TRANSFUSION INTERREGIONALE CRS
INTERREGIONALE BLUTSPENDE SRK

What can « omics » bring to transfusion medicine?

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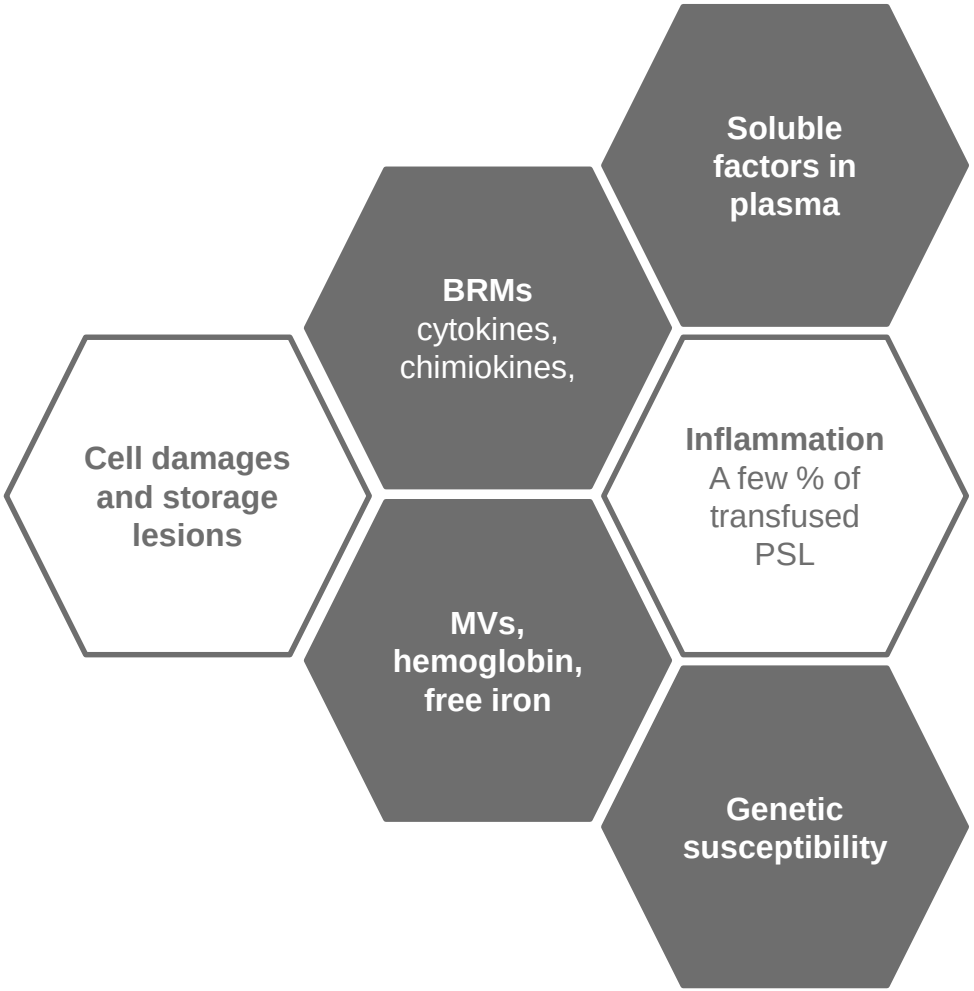
Transfusion and inflammation



Transfusion and inflammation

Examples

Platelet concentrates

- **Soluble CD40 ligand** and **TRALI** (Khan *et al*, *Blood*, 2006; Cognasse *et al*, *Blood*, 2008)
- sCD40L, interleukin-27 and sOX40L **elevated in inflammatory ATRs** (Hamzeh-Cognasse *et al*, *Transfusion*, 2014)
- **Microvesicles** can participate to **inflammation** (Boilard *et al*, *Science*, 2010; Cloutier *et al*, *EMBO mol med*, 2013; Cognasse *et al*, *TRASCI*, 2015)

Erythrocyte concentrates (ECs)

- **Extravascular hemolysis** of long-term-stored ECs (Hod *et al*, *Blood*, 2010; Spitalnik, *Transfusion,* 2014)
- **Microvesicles** amplify systemic **inflammation (**Zecher *et al*, *Arterioscler Trhomb Vasc Biol*, 2014)
- Accumulation of **metabolites** (hypoxanthine degraded by xanthine oxidase) may initiate a cascade of **inflammatory reactions** (Casali *et al*, *Blood Transfus*, 2016)

Transfusion and inflammation

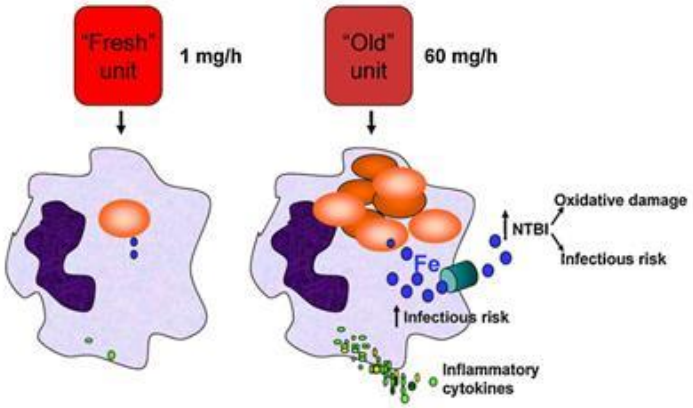
Hemolysis and clearance of RBCs

- **Canine model**, infection with *Staphylococcus aureus* + transfusion of “young” or “old” ECs (Solomon *et al*, *Blood*, 2013)
  - *In vivo* hemolysis after transfusion and free Hb increased pulmonary hypertension and mortality

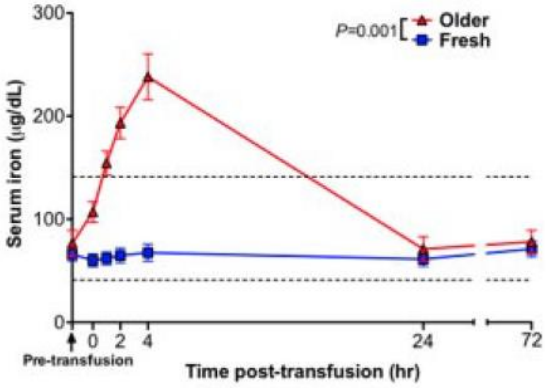
- **Mouse model**, transfusion of “young” or “old” ECs (Hod *et al*, *Blood*, 2010)
  - Accumulation of **iron in spleen and liver** (due to RBCs)
  - Markers of **inflammation**: IL-6, KC (due to RBCs too)
  - Increase of NTBI in plasma: **risk of infection**

  Increase of bacteria growth *in vitro*

- Clinical study on **healthy volunteers** (Hod *et al*, *Blood*, 2011)
  - Only iron parameters influenced by the transfusion of “old “ ECs



Iron hypothesis



Iron level post-transfusion

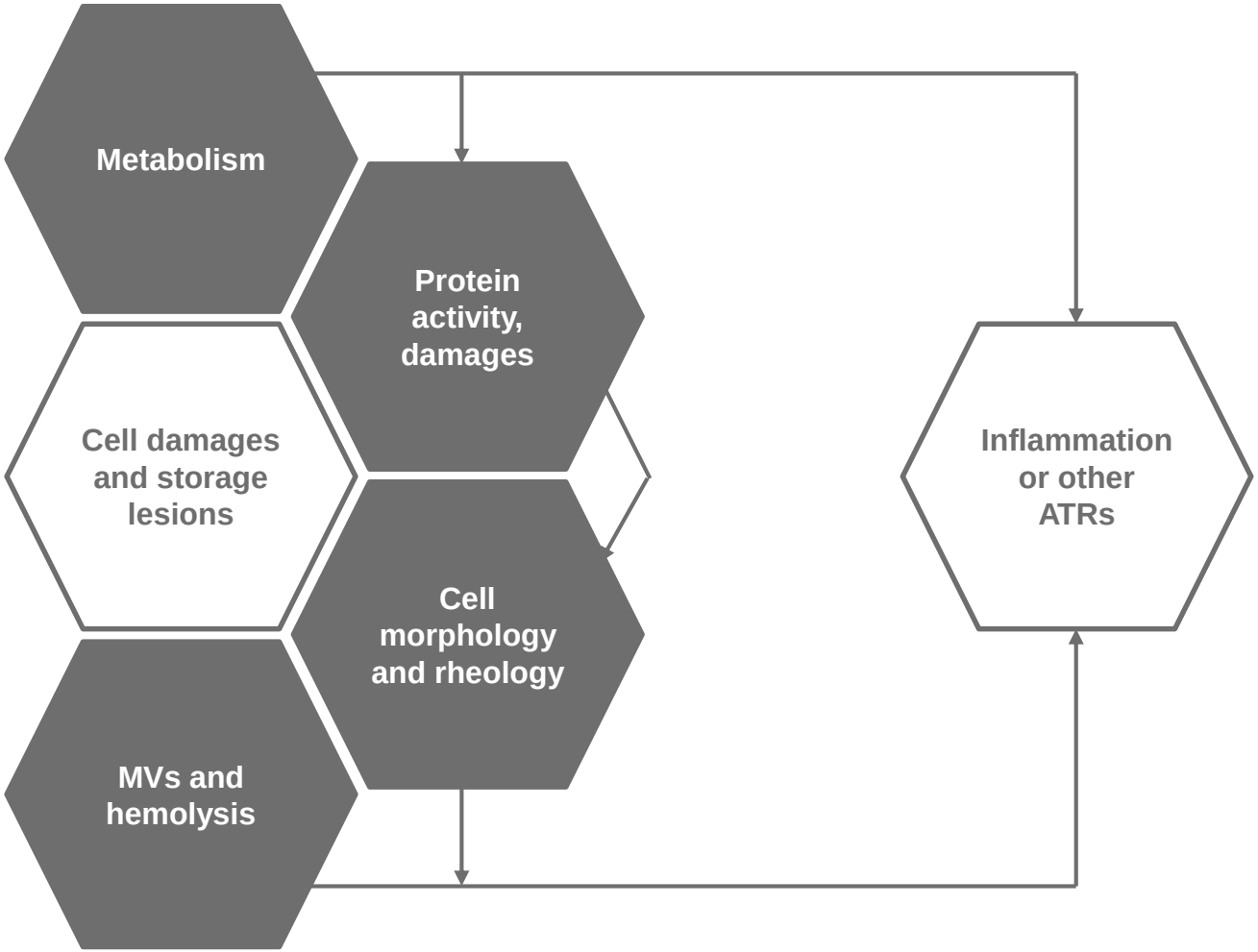
Erythrocytes concentrates

- Collected from whole blood donation
- Processed (eg filtration, leukoreduction)
- Stored at 4°C in additive solution (SAGM, PAGGSM, AS-3, MAP…) up to 42-49 days

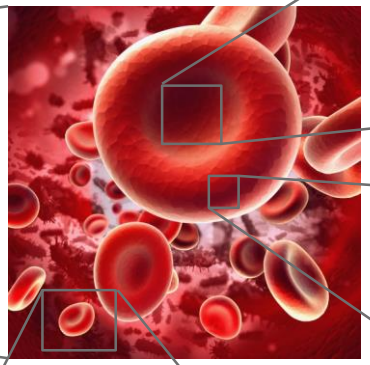
Storage

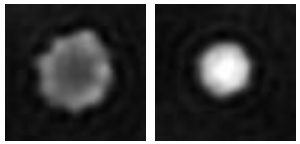
- **Reversible lesions (*in vivo* or *in vitro*)**
  - Energy metabolism (decrease in ATP, 2,3-DPG)
  - Proteins (enzymatic activity, transport…)
  - Morphology change
- **Irreversible lesions**
  - Protein oxidation
  - Ion release (K^+)
  - Morphology change
  - Expression of aging markers (PS)
  - Hemolysis and microvesiculation

Erythrocyte concentrates



Investigation of cell lesions





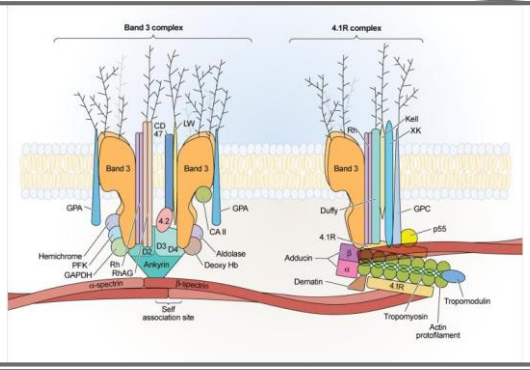
Morphology and microvesicles

Metabolism

Glycolysis | **PPP** | **Purine metabolism**

GSH synthesis | **Anti-oxidants**

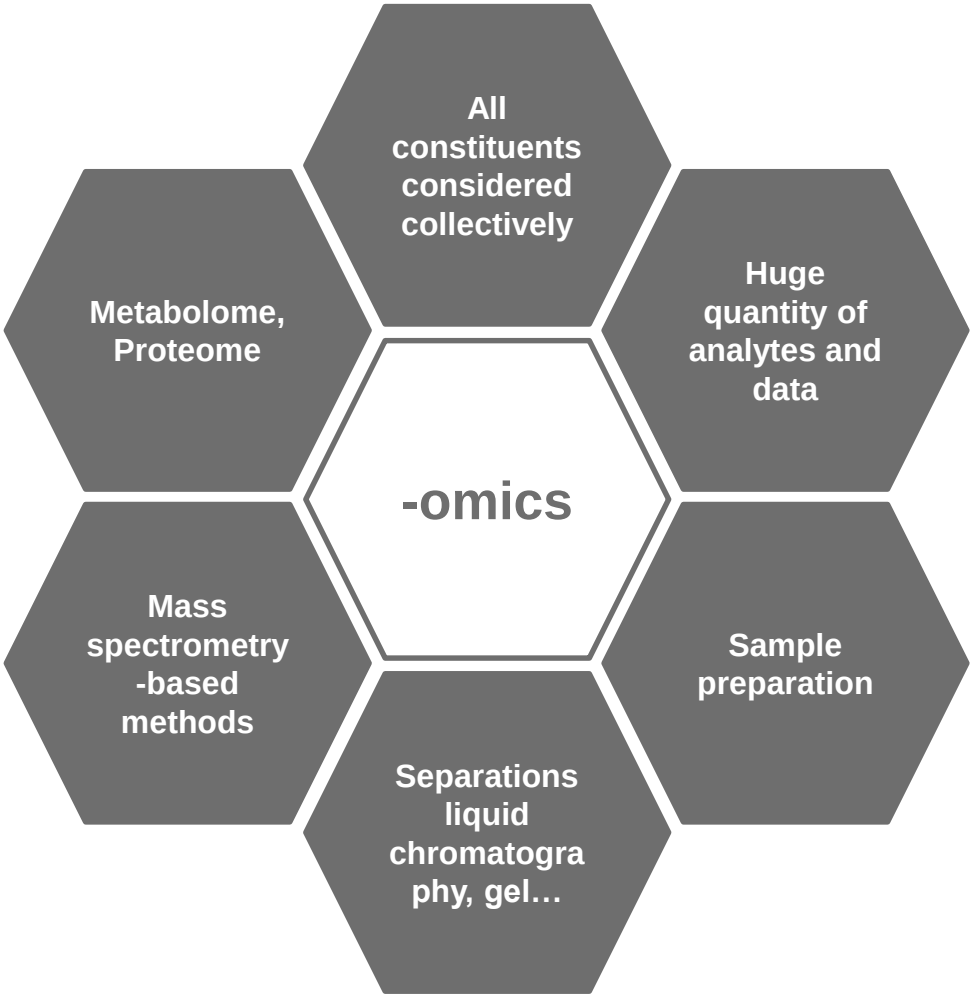
S-adenosylmethionine cycle



Proteins:
phosphorylation and oxidation

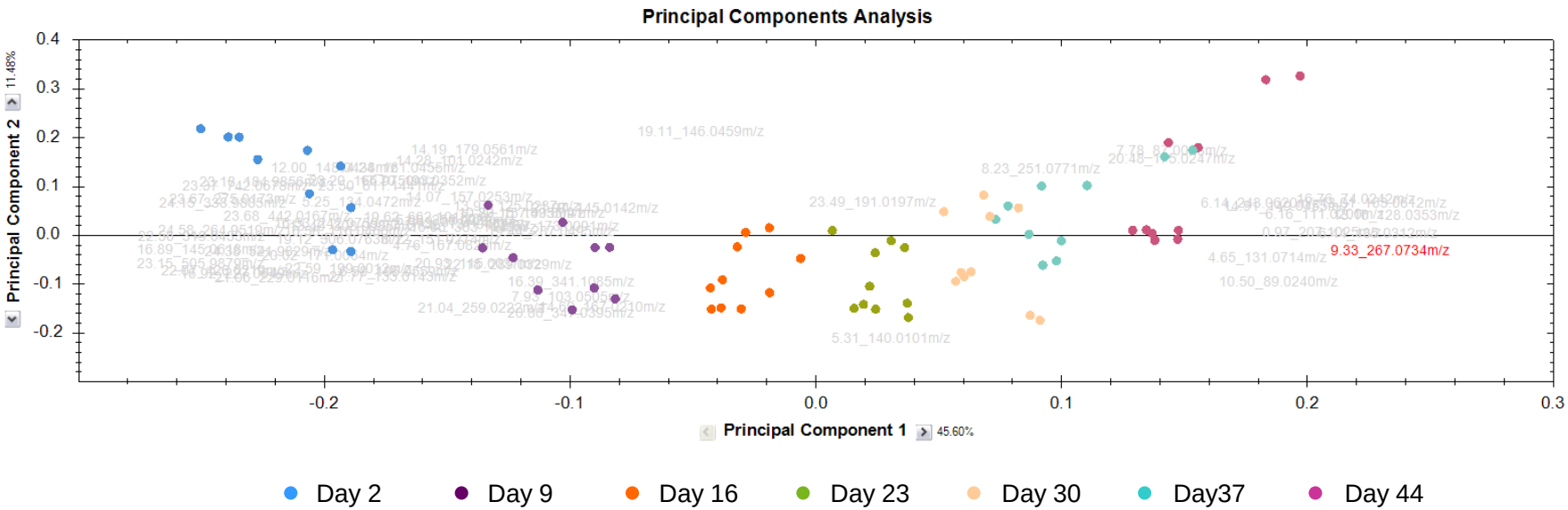
Salomao *et al*, *PNAS*, 2008
Larousse.fr

Omics


All constituents considered collectively
Metabolome, Proteome
Huge quantity of analytes and data
-omics
Mass spectrometry -based methods
Sample preparation
Separations liquid chromatogra phy, gel…

Metabolism

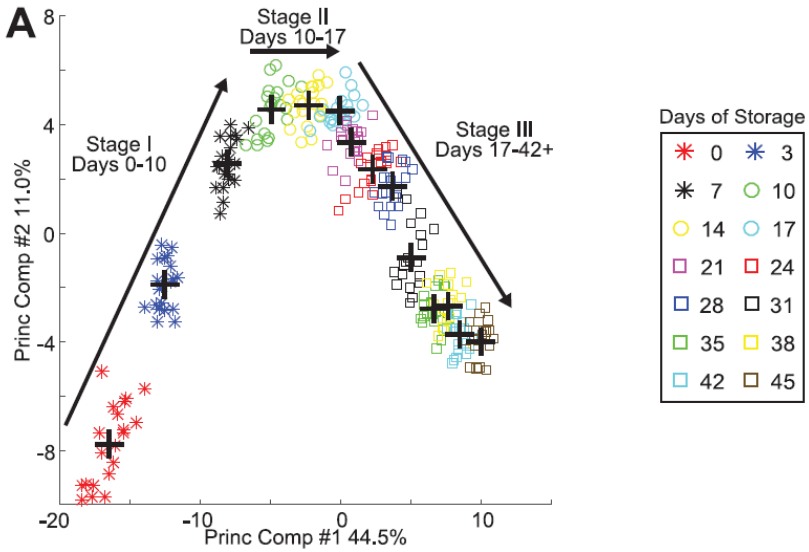
Targeted metabolomics of RBCs stored in SAGM



PCA of metabolomic data from RBC storage

Metabolism

Targeted metabolomics of RBCs stored in SAGM (Brodbar *et al*)



*PCA of metabolomic data from RBC storage (*Bordbar *et al*, *Transfusion*, 2016*)*

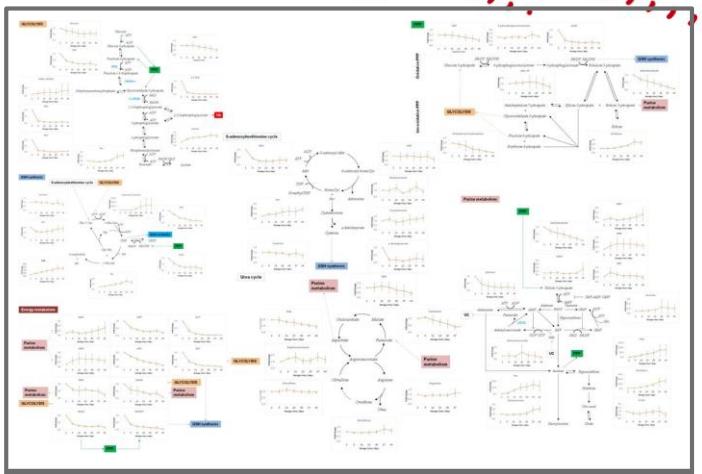
Metabolism

Energy metabolism affected

- Decrease in glycolysis (PFK and other enzymes)
- Pentose phosphate pathway (PPP) produces NADPH to enable GSSG reduction
- GSH synthesis works but not efficient enough to maintain GSH level
- Changes observed around the second week (day 9) and 4th-5th week of storage.

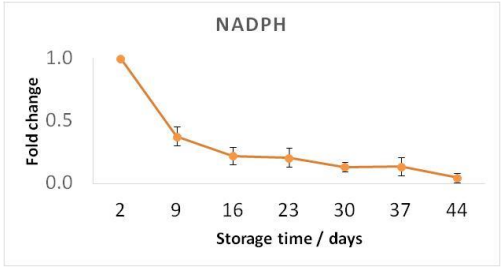
Protection against oxidants, eg H_2O_2 from hemoglobin auto-oxidation

$$H_2O + 1/2O_2 \xleftarrow[\text{CAT}]{NADP^+ \; NADPH} H_2O_2 + 2GSH \xrightarrow[\text{GSH peroxidase}]{NADPH \; NADP^+} 2H_2O + GSSG$$

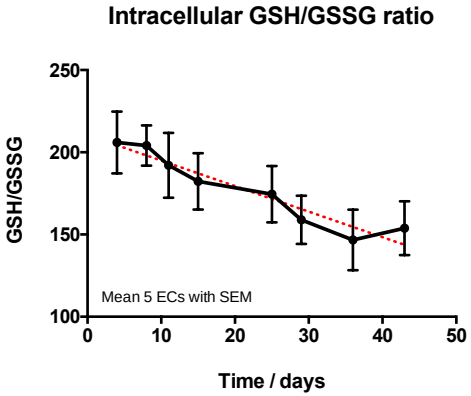


Metabolomic map

$$GSH \rightleftharpoons GSSG \quad (NADP^+ \; NADPH)$$



Evolution of NADPH (metabolomics, n = 5)



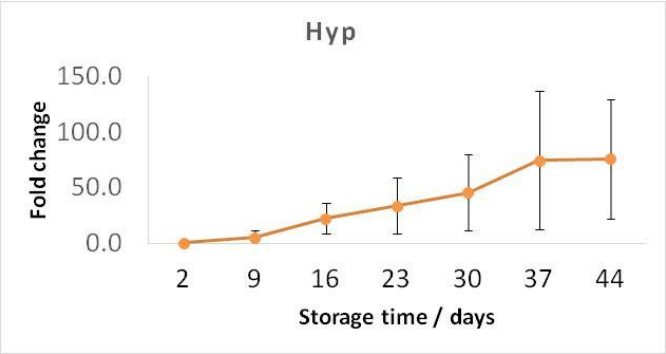
Ratio GSH/GSSG (spectrophotometric, n = 5)

$$\text{Glucose 6-phospate} \rightleftharpoons \text{6-phosphogluconolactone} \quad (NADP^+ \; NADPH)$$

PPP G6PD

Prudent *et al*, Swisstransfusion (*Clin Lab*), 2014
Müller *et al*, in preparation

Excreted metabolites



Evolution of hypoxanthine (metabolomics, n = 5)

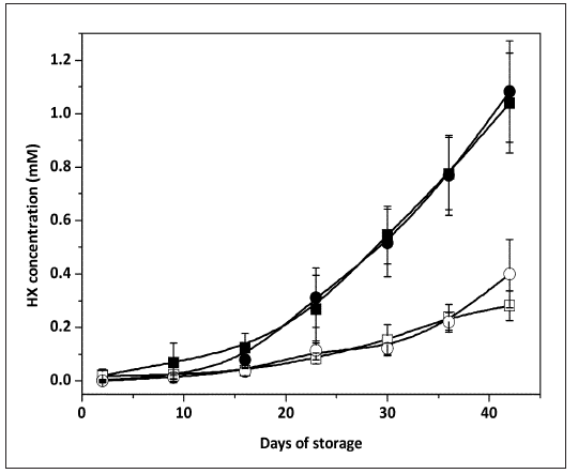


Figure 1 - Hypoxanthine (HX) quantification by ^{1}H-NMR in RBC units during storage.
Leucodepleted RBC (n=8): (□) HX inside RBC and (■) HX in the suspension medium. Non-leucodepleted RBC (n=8): (○) HX inside RBC and (●) HX in the suspension medium. Data are expressed as the mean ± standard deviation.
RBC: red blood cells; ^{1}H-NMR: proton nuclear magnetic resonance.

$$Hyp + O_2 \xrightarrow[\text{Xanthine oxidase}]{H_2O} Xanthine + H_2O_2$$

- In case of transfusion of long-term-stored ECs, **oxidation of Hyp** by recirculating **XO** will increase ROS that may **initiate inflammatory reactions** (Casali *et al*, *Blood Transfus*, 2016)

Prudent *et al*, Swisstransfusion (*Clin Lab*), 2014

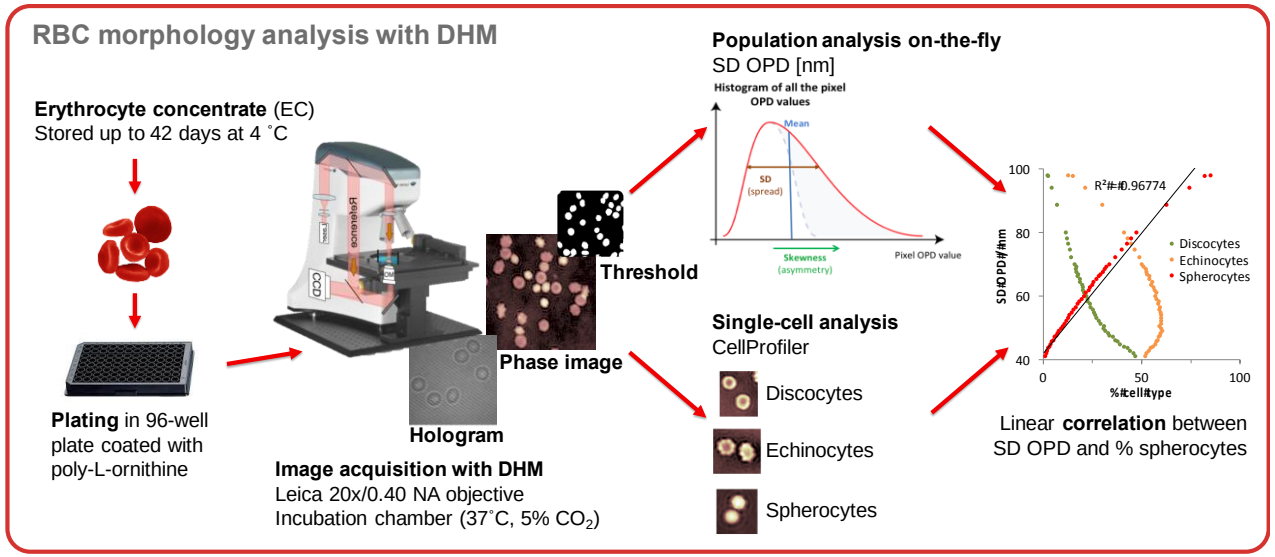
Protein activity

Example of membrane protein phosphorylation

- Phosphorylation is involved in signaling pathways, enzymatic activity and protein-protein interactions

Effect of phosphorylation on RBC morphology

- Cellular level: digital holographic microscopy



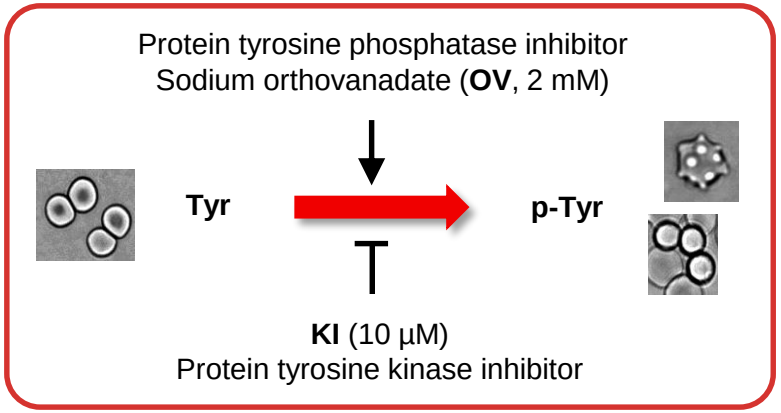
Prudent *et al*, Protein phosphorylation and RBC morphology, in preparation
Prudent *et al*, AABB (*Transfusion*), 2014

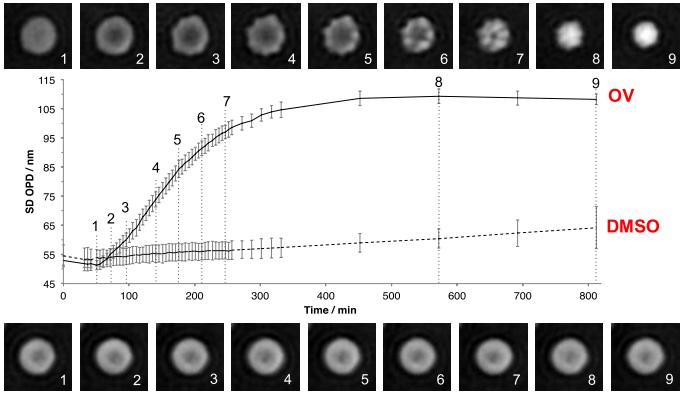
Protein activity

Example of membrane protein phosphorylation

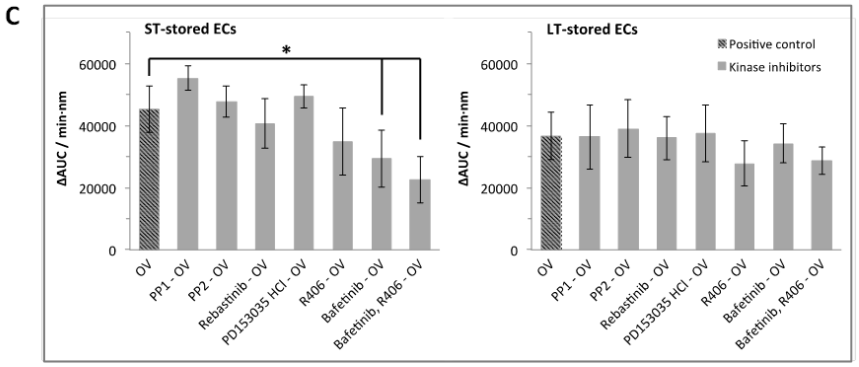
Effect of phosphorylation on RBC morphology

- Cellular level: morphology change less important at expiration date

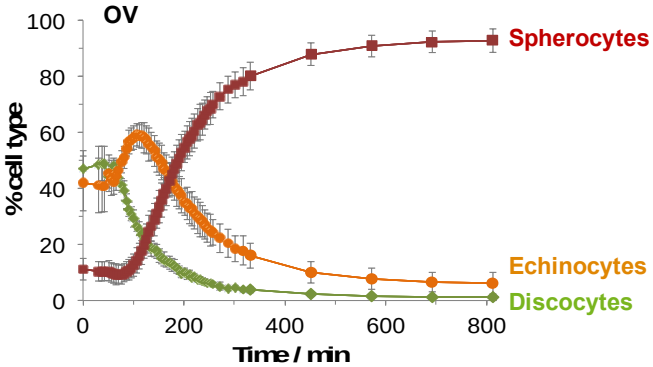




Shape change upon OV treatment



Effect of kinase inhibitors



Single cell analysis of OV-treated RBCs

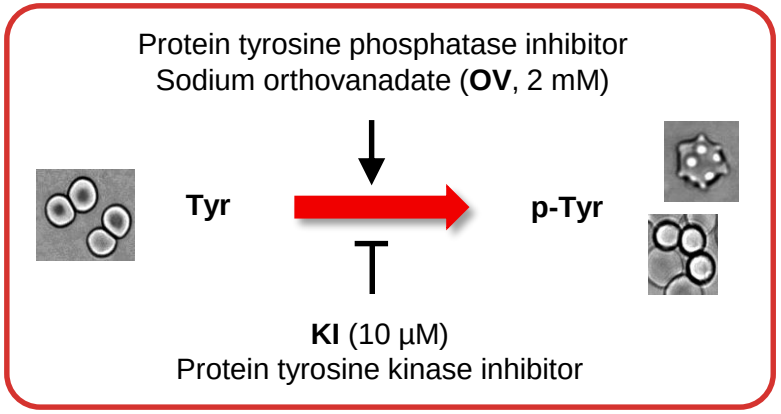
Prudent *et al*, Protein phosphorylation and RBC morphology, in preparation
Prudent *et al*, AABB (*Transfusion*), 2014

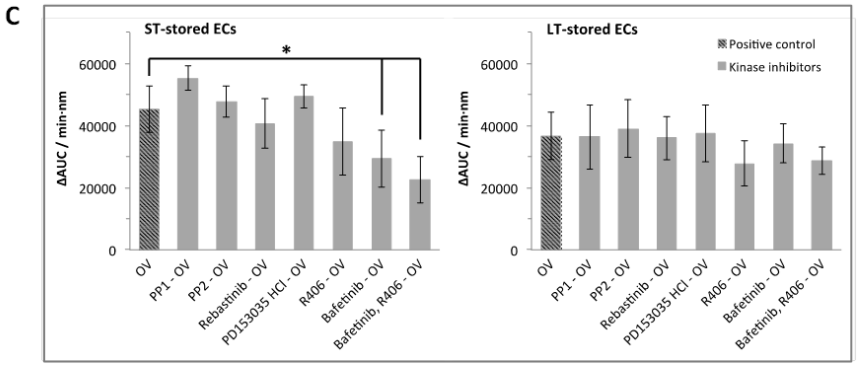
Protein activity

Example of membrane protein phosphorylation

Effect of phosphorylation on RBC morphology

- Cellular level: morphology change less important at expiration date





Effect of kinase inhibitors

- **Phosphorylation plays a role in cell shape up to 50% only in "young" ECs**

Prudent *et al*, Protein phosphorylation and RBC morphology, in preparation
Prudent *et al*, AABB (*Transfusion*), 2014

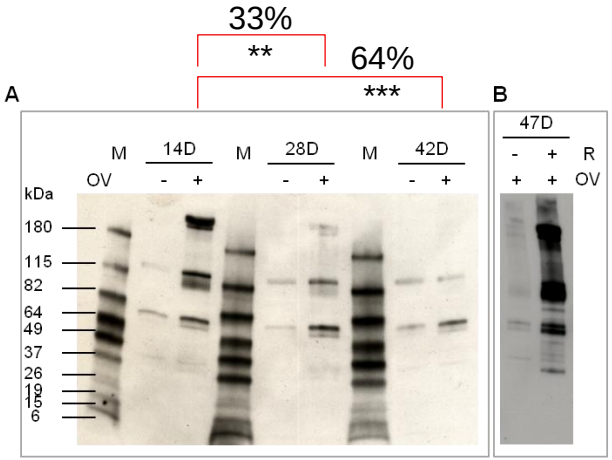
Protein activity

Example of membrane protein phosphorylation

Effect of phosphorylation on RBC morphology

- Biochemical level: loss of protein tyrosine phosphorylation capacity during storage of ECs

  (restored by rejuvenation treatments, based on inosine, pyruvate and adenine)



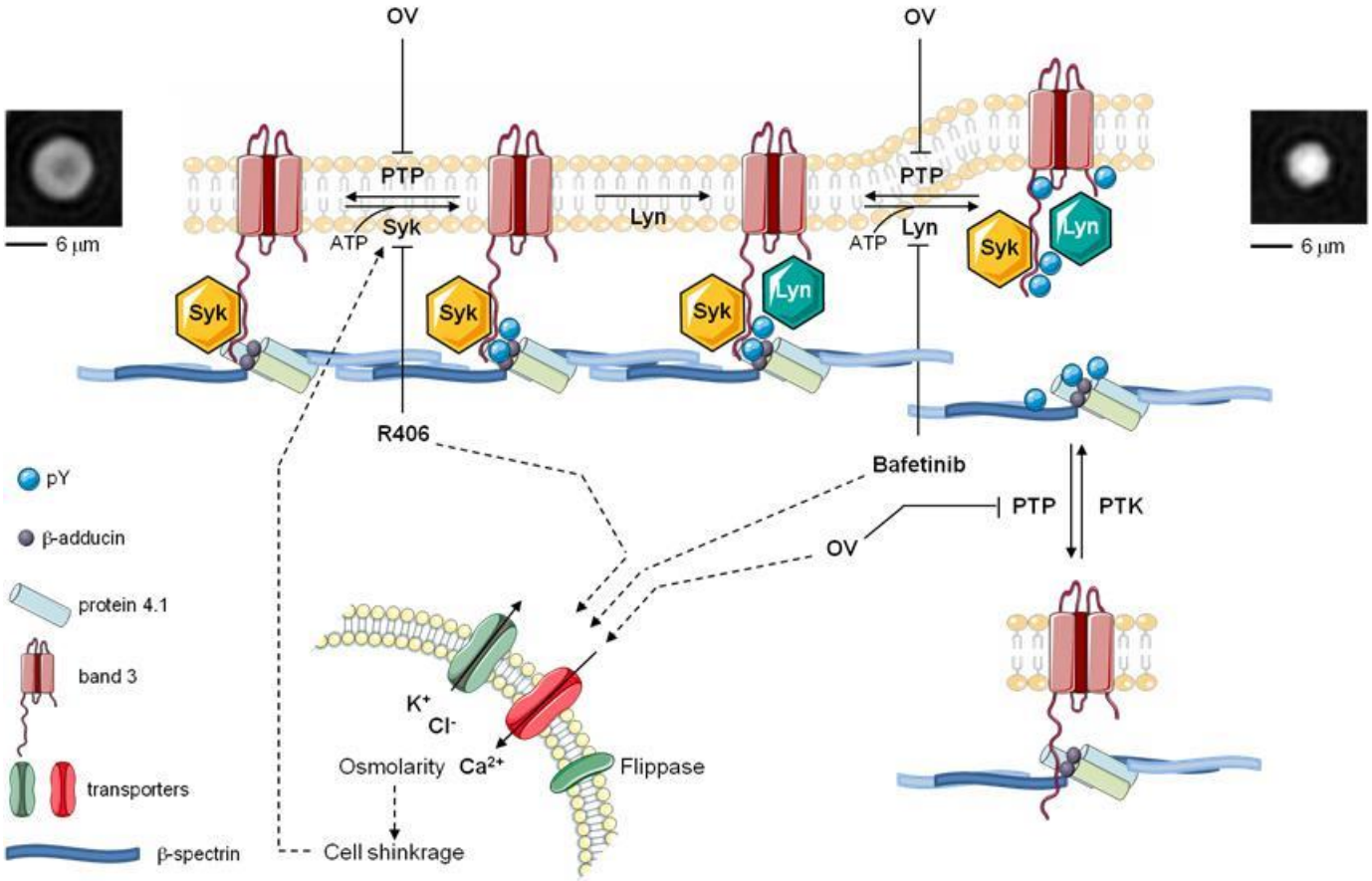
Western blot analysis of protein phosphorylation (pY). Effect of storage (A) and rejuvenation (B)

- **Loss of phosphorylation capacity during storage (linked to energy metabolism)**
- **Phosphoproteomics: band 3, β-spectrin, protein 4.1 et β-adducin significantly phosphorylated**

Prudent *et al*, Protein phosphorylation and RBC morphology, in preparation
Prudent *et al*, AABB (*Transfusion*), 2014

Protein activity

Example of membrane protein phosphorylation

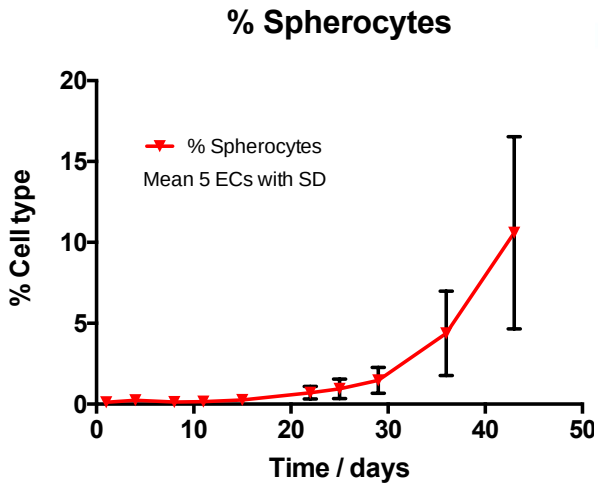


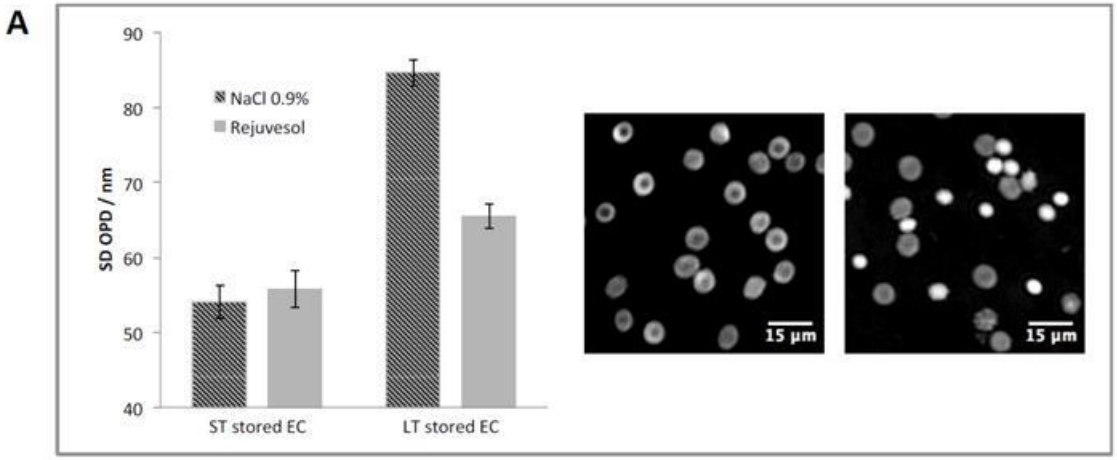
Protein phosphorylation and shape change

→ **Loss of protein phosphorylation may affect cell deformability and transfusion efficiency**

Prudent *et al*, Protein phosphorylation and RBC morphology, in preparation
Prudent *et al*, AABB (*Transfusion*), 2014

Irrevesible lesions





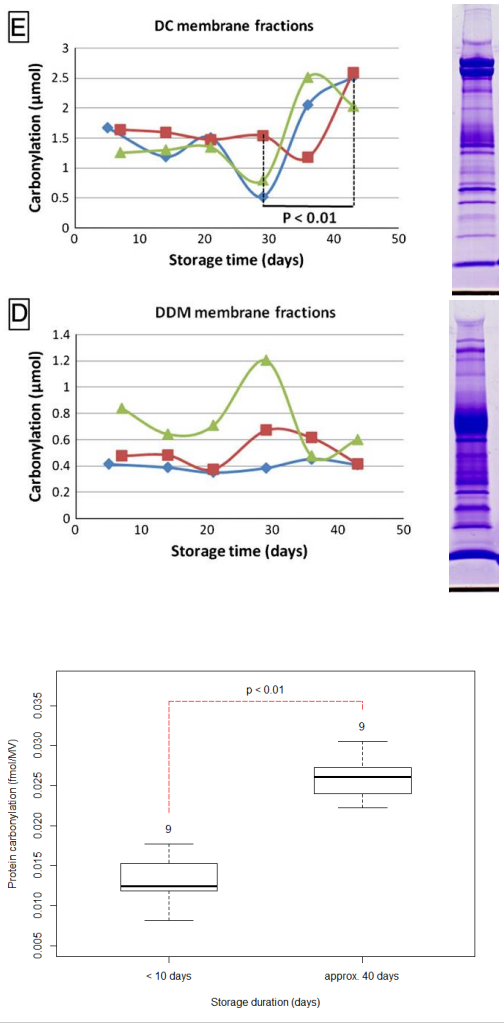
RBC morphology by digital holographic microscopy

- Rejuvenation (based on inosine, pyruvate and adenine) restores *in vitro* metabolite levels such as ATP
- However, the benefit at the cellular level is not total on long-term stored ECs

Prudent *et al*, Protein phosphorylation and RBC morphology, in preparation
Prudent *et al*, AABB (*Transfusion*), 2014

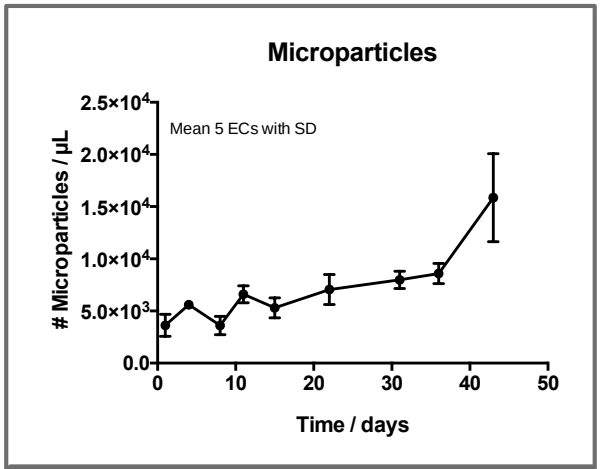
RBC and redox proteomics

Carbonylation



Protein carbonylation

- Carbonylation of proteins is a hallmark of oxidative stress (addition of carbonyl functions (C=O))
- Accumulation of oxidized proteins at the cytoskeleton after 4th week of storage
- Several pathways affected (Glycolysis, PPP, antioxidant enzymes, proteasome). Carbonylation of TALDO during 2nd half of storage can explain TALDO/SOD complex dissociation during storage (Pallotta *et al*, *Transfusion*, 2015)
- Accumulation of microvesicles during storage



Microvesiculation

Delobel *et al*, *J Proteomics*, 2012
Delobel et al. Proteomics Clin appl, 2016

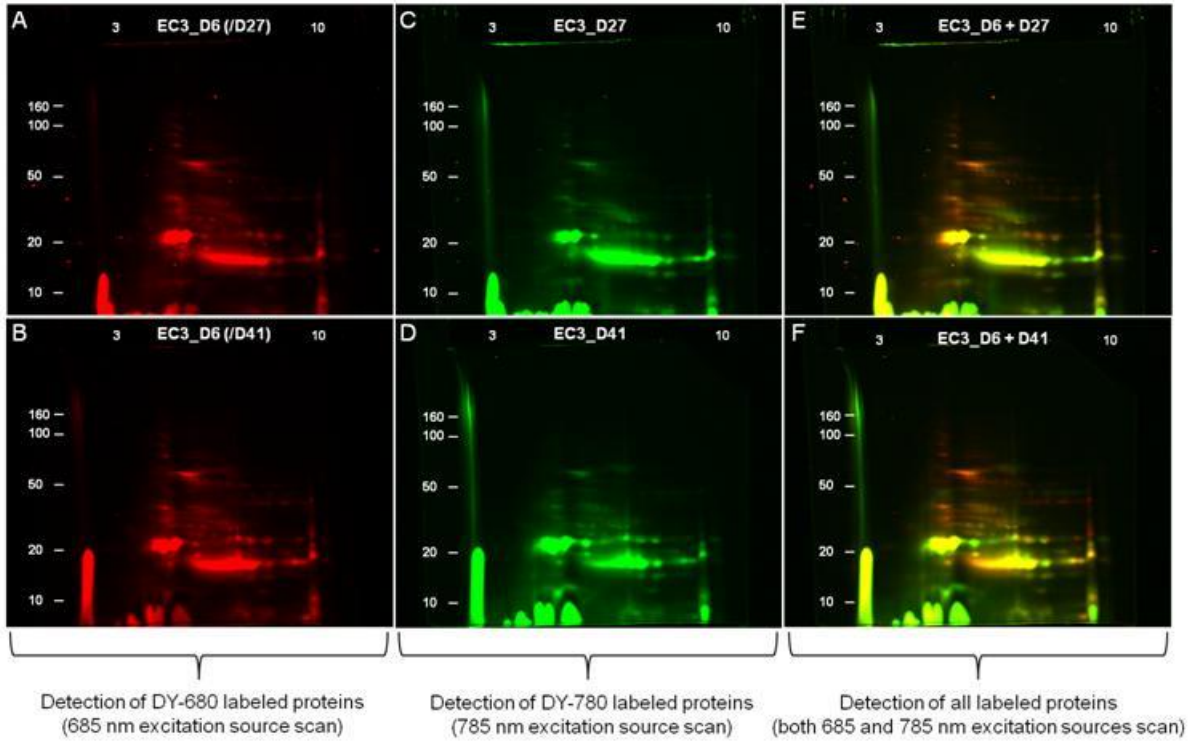
RBC and redox proteomics

Cysteine oxidation

R-SOH
R-S-SR + **red dye**

R-SOH
R-S-SR + **green dye**

Merged

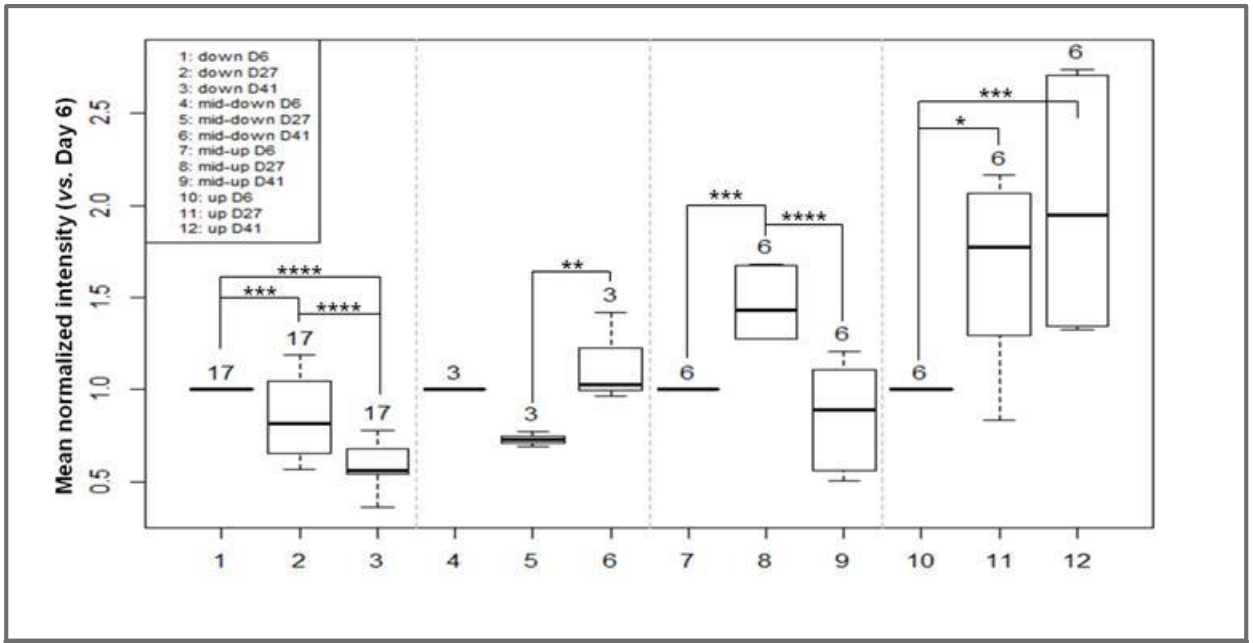


2D gels of IR-labeled proteins

Delobel *et al*, *Proteomics Clin Appl*, under revision

RBC and protein oxidation

Cysteine oxidation

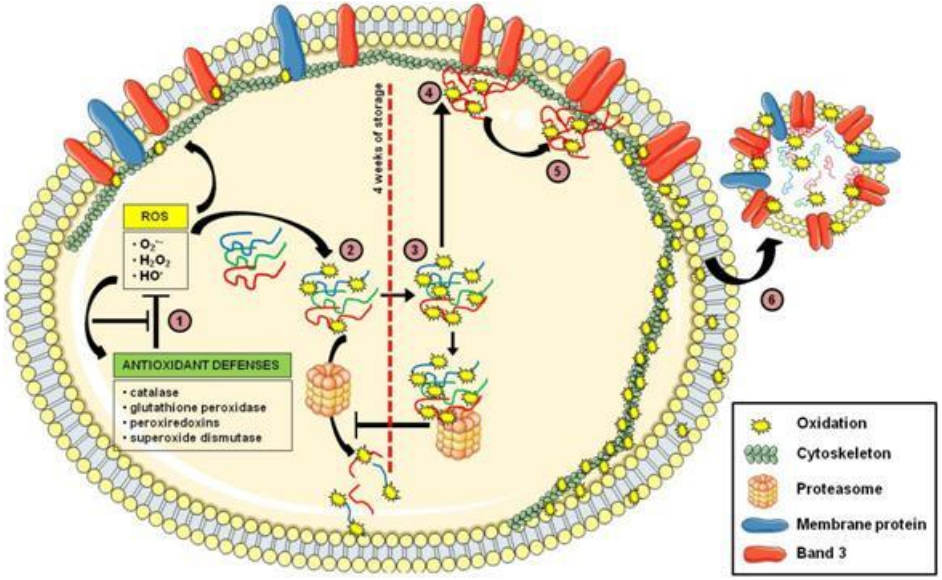


2D spot intensities of labeled proteins as a function of storage

- Different trends were observed during storage
- 43 cysteine-containing proteins were identified as oxidized in soluble extracts
- Catalytic and antioxidant activities particularly affected

Delobel *et al*, *Proteomics Clin Appl*, under revision

RBC and oxidative stress

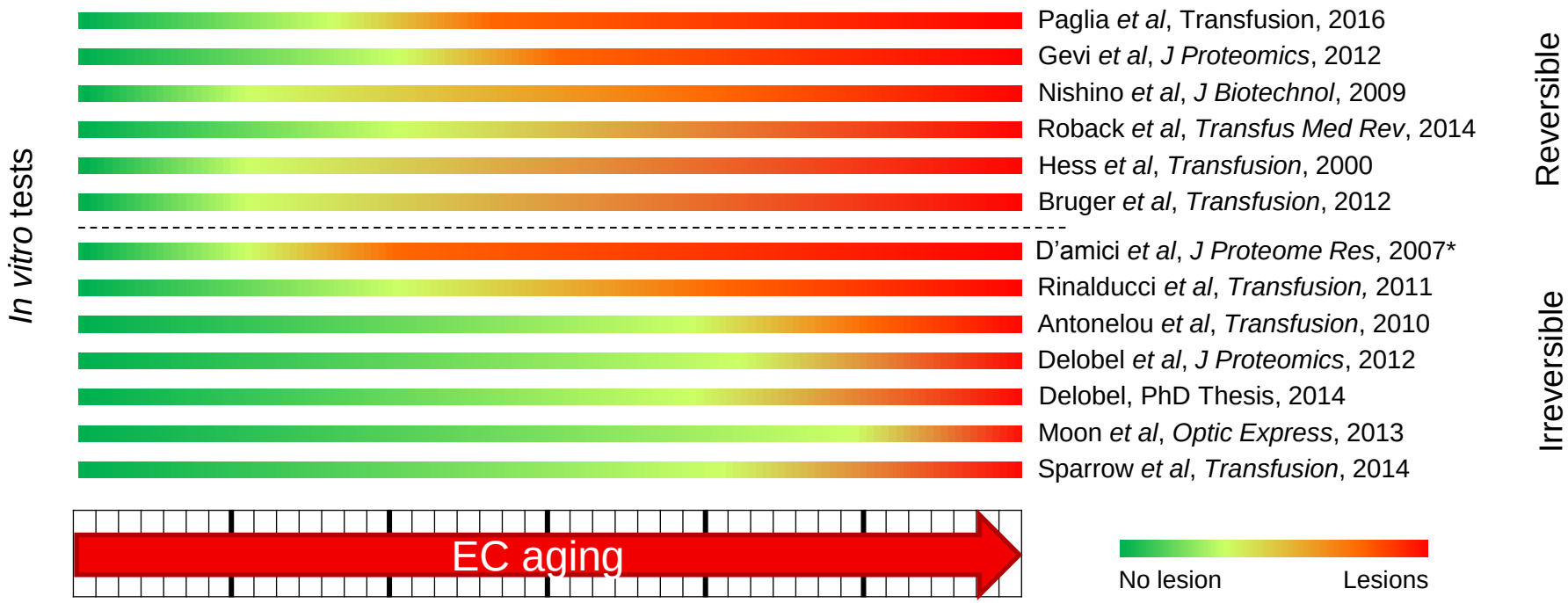


RBCs fight against oxidative stress as follows:

- Antioxidants (chemical or enzymatic) decrease after the 1st weeks
- Proteasome expected to decrease its activity after the 4th week of storage
- Microvesiculation allows to eliminate “aging” surface markers and oxidized proteins
- Accumulation of damaged materials can affect RBC deformability

Delobel *et al*, *J Proteomics*, 2012
Delobel, PhD thesis, Lausanne, 2015

Summary storage lesions



RBCs *ex vivo* « aging » analyses showed that:

- Two limits around 2nd and 4-5th week of storage
- Reversible lesions are *per se* not permanent, and RBC functions can be restored once transfused
- Irreversible lesions are potentially deleterious

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

Transfusion of end-of-storage ECs

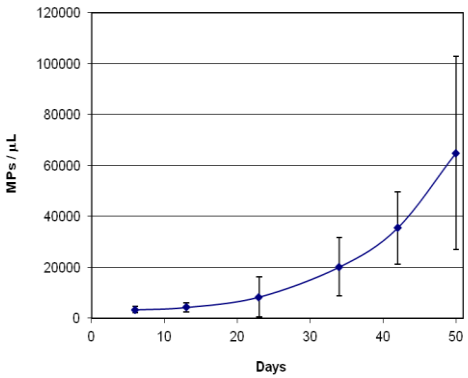
What we know:

- Detection of irreversible lesions since the 4th week of storage
- Accumulation of oxidized products (proteins, lipids, metabolites)
- Formation of spherocytes and membrane rigidification
- Accumulation of microvesicles (up to 10x the physiological concentration) exposing senescence markers (PS)
- Hemolysis

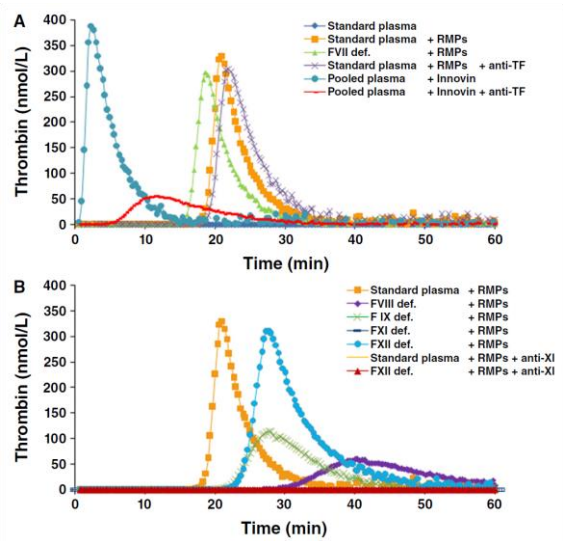
What are the impacts on patients?

- PS-positive MVs and hemostasis, inflammation?
- Free iron, cell clearance and inflammation?

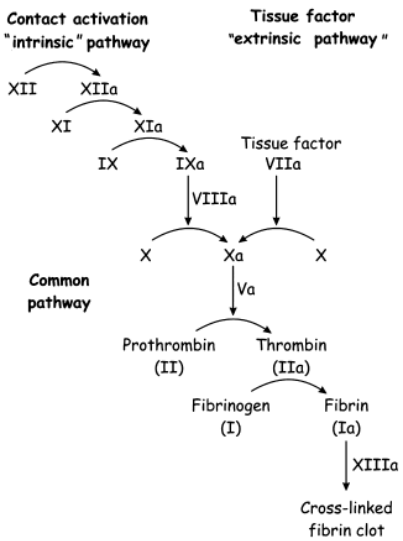
Microvesicles, hemostasis and inflammation



Increase of MVs during storage



Microvesicles and thrombin generation



Coagulation cascade

Microvesicles: Irreversible lesions that cannot be eliminated *ex vivo*

- Pro-coagulant effect induces factor XI-dependent thrombin generation
- MVs improve thromboelastogram of patients and decrease bleeding in animal models (Jy *et al, Thromb Haemos*, 2013)
- Microvesicles amplify systemic inflammation in a mouse model (Zecher *et al*, *Arterioscler Trhomb Vasc Biol*, 2014)

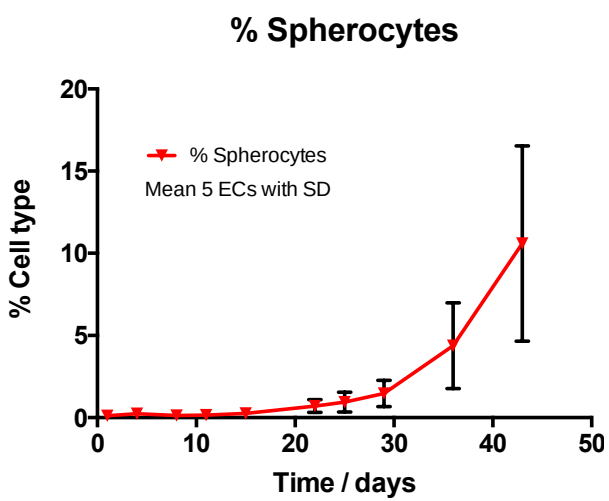
Rubin *et al*, *Vox sanguinis*, 2008
Rubin *et al*, *Transfusion*, 2013

Extravascular hemolysis and inflammation

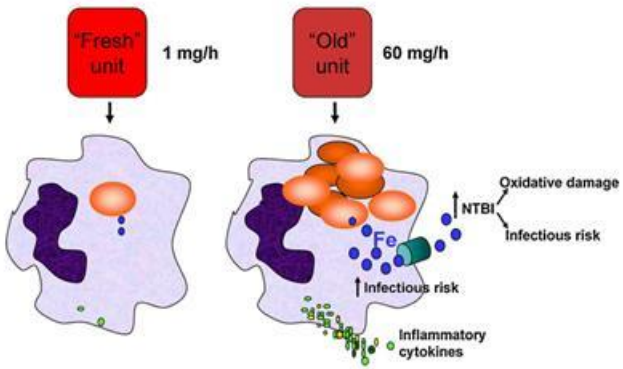
Macrophages eliminate targeted RBCs that

- exposed surface markers such as PS or βGal.
- are spherocytes and exhibited a surface loss > 18%

  (Safeukui *et al, Blood*, 2012)



Increase of spherocytes during storage



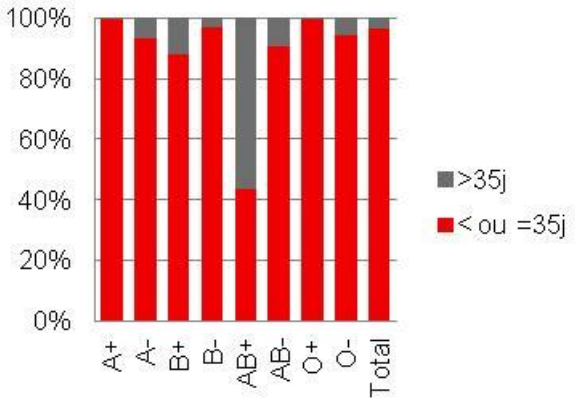
Iron hypothesis

Hod EA *et al*, *Blood*, 2010
Müller *et al*, in preparation

Risks or benefits for patients

Clinical trials

- Recent trials reinsured us on transfusion safety, even though irreversible lesions have not been specifically considered
- Does it really matter? 95% of ECs are transfused before 35 days of storage (CHUV)
- Important in specific cases, such as rare blood groups



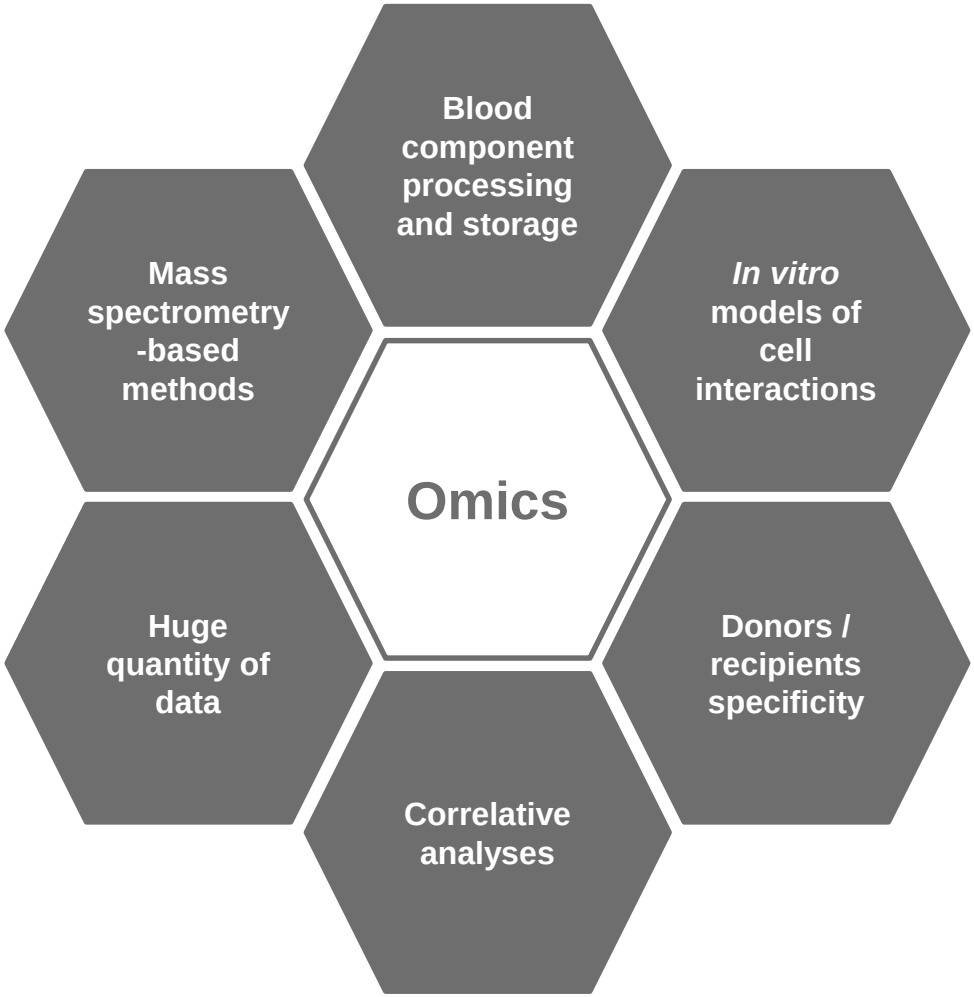
EC transfusion at the University Hospital of Vaud (CHUV, 2011-2012)

Storage lesions

- Cascade of events that leads to lesions and may lead to ATRs
- Linked to long-term-stored ECs
- Risk in case of infection
- Benefits in case of hemostatic troubles?
- Limitations
  - Study complex on human subjects
  - Animal models have limitations (biochemistry of RBCs, effect of storage, volume transfused…)

Steiner *et al, NEJM*, 2015 (RECESS)
Lacroix *et al, NEJM*, 2015 (ABLE)

Omics in transfusion medicine



- Sensitive methods (sample preparation, fractionation of molecules, quantitative approaches, data treatment…)
- Revised markers of lesions and references in clinical trials (beyond gold standards)
- In depth study of new storage strategies
- Donor and recipient specificity (“good storers”, “sensitive recipients”?)
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Omics in transfusion medicine

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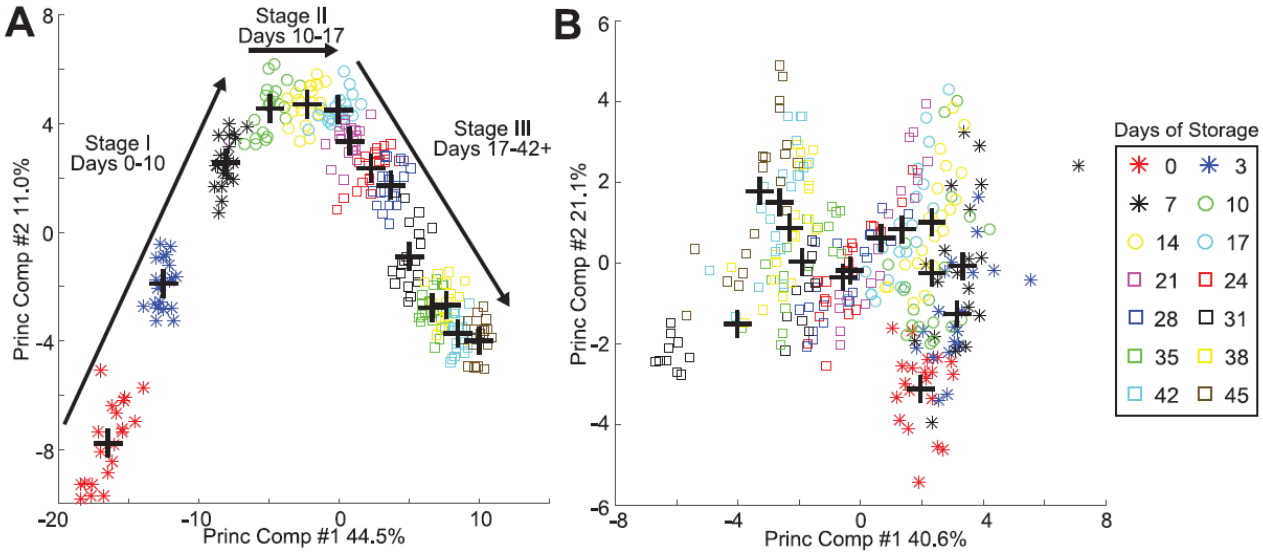
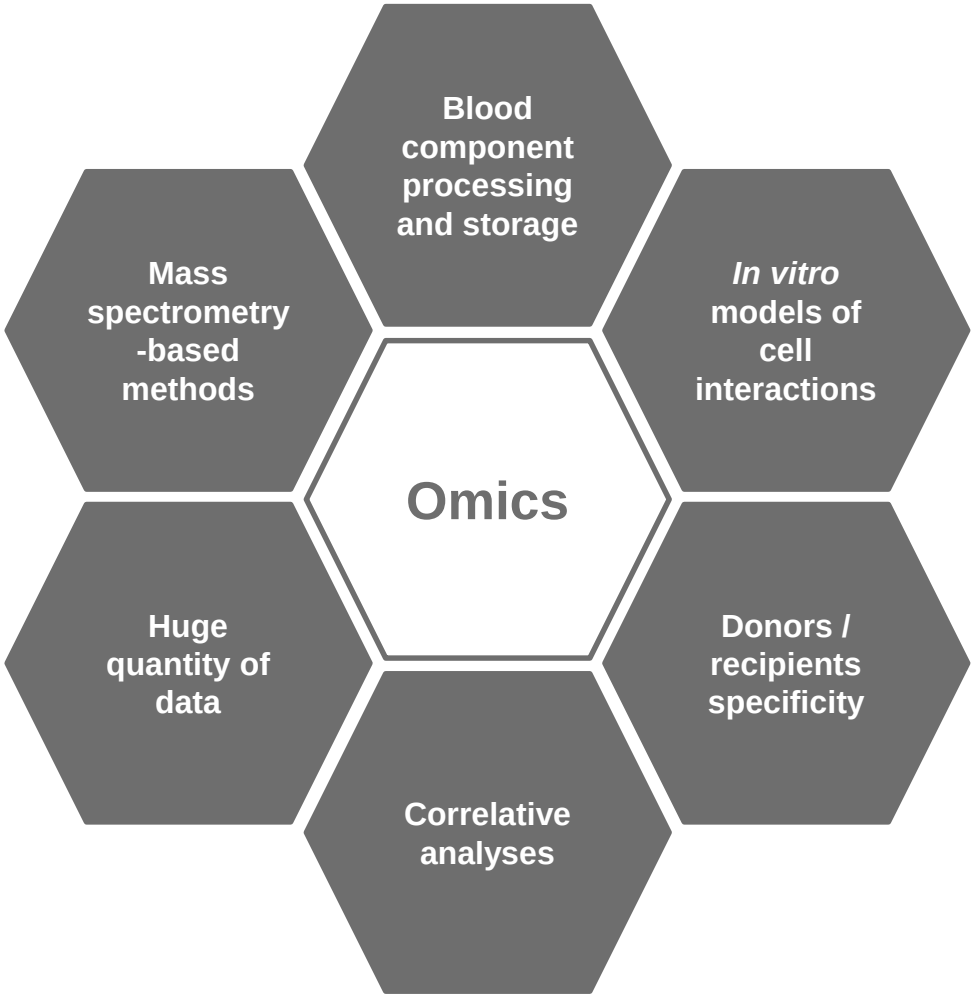


*PCA of metabolomic (A) and hematologic data from RBC storage (*Bordbar *et al*, *Transfusion*, 2016*)*

- Traditional RBC physiology variables could not clearly differentiate the three stages

Omics in transfusion medicine



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Omics in transfusion medicine

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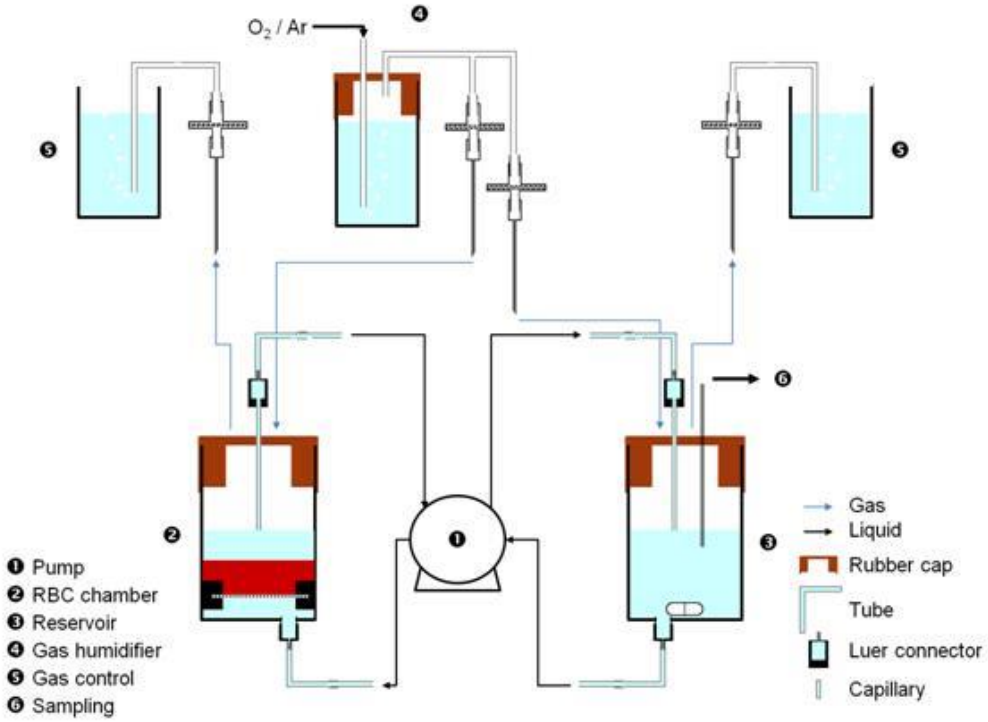
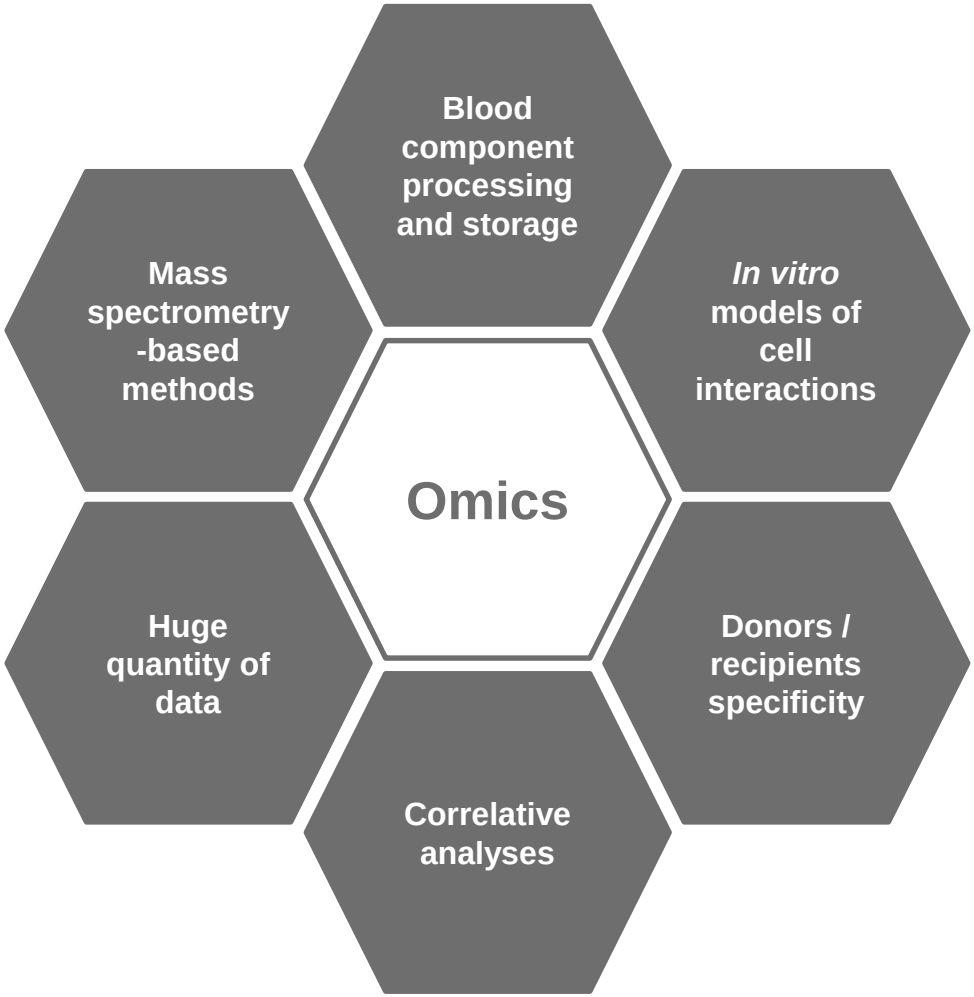


*Small-scale perfusion bioreactor (*Prudent *et al*, *Frontiers in Mol Biosci*, accepted*)*

Omics in transfusion medicine



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Thank you for your attention

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