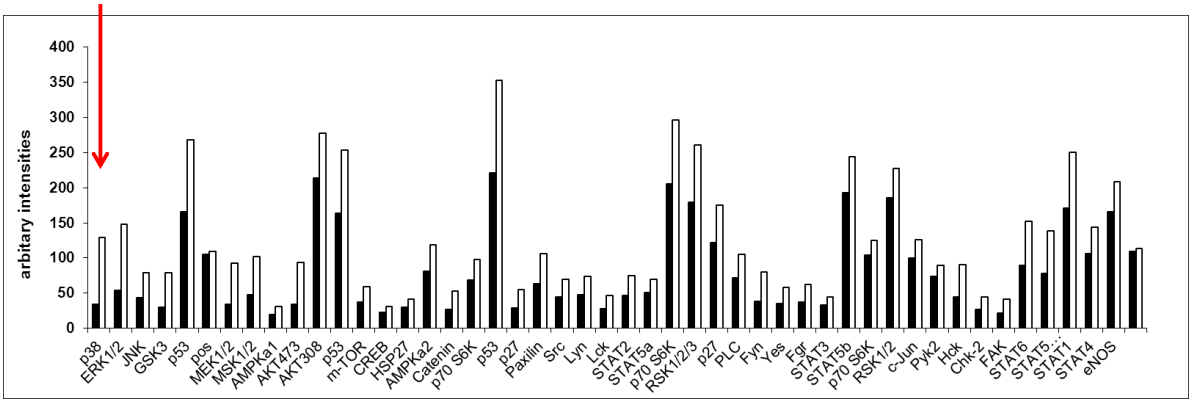
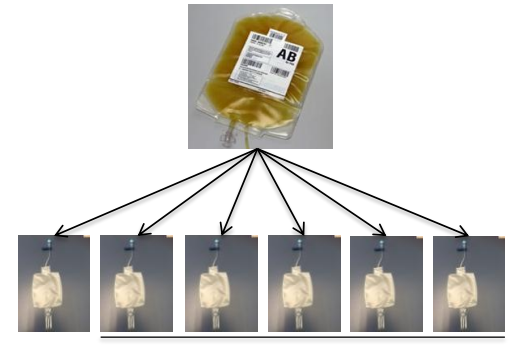
PI treatment: protein kinase profiling



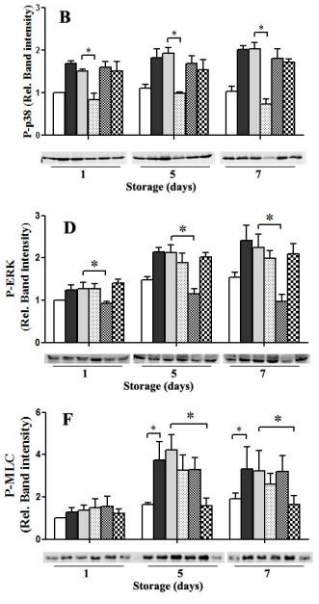
Schubert P *et al*. Transfusion 2015;55:815–823



Towards the elucidation of molecular mechanism triggered by PI



Sample collection
Day 0, 1, 4 & 6
storage



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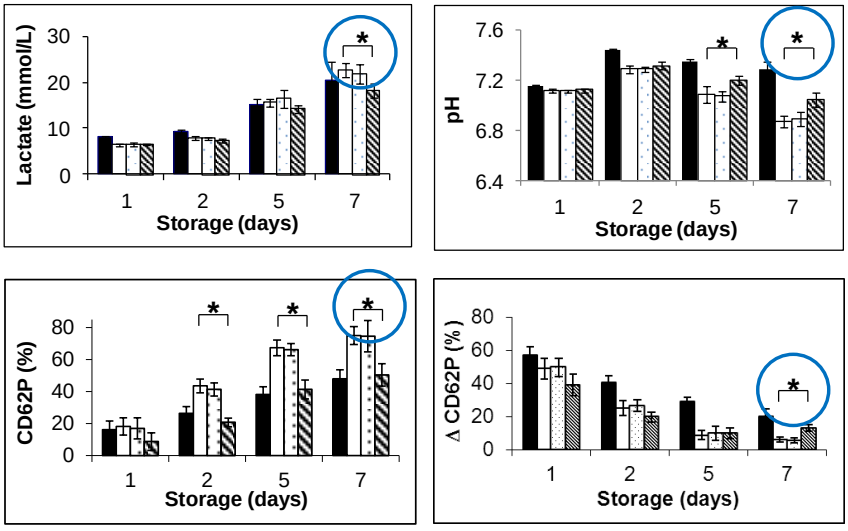
Kinase inhibitor study



Schubert P *et al*. Transfusion 2015;55:815–823

p38 inhibition significantly improves platelet *in vitro* quality.

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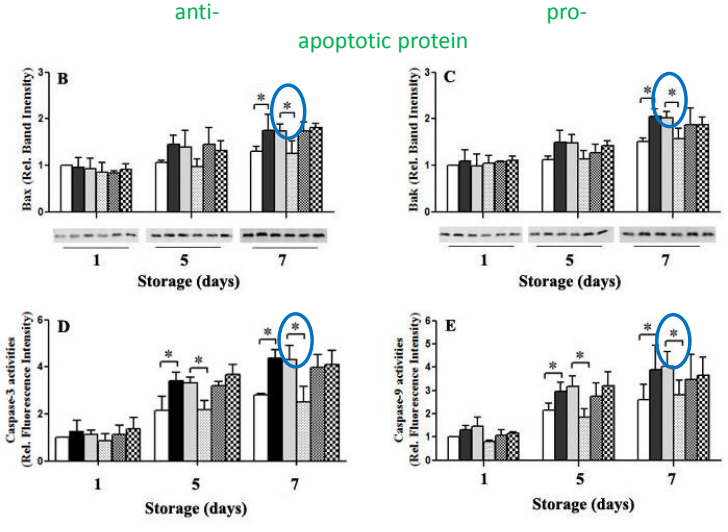
Kinase inhibitor study II

anti- pro-

apoptotic protein



caspase 3 caspase 9

Chen *et al*. Transfusion 2015;53:848-57

p38 inhibition significantly reduced apoptosis development

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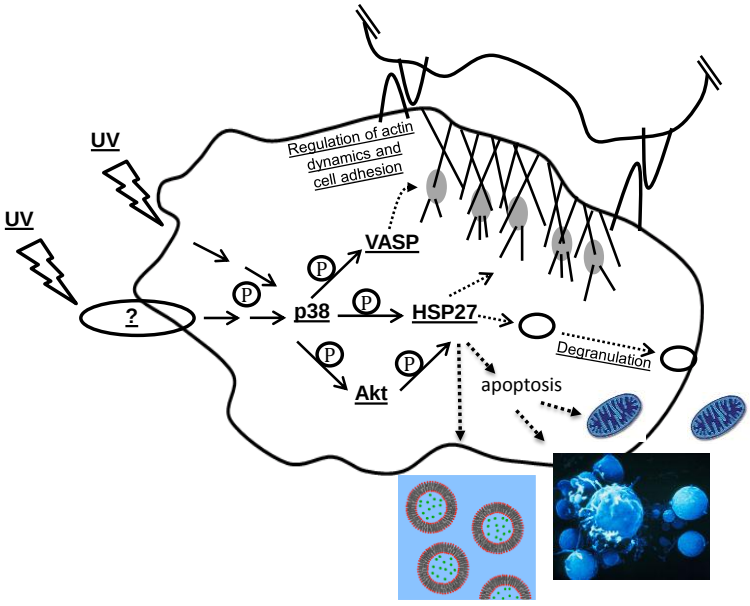
A current signaling model



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Pathogen inactivation of red blood cell concentrates

Optical density issue → high dose of UV light

Cerus: S-303 + GSH (quencher) [Henschler et al. (2011) Transfus Med Hemother, 38:33-42]

- 24h recovery ~ untreated study arm
- >35 days of storage: impact on quality

Pathogen inactivation of whole blood

Benefits: - early pathogen inactivation (from donor)
- protection against TA-GvHD due to WBC inactivation
- all productions pathogen inactivated

TerumoBCT: riboflavin + UV (Mirasol) [Goodrich et al. (2010) Biologicals, 38:20-30]

- Feasibility studies
- Recent study in Ghana/Africa: malaria inactivation in WB [Qwusu-Ofori et al. (2015) Shock, 44S1:33-38]

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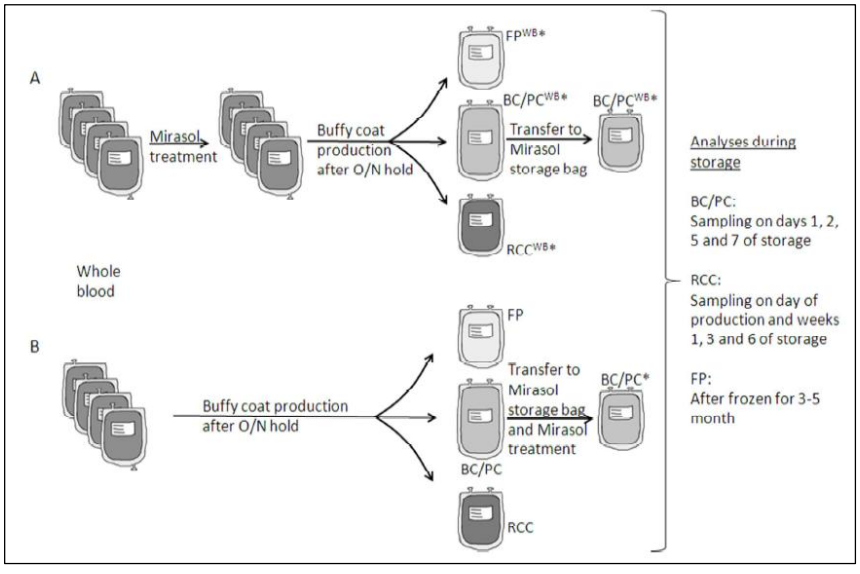
From individual blood component treatment to whole blood treatment

Treatment efficacy

Pathogens	Publication
WBC	Goodrich RP et al. (2010) Biologicals, 38:20-30.
HIV (intracellular)	Keil SD et al. (2012) Vox Sang, 103:144.
Trypanosoma cruzi	Tonnetti L et al. (2012) Transfusion; 52:409-16.
Babesia microti	Tonnetti L et al. (2013) Transfusion, 53:860-7.
Plasmodium falciparum	El Chaar M et al. (2013) Transfusion, 53:3174-83.

- What effect does treatment have on RBC, platelet and plasma quality?
- Is there any difference in product quality between components produced by 'PRP' manufacture [Cancelas et al. (2011) Transfusion, 51(7):1460-8] and buffy coat production used outside of the US (including by Canadian Blood Services)?
- How do platelets derived from Mirasol-treated whole blood compare to buffy coat derived platelet concentrates treated with Mirasol after component production?

Pathogen inactivation: study design



Schubert P *et al.* Transfusion 2015;53:3164-73

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The effect of PRT on RBC *in vitro* quality

Assay	Week 0			Week 1			Week 3			Week 6		
	RCC		RCC^{WB*}	RCC		RCC^{WB*}	RCC		RCC^{WB*}	RCC		RCC^{WB*}
Red cell concentration ($\times 10^9$ L^{-1})	6.2 ± 0.3		6.3 ± 0.3	6.2 ± 0.2		6.2 ± 0.2	6.3 ± 0.2		6.1 ± 0.2	6.1 ± 0.2		6.0 ± 0.2
Mean corpuscle volume (fL)	101.5 ± 0.8		100.2 ± 0.8	101.5 ± 1.7		101.5 ± 1.7	102.7 ± 1.5	#	107.5 ± 1.3	107.5 ± 1.4	#	113.1 ± 1.5
pH	7.13 ± 0.02		7.11 ± 0.03	7.00 ± 0.05		6.96 ± 0.06	6.80 ± 0.06		6.80 ± 0.07	6.60 ± 0.02		6.66 ± 0.08
Glucose (mM)	25.4 ± 3.2		25.0 ± 4.1	21.6 ± 2.0		21.8 ± 1.7	18.5 ± 3.02		18.4 ± 1.7	14.3 ± 0.6		15.2 ± 0.9
Lactate (mM)	3.8 ± 0.0		4.2 ± 0.1	8.8 ± 1.6		9.0 ± 1.6	14.4 ± 2.9		13.7 ± 1.8	17.6 ± 3.0		16.0 ± 2.2
Hemolysis (%)	0.04 ± 0.01		0.05 ± 0.01	0.06 ± 0.01	#	0.40 ± 0.01	0.12 ± 0.03	#	0.47 ± 0.06	0.27 ± 0.06	#	1.04 ± 0.09
Potassium (mM)	1.1 ± 0.2	#	1.7 ± 0.5	9.6 ± 0.2	#	27.1 ± 1.3	20.1 ± 0.9	#	35.4 ± 1.3	28.4 ± 1.4	#	36.7 ± 1.3
ATP (µmol/g Hb)	4.25 ± 0.36	#	4.14 ± 0.31	4.51 ± 0.28	#	4.15 ± 0.32	3.81 ± 0.29	#	3.23 ± 0.28	2.67 ± 0.49	#	2.00 ± 0.37
MP count ($\times$ µL^{-1} SN)	749 ± 220		765 ± 220	838 ± 318		2227 ± 929	1759 ± 512	#	19900 ± 4819	6957 ± 1860	#	89809 ± 39421

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Reduced red cell quality
→ FDA: 28 day shelf life?

The effect of PRT on plasma *in vitro* quality

Assay			
	FP		FP^{WB*}
Factor V (IU/mL)	0.81 ± 0.07	#	0.60 ± 0.05
Factor VII (IU/mL)	1.03 ± 0.07	#	0.78 ± 0.05
Factor VIII (IU/mL)	0.83 ± 0.13	#	0.47 ± 0.07
Factor XIII (IU/mL)	1.12 ± 0.14		1.05 ± 0.15
vWF (IU/mL)	0.90 ± 0.18		0.87 ± 0.19
Protein S (IU/mL)	0.84 ± 0.08	#	0.73 ± 0.04
α2-Antiplasmin (IU/mL)	0.88 ± 0.06	#	0.79 ± 0.04
Fibrinogen (mg/mL)	2.62 ± 0.20	#	1.85 ± 0.14
Prothrombin (sec)	13.17 ± 0.26	#	15.19 ± 0.35
APTT (sec)	34.17 ± 0.73	#	42.97 ± 1.00

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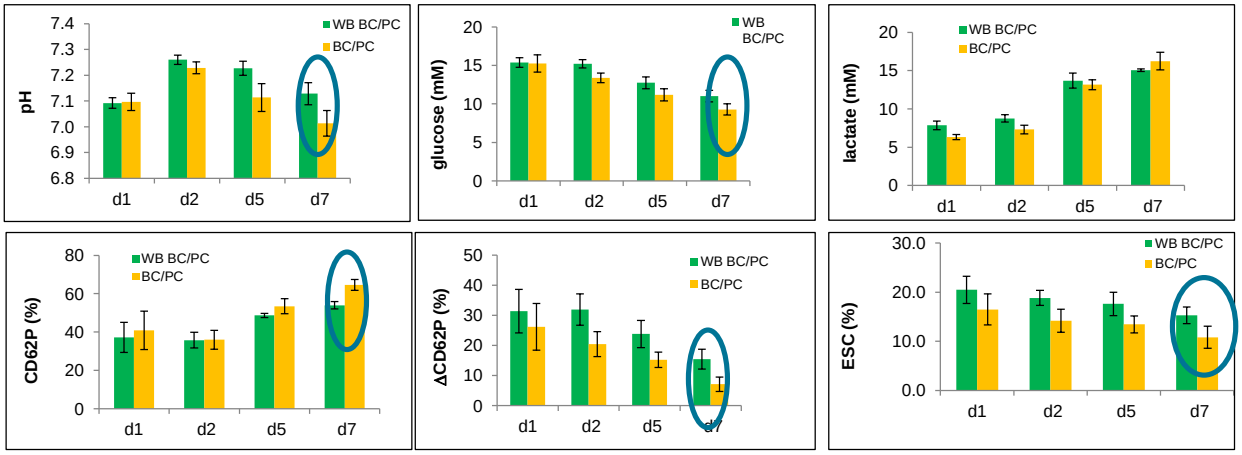
- Significant reduction of activity of several coagulant and anticoagulant proteins
- Unclear clinical relevance

The effect of PRT on platelet *in vitro* quality



Platelet units derived from whole blood treatment showed better quality then treated platelet concentrate on day 7 of storage.

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Conclusion and future perspective

Reduction in component effectiveness
Acute recipient adverse reaction
Chronic toxicity

TTI reduction (EIA, CMV)
Leukocyte reduction
→ TA-GvHD
→ Reduction of Tx reactions

- Two sides of a coin: balancing safety vs quality
  → Research to understand molecular mechanism triggered by PI
- Outcome of clinical trial is essential for decision-making for implementation
- Whole blood pathogen inactivation seems best option, but RBC in vitro quality still significantly impacted
  → Research to understand molecular mechanism triggered by PI

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- Thanks to all netCAD donors
- Brana and our lab
- netCAD staff

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