TRANSFUSION INTERREGIONALE CRS
INTERREGIONALE BLUTSPENDE SRK

Lésions de stockage des PSL
Une revue générale

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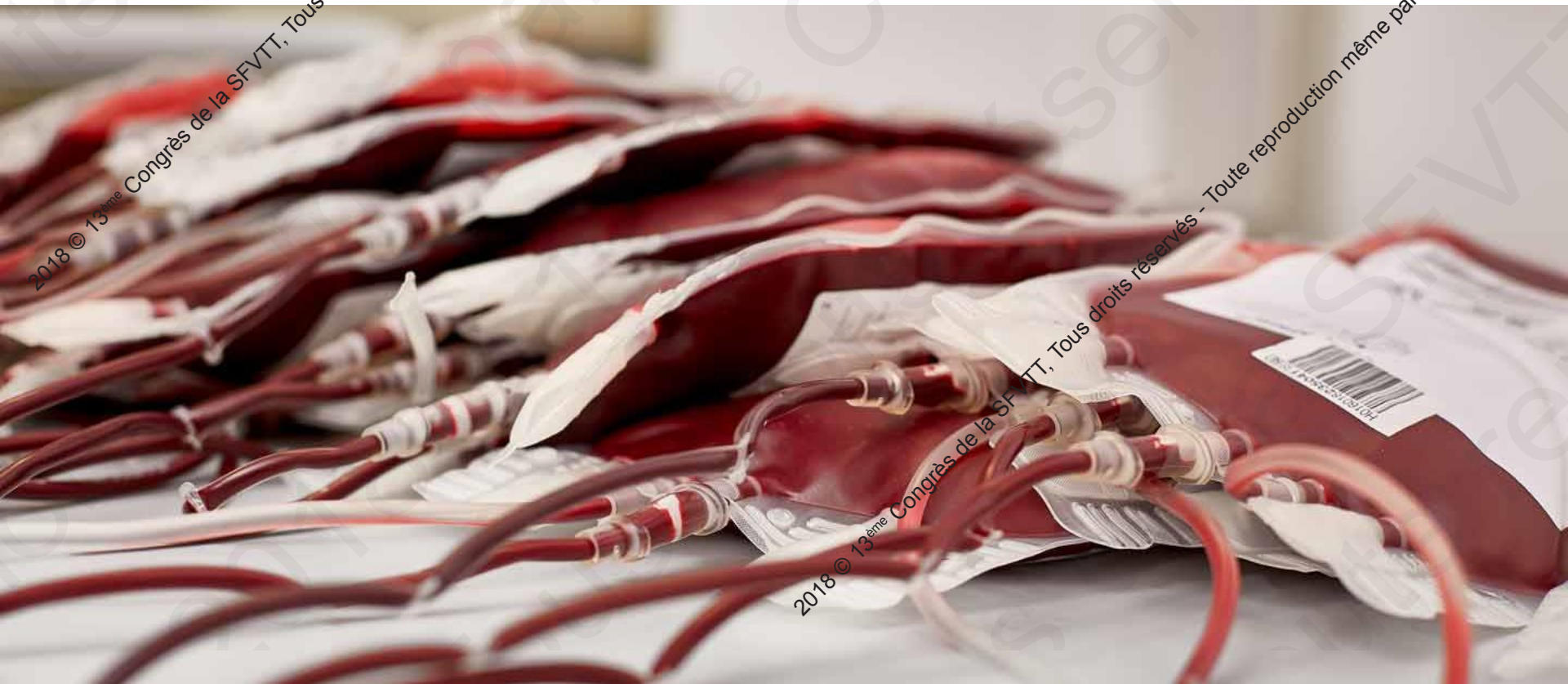
SFVTT Saint-Malo
22 novembre 2018



Introduction

- Revue générale sur la **qualité *in vitro*** des **produits sanguins labiles (PSL)**
- Lésions dues à la **fabrication** et à la **conservation**

CE

= Concentré érythrocytaire
= Concentré de globules rouges (CGR)

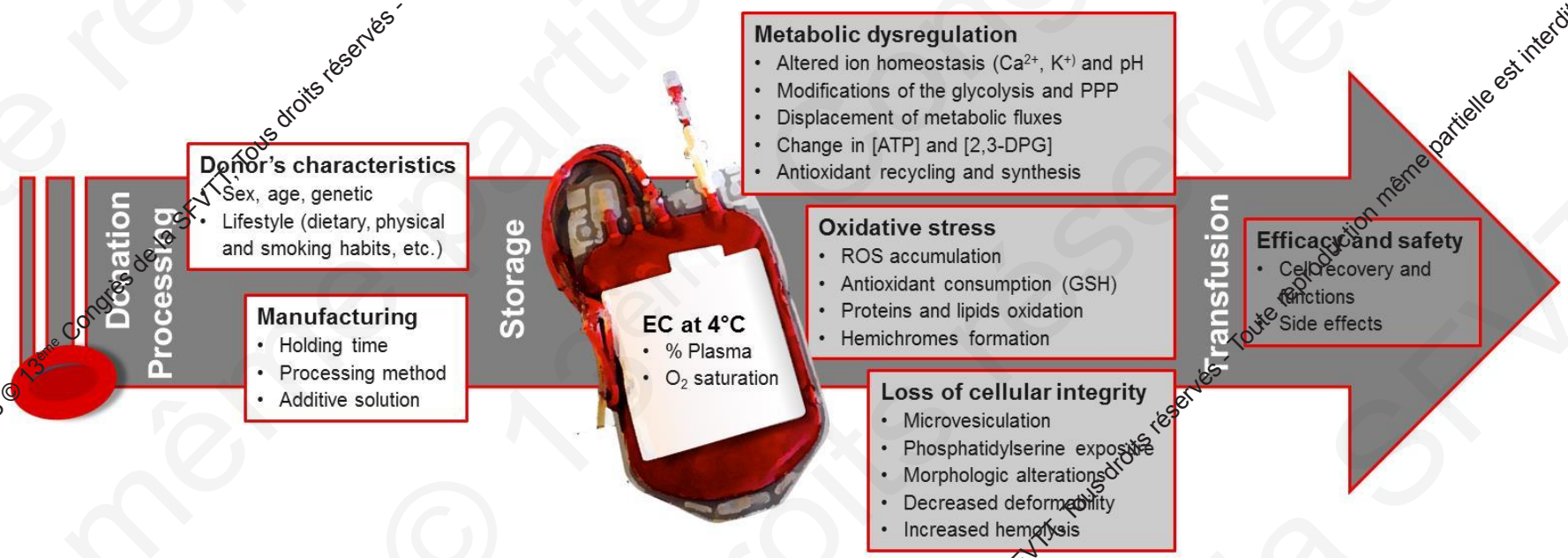
CP

= Concentré plaquettaire
CPA = CP d'aphérèse
MCP = Mélange de CP

Plasma

Plasma «thérapeutique»
Plasma pour fractionnement

The *ex vivo* journey of a RBC



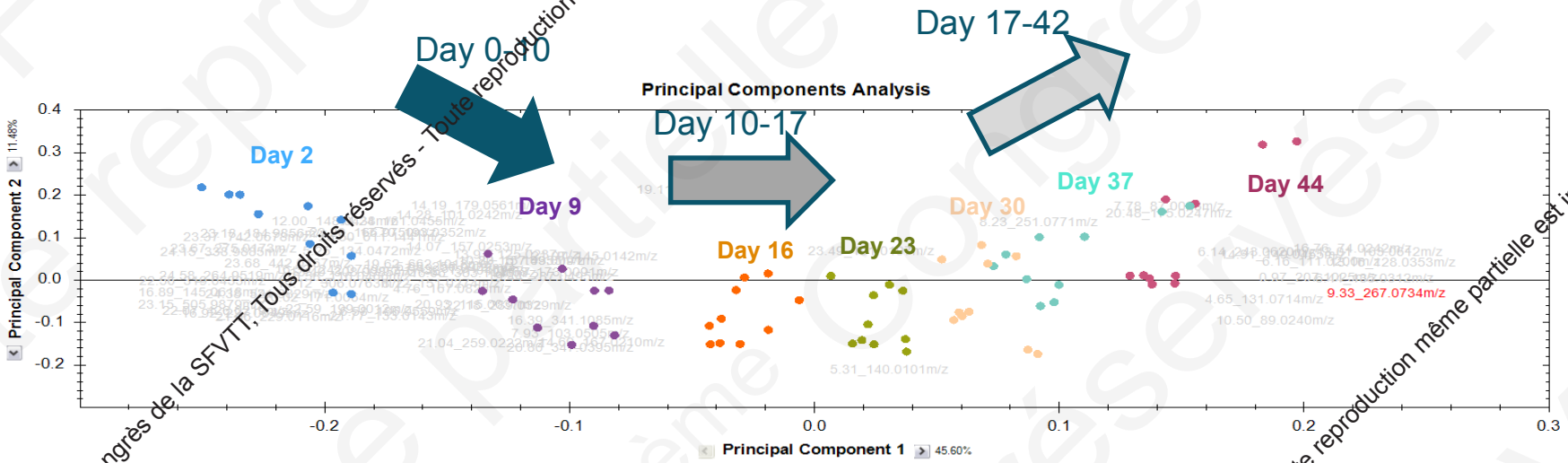
Max. 42-49 days

Bardyn *et al*, *Transfus. Clin. Biol.*, 2017

Metabolic dysregulation

Metabolic dysregulation

- Altered ion homeostasis (Ca^{2+}, K^{+}) and pH
- Modifications of the glycolysis and PPP
- Displacement of metabolic fluxes
- Change in [ATP] and [2,3-DPG]
- Antioxidant recycling and synthesis



Phase I: Glycolytic activity/ ATP production/ glutathion synthesis

Phase II: transition / Displacement of metabolic fluxes

Phase III: accumulation of purine end-products

Cold storage:

- Inactivation of membrane ionic pumps
- Slows down enzymatic activities

Bordbar *et al*, *Transfusion*, 2016; Kinoshita *et al*, *J Biotechnol*, 2009; Gevi *et al*, *J Proteomics*, 2012
Prudent *et al*, *Clin Lab*, 2014

Extracellular metabolites

➢ Biomarkers of erythrocyte concentrate age

Paglia et al. identified **8 extracellular metabolites** able to discriminate the age of ECs in the three different phases

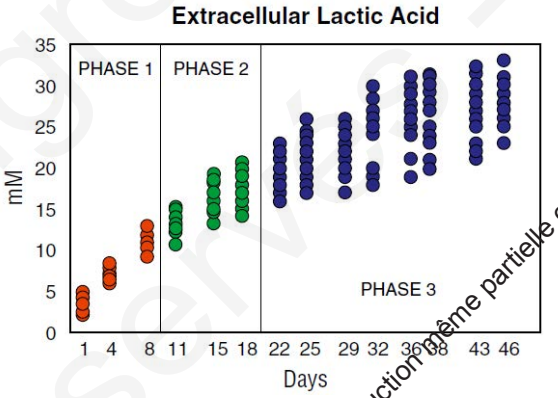


Table 2. Selected extracellular biomarkers with AUC higher than 0.75

		SAGM (extracellular metabolites)					
		Phase 1 vs phase 2		Phase 1 vs phase 3		Phase 2 vs phase 3	
Metabolites	**HMDB ID**	**AUC**	**Student *t* tests**	**AUC**	**Student *t* tests**	**AUC**	**Student *t* tests**
Lactic acid	HMDB00190	0.99	9.0E-25	1.00	2.4E-72	0.93	1.8E-31
Nicotinamide	HMDB01406	0.98	3.2E-25	1.00	3.4E-81	0.97	6.7E-41
Glucose	HMDB00122	0.97	9.5E-27	1.00	2.3E-51	0.93	9.1E-25
5-oxoproline	HMDB00267	0.94	7.7E-20	1.00	6.7E-66	0.89	1.2E-25
Malic acid	HMDB00156	0.90	3.0E-16	0.98	3.4E-53	0.84	2.6E-17
Adenine	HMDB00034	0.89	3.0E-14	0.98	3.2E-56	0.78	3.9E-11
Hypoxanthine	HMDB00157	0.86	2.7E-14	1.00	1.6E-88	0.98	2.6E-50
Xanthine	HMDB00292	0.86	2.3E-13	0.99	2.1E-57	0.92	1.3E-30

Paglia *et al*, *Blood*, 2016

Hypoxanthine and hypoxia

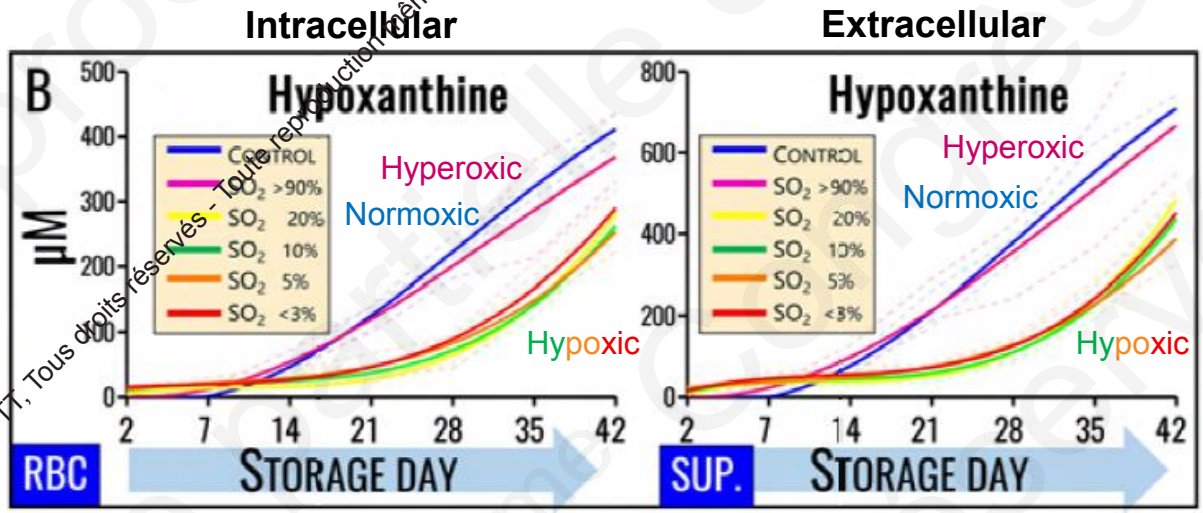


Figure 4 – Human RBCs were donated by healthy volunteers (n=4) prior to refrigerated storage in AS-3 under control (normoxic), hyperoxic (SO2>95%), or hypoxic (SO2 = 20%, 10%, 5% or <3%) conditions for up to 42 days (**A**). Hypoxanthine concentration was determined in cells and supernatants, with significant decreases in the presence of hypoxia (**B**). In **B**, all data points shown on the x axis were tested and interpolated with third order polynomial curves (not assuming linear evolution of hypoxanthine accumulation during storage) and median ± ranges (n=4) are shown (dark blue line and light blue areas, respectively).

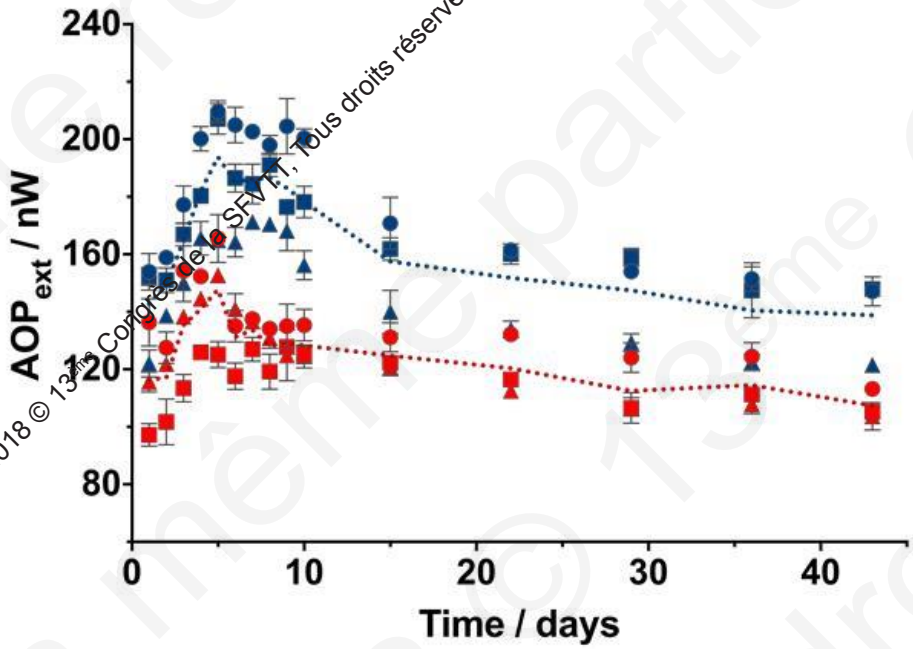
- **Accumulation of intra- and extra-cellular hypoxanthine** (end-product of purine metabolism)
- Level of hypoxanthine is **decreased by hypoxia,** both *in vivo* and *in vitro*
- **Negative correlation between intracellular level of hypoxanthine and post-transfusion recovery** in mouse and humans

Nemkov *et al*, *Haematologica*, 2017

Oxidative stress

Oxidative stress
- ROS accumulation
- Antioxidant consumption (GSH)
- Proteins and lipids oxidation
- Hemichromes formation

- **Accumulation of ROS during storage**
- **Antioxidant consumption (GSH)**



Variation of antioxidant defenses in ECs

- **Excretion of uric acid at the beginning** (probably due to dilution of plasma and homeostasis effect).
- **Antioxidant consumption**

Hallmarks of oxidative stress

Oxidative stress
- ROS accumulation
- Antioxidant consumption (GSH)
- Proteins and lipids oxidation
- Hemichromes formation

Reviewed in: D'Alessandro *et al*, *Transfusion*, 2015

➢ Protein fragmentation, membrane migration or externalization

- → Accumulation of **flotillin-2** in band 3 complexes Prudent et al, accepted for publication
- → Accumulation of **Peroxiredoxin-II** on RBC membrane

Rinalducci et al., Transfusion 2011

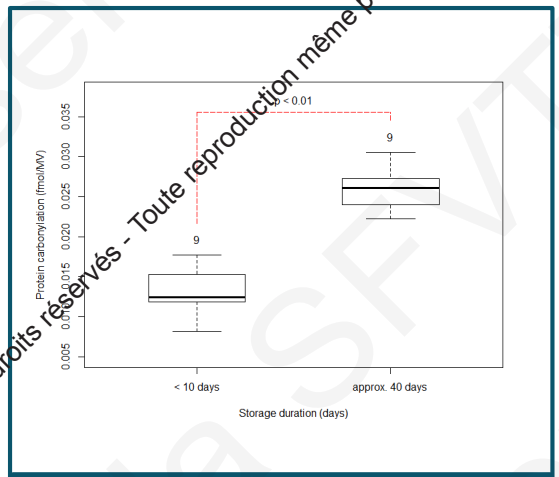
➢ Protein carbonylation (addition of carbonyl functions C=O)

- → Accumulation of oxidized proteins at the cytoskeleton after 4th week of storage

Delobel *et al*, *J Proteomics*, 2012

➢ Oxidation of lipids

- → Lipid peroxydation

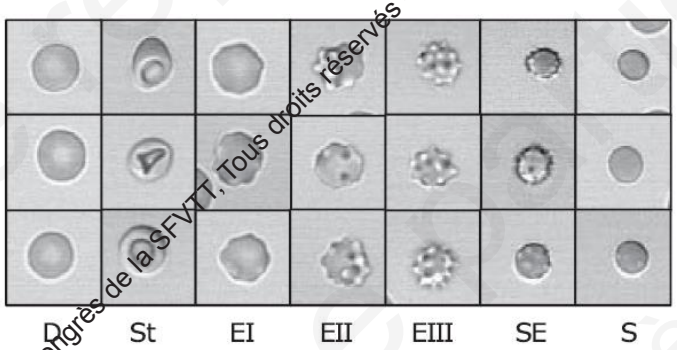


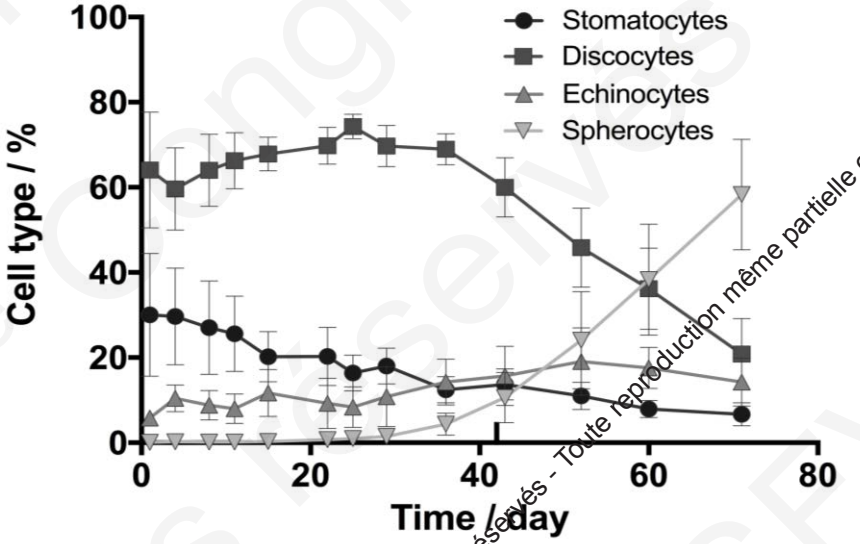
Protein carbonylation

Loss of cellular integrity

Loss of cellular integrity
- Microvesiculation
- Phosphatidylserine exposure
- Morphologic alterations
- Decreased deformability
- Increased hemolysis

➢ Morphology alterations





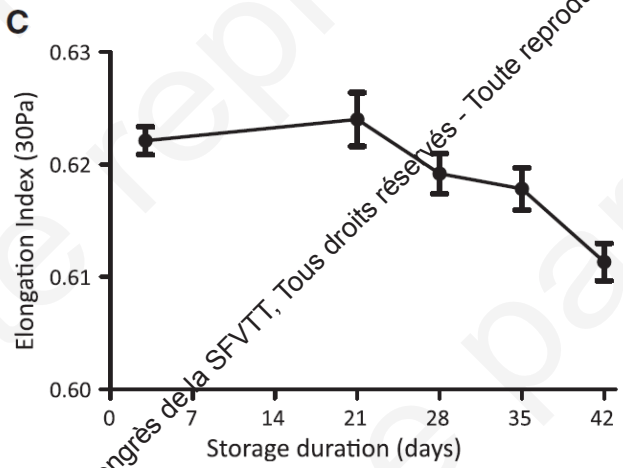
- Morphology is affected with a significant effect during the last two weeks of storage
- The percentage of **discocytes** (or **other reversible cell morphology**) decreases
- **Spherocytes** are formed
- Formation of **small RBC** were reported after 28 days of storage

Roussel *et al*, *Transfusion*, 2017, Blasi *et al*, *Transfus. Med.*, 2012
Bardyn *et al*, *Blood Transfus.*, 2017

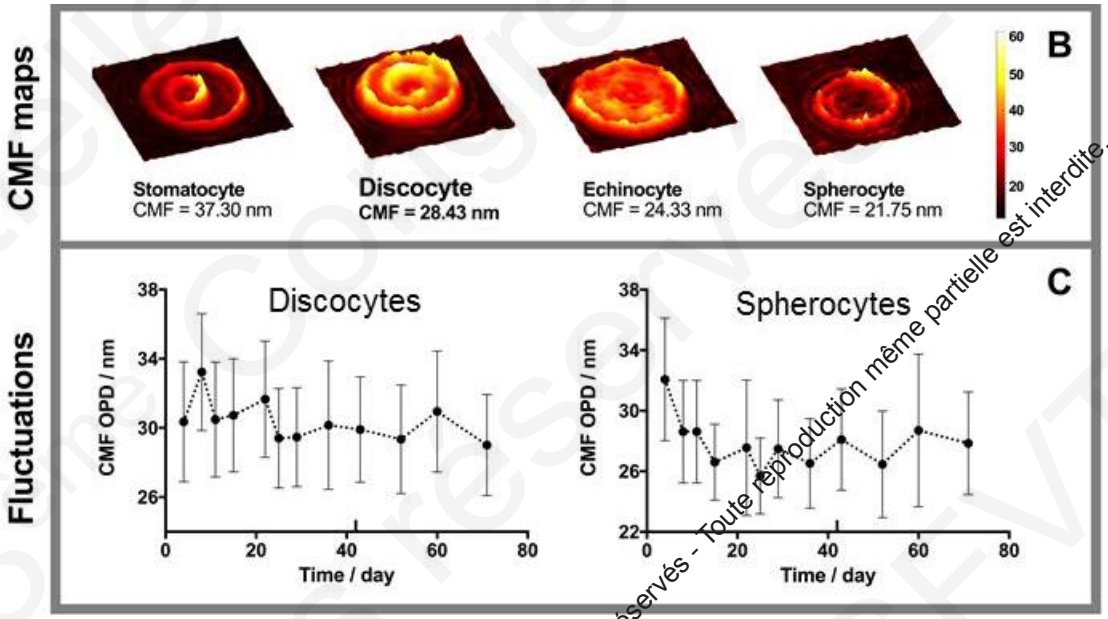
Loss of cellular integrity

Loss of cellular integrity
- Microvesiculation
- Phosphatidylserine exposure
- Morphologic alterations
- Decreased deformability
- Increased hemolysis

➢ Deformability / Membrane fluctuation



Deformability - Ektacytometry



Membrane fluctuation - DHM

- **Decreased deformability** during the storage
- **Lower membrane fluctuation** in spherocytes compared to discocytes
- RBC are **less susceptible to flow through the capillary microcirculation** and will decrease the tissue oxygenation power.

Roussel *et al*, *Transfusion*, 2017
Bardyn *et al*, *Blood Transfus.*, 2017

Loss of cellular integrity

Loss of cellular integrity
- Microvesiculation
- Phosphatidylserine exposure
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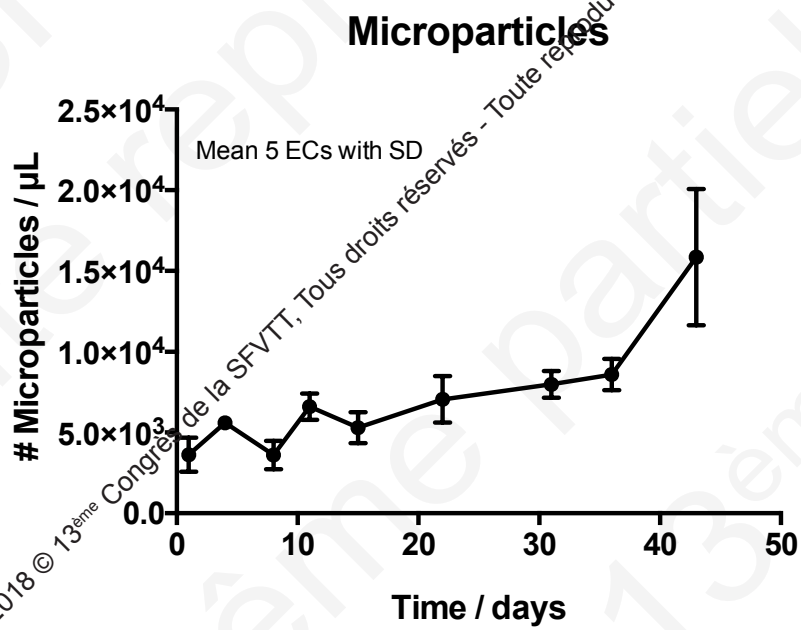
➢ Microvesiculation



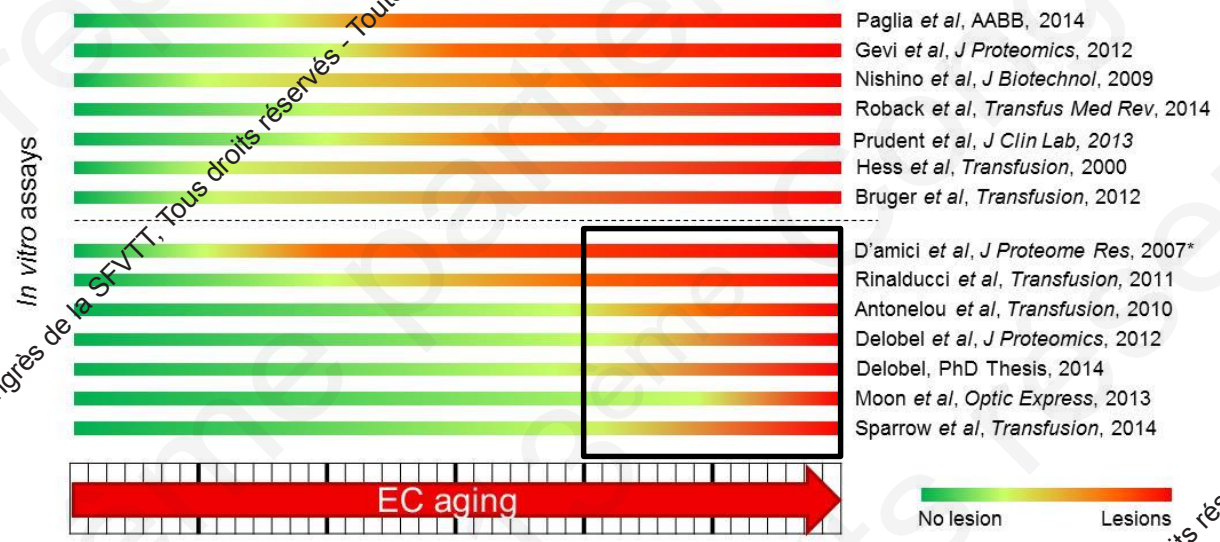
- **Microvesicles: Irreversible lesions** due to membrane perturbations, cannot be eliminated *ex vivo*
- **View as a protective phenomenon** since altered Band-3 are enriched in MVs.
- **Pro-coagulant** and might contribute to **inflammation** in some patients

➢ Increased hemolysis

Rubin *et al*, *Vox sanguinis*, 2008
Rubin *et al*, *Transfusion*, 2013
Prudent *et al*, *Transfus Aph Sci*, 2015
Bardyn *et al*, *Blood Transfus.*, 2017; Gov et al, Adv Plan Lip Bil Lip, 2009

Summary (Red Cells)

- **Cascade of events (3 phases) that leads to irreversible lesions**
- The **last two weeks** exhibit the **more pronounced irreversible lesions**



- **Reversible lesions (*in vivo* or *in vitro*)**
  - Energy metabolism (decrease in ATP, 2,3-DPG)
  - Proteins (enzymatic activity, transport…)
  - Morphology change

- **Irreversible lesions**
  - Protein oxidation
  - Ion release (K^+)
  - Morphology change
  - Expression of aging markers (PS)
  - Hemolysis and microvesiculation

Prudent M *et al*, *Transfus Aph Sci*, 2015

The *ex vivo* journey of a platelet

Donor's characteristics
- Sex, age, genetic
- Lifestyle (dietary, physical and smoking habits etc.)

Donation

Processing

Manufacturing
- Apheresis vs. Buffy-coat
- Holding time
- Centrifugation /Filtration steps
- Platelet additive solution
- Pathogen reduction

Storage

Max 5-7days

Functional decline

Morphological change

Secretome

Metabolic dysregulation

Oxidative lesions

Proteome alteration

Transfusion

Efficacy and safety
- Cell recovery and functions
- Side effects

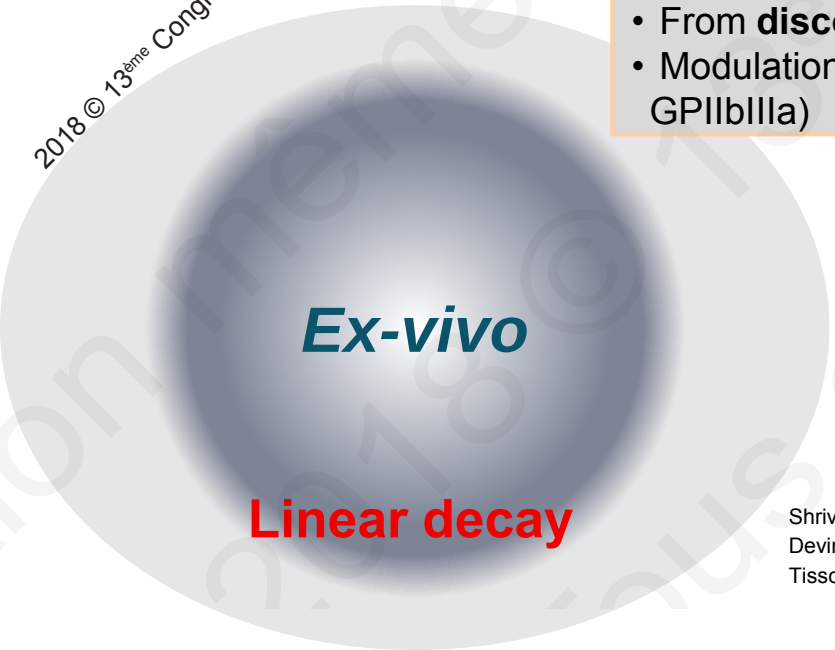
Platelets stored at 22°C under agitation

Functional decline

- **Platelet activation** (CD62P, active configuration of GPIIbIIIa, enhanced fibrinogen binding)
- **Decrease sensitivity to agonist activation** (lower aggregation capacity)
- **Lower response to hypotonic shock (HSR)** (membrane integrity)
- **Mitochondrial dysfunction** (loss of mitochondrial transmemrane potential)
- **Markers of pre-apoptotic events** (Annexin V)

Morphological change

- From **discoid to spheroid** (swirling)
- Modulation of **surface glycoproteins** (loss GPIbα, active form of GPIIbIIIa)



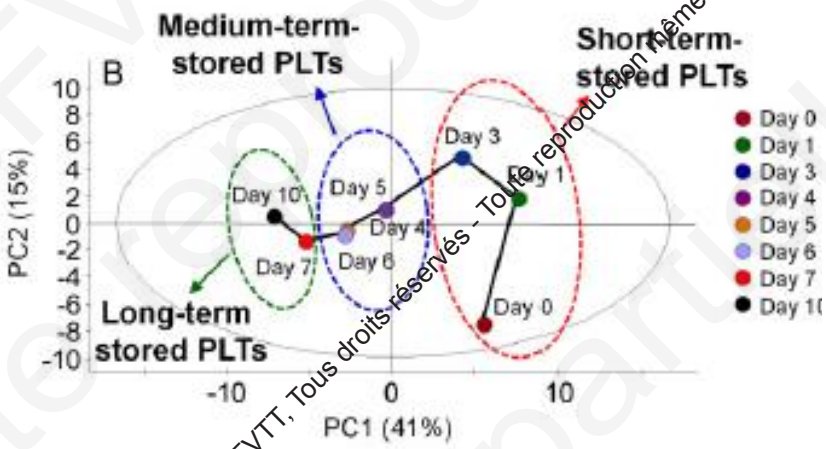
Secretome

- **α-granule secretion**
- **Release of immunomodulatory cytokines, chemokines and associated molecules**
- **Release of mtDNA**

Shrivastava et *al*, *Transfus Aph Sci*, 2009
Devine DV et al., Clin Lab Med 2010
Tissot JD et al, Transfus Clin Bio, 2017

Platelets stored at 22°C under agitation

Metabolic dysregulation
- Linear vs. discrete metabolic phenotypes
- Mitochondrial dysfunction
- Increased glycolysis (+++ upon PRT)
- ATP/ADP changes
- Alteration of purine and gluthatione metabolism upon PRT



Metabolomics approach

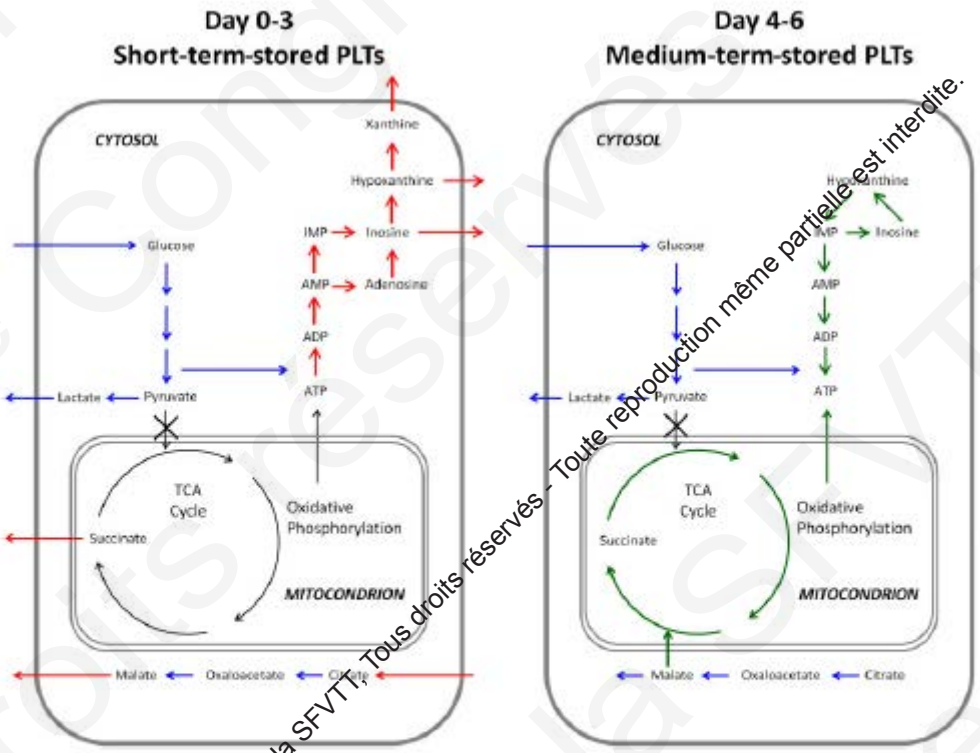


Fig. 6. Proposed metabolic phenotypes for short-term- and medium-term-stored PLTs. Blue lines represent active metabolic pathways in both phenotypes; black lines represent down regulated metabolic pathways; red lines represent metabolic pathway characteristic of short-term-stored PLTs; and green lines represent metabolic pathway characteristic of medium-term-stored PLTs.

Ex-vivo

Discrete metabolic phenotype

Paglia *et al*, *Haematologica*, 2014

Pathogen reduced platelets (22°C, under agitation)

Intercept (Cerus)

Mirasol (Terumo BCT)

Theraflex (MacoPharma)

→ Photo-(chemical) treatments

Schubert P. *et al*, *Frontiers , 2018*

Pathogen reduced platelets (22°C, under agitation)

Platelet storage feature	PI system		
	INTERCEPT	MIRASOL	THERAFLEX
Metabolic activity	± (96); ↑ (97)	↑ (98)	↑ (99)
Platelet activation (CD62P expression)	↑ (96, 100)	↑ (98)	↑ (99)
Platelet adhesion (under flow)	± (101); ↑ (102)[a]	↓ (102); ±(103)	n.d.
Clot formation (thrombo-elastography)	↓ (104)	↑[b], ↓[c] (105)	↓ (99)
Responsiveness (to agonists)	↓ (102); ±↓[d] (106)[c]	↓ (98)	± (99)
Platelet apoptosis (PS exposure)	± (107); ↑ (108)[a]	↑ (109)	↑ (99)
Platelet microparticle release	↑ (110)	↑ (111)	↑ (112)
Free mitochondria release	n.d.	↑ (95)	n.d.

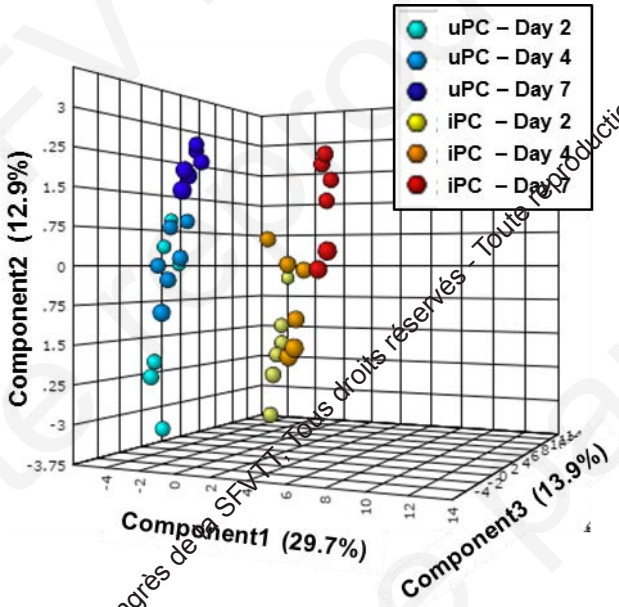
↓ = decrease; ± = similar; ↑ = increase; n.d. = not determined. The references are only examples of published studies, but are not comprehensive. Differences in some study outcomes could be due to variations in production methods used (platelet-rich plasma vs BC/PCs or apheresis PCs), composition in storage solution—plasma vs platelet additive solution (in different concentration)—and assay procedures.

[a]At end of storage.
[b]Thrombus stability.
[c]Aggregation.
[d]Agonist-dependent.

→ In general, **acceleration of the platelet storage lesions** following pathogen reduction treatments

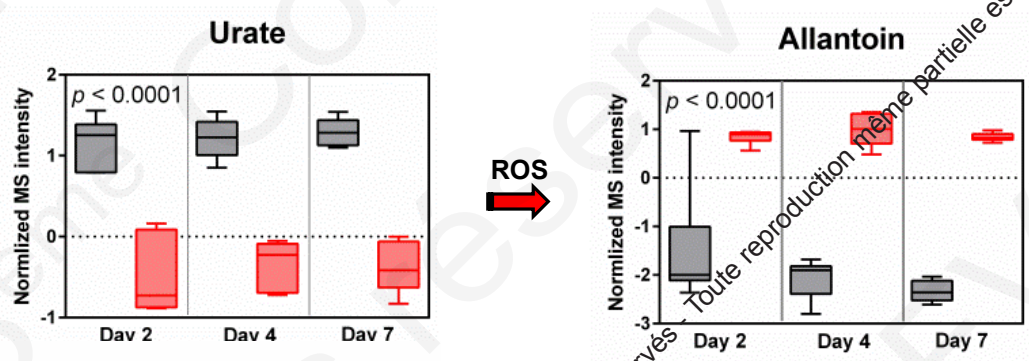
Schubert P. *et al*, *Frontiers , 2018*

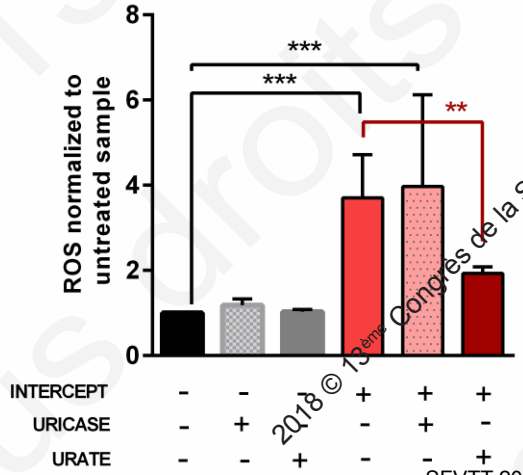
Pathogen reduced platelets (22°C, under agitation)

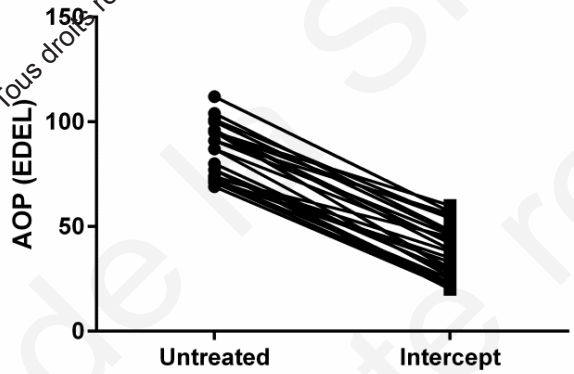


Oxidative lesions at the molecular level

- Discrete metabolic phenotypes depending on **storage time** and **PRT**
- Alteration of **purine** and **gluthatione metabolism**







Abonnenc *et al*, Metabolomics, 2016

Pathogen reduced platelets (22°C, under agitation)

➢ **Minor impact on the overall proteome**

INTERCEPT:
Intracellular pathway

MIRASOL:
Adhesion and platelet shape change

THERAFLEX (! Few data):
Platelet shape change
Aggregation

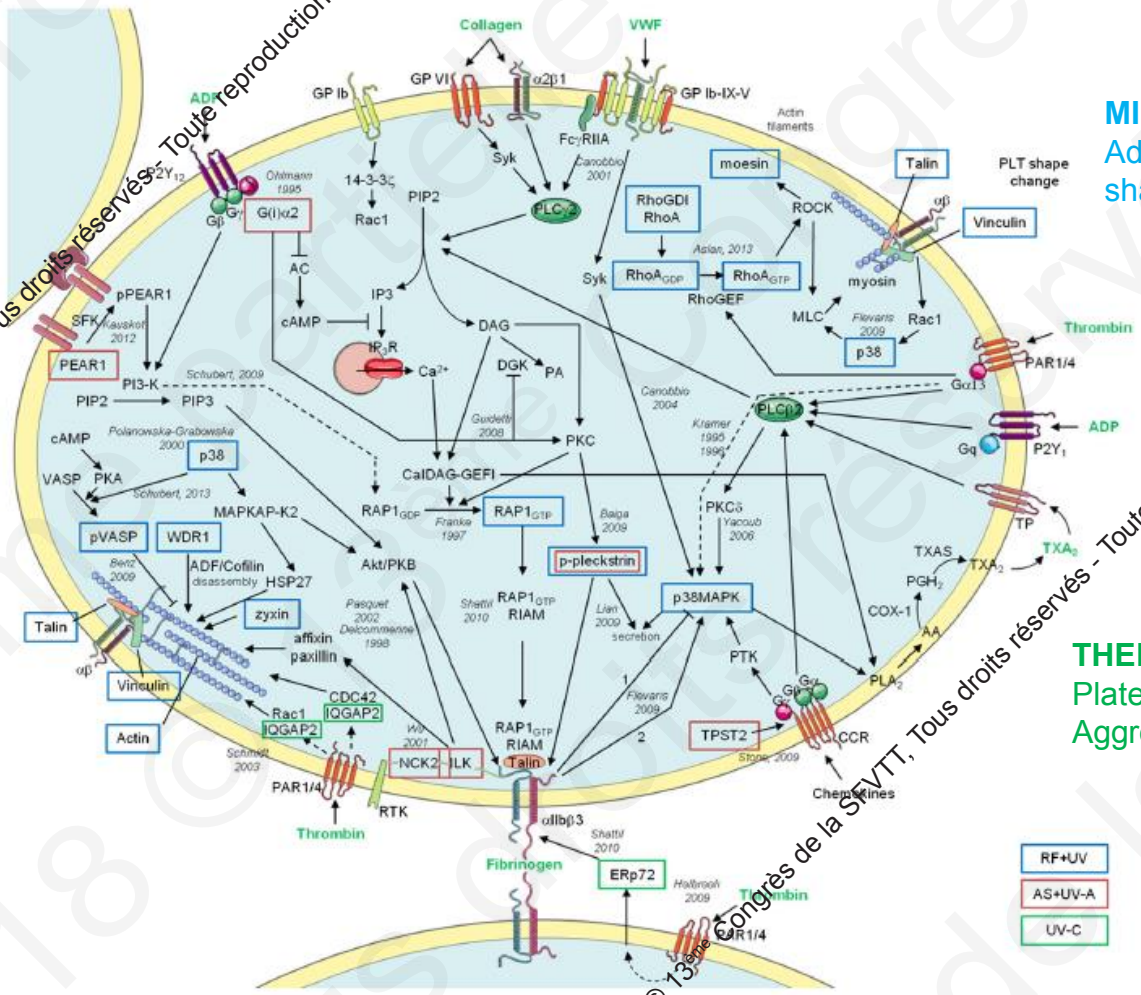


Fig 3. Platelet activation/aggregation pathways and proteins potentially affected by PI. Only potentially affected pathways are pictured. Part of the mechanisms was based on Ref [101,111], and cited references in italic. Illustrations used elements from Servier Medical Art [102].

Prudent *et al*, Transfusion Medicine Reviews, 2014

Summary (Platelets)

- **«Standard» platelets stored at +22°C, under agitation**
  - Functional decline
  - Importance of the composition of the platelet additive solution (PAS)

- **Pathogen reduction technologies**
  - Oxidative lesions (due to the nature of these photo-(chemical) treatments)
  - Accelerate the apparition of platelet storage lesions
  - Low impact on the overall proteome

- **Emerging concept:**

Central role of the p38 MAPK

Schubert *et al*, Frontiers in medicine, 2018

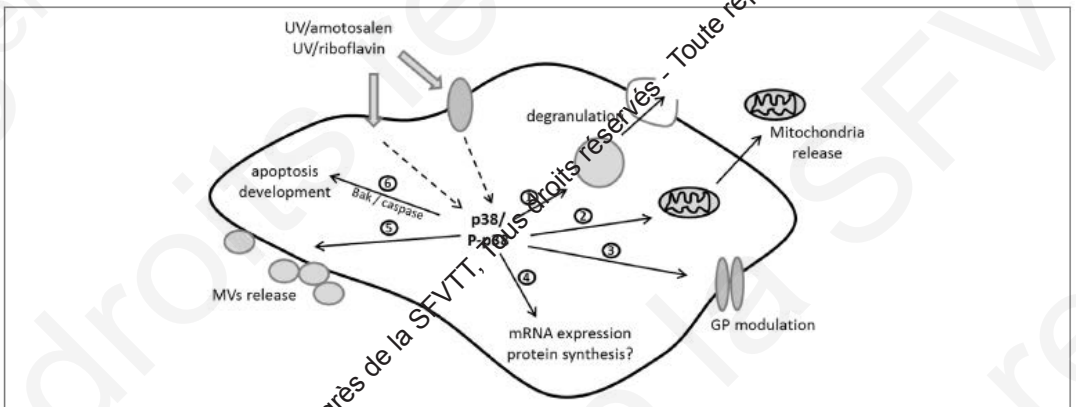


FIGURE 1 | Current molecular model of signaling triggered by ultraviolet (UV)/riboflavin (MIRASOL) and UV/amotosalen (INTERCEPT) in platelets: UV can penetrate either directly or *via* surface/receptor proteins to activate p38MAPK kinase as one of the central players in the signaling cascade. Thus far it has been shown that p38 activation/phosphorylation (P-p38) is involved in regulating (1) degranulation, (2) release of free mitochondria, (3) the modulation of glycoproteins (GPs), (4) the expression levels of mRNAs and potentially protein synthesis, (5) microvesicle (MVs) release, and (6) the development of apoptosis via proapoptotic protein expression and caspase activation. This figure was modified from Ref. (150).

The *ex vivo* journey of plasma

Donor's characteristics
- Sex, age, genetic
- Lifestyle (dietary, physical and smoking habits, etc.)

Donation

Processing

Manufacturing
- Apheresis vs. whole blood
- Time-to-freezing
- Pathogen reduction
- Lyophilization

PFC

PF24

Lyo-plasma

Storage

Thawing

Coagulation factors
- FV
- FVII
- FVIII
- FX

Proteins
- A2-antitplasmin
- Protein S
- Antithrombin

Transfusion

Efficacy and safety

Different types of plasma

Fresh Frozen Plasma (FFP)
(frozen in <8h post-don)

Frozen plasma 24h (FP24)
(frozen in <24h post-don)

Quarantine

S/D Plasma
(pooling of FP24)

Pathogen-Inactivated Plasma (PI-FFP or PI-FP24)
Mirasol (Terumo BCT), Theraflex-MB (Macopharma), Intercept (Cerus)

Thawed plasma (FFP or FP24)
(should be used ASAP but can be shortly stored at 22°C (4h) or at 4°C (24h)

Lyophilized plasma (FFP, S/D plasma or PI-FFP)

Summary (Plasma)

Review of literature (> 30 publications)

- *In vitro* quality parameters (Coagulation factors, fibrinogen, proteins)

The more the plasma is processed, the worst the impact

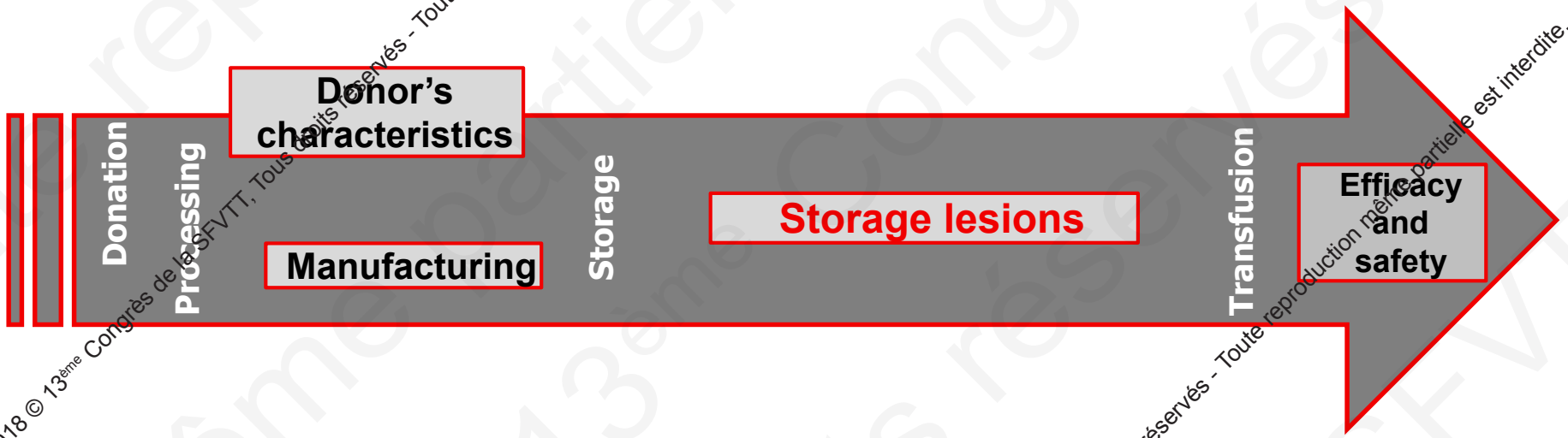
- **Time before processing and freezing**
  - **FFP** is the **less affected product** (but heterogeneous and donor-dependent)
  - **FP24**: **labile factors are decreased**
- **S/D plasma**: **Factor VIII**, **α2-antiplasmin** and **Protein S** are clearly **decreased** compared to FFP (but homogeneous and standardized product, higher level of safety)
- **PI-plasma**: **Factor VIII** is decreased compared to FFP (homogeneous and standardized product – but less that S/D plasma, higher level of safety)
- **Lyo-plasma**: depends on the **plasma used** for freeze-drying (advantages: storage and reconstitution time)

- **Leukoreduction is negligible** (Gosselin *et al*, *Transfusion*, 2013)

Prudent *et al*, Review not published yet

General conclusion

The *ex vivo* journey of blood components



There is still room for improving the quality of blood products by developing our knowledge on storage lesions

Transfusion Interrégionale CRS, Biopôle, Epalinges

Thank you
for your attention

Laboratoire de Recherche
Dr Michel Prudent
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Dr Julien Delobel
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