TRANSFUSION INTERREGIONALE CRS
INTERREGIONALE BLUTSPENDE SRK

The storage lesions: from past to future
Les lésions de stockage: qu'avons-nous appris ?

Jean-Daniel Tissot

Transfusion Interrégionale CRS

Laboratoire de Recherche sur les Produits Sanguins

Epalinges, Switzerland

&

Faculté de Biologie et de médecine, Université de Lausanne

SFTS 2017
SOCIÉTÉ FRANÇAISE DE TRANSFUSION SANGUINE

SFTS, Bordeaux, 22.09.2017

Déclaration de conflits d'intérêts

Nom : Tissot Jean-Daniel

Je n'ai pas de conflit d'intérêt en relation avec la thématique traitée sauf:

J'ai dirigé un établissement de transfusion (Fondation de droit privé, puis SA à but non lucratif), avec cession (vente) de produits sanguins labiles aux hôpitaux, cliniques à charge des assurances sociales/financements publics

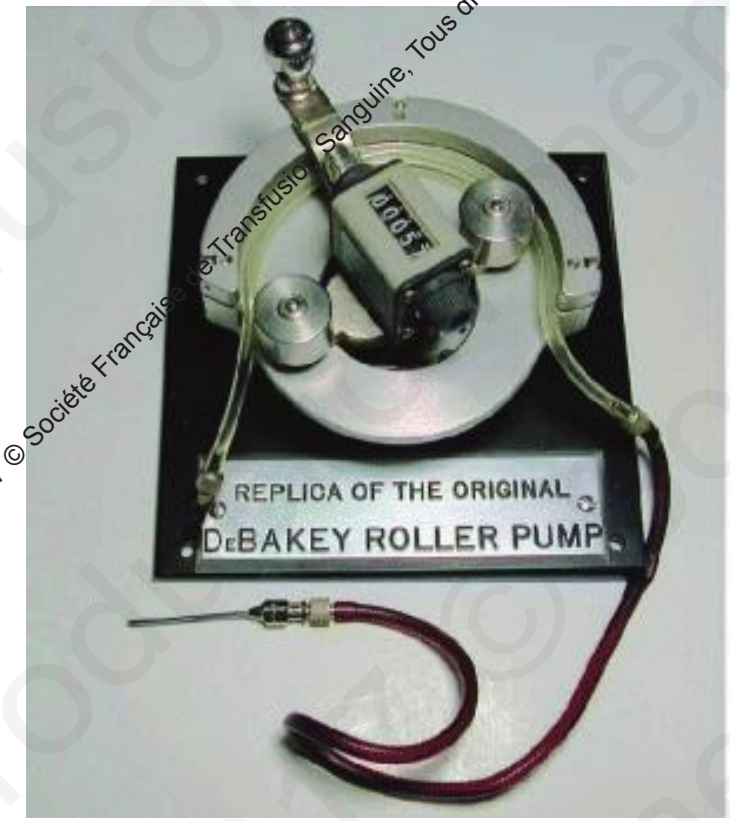
Un monde nouveau: fiction

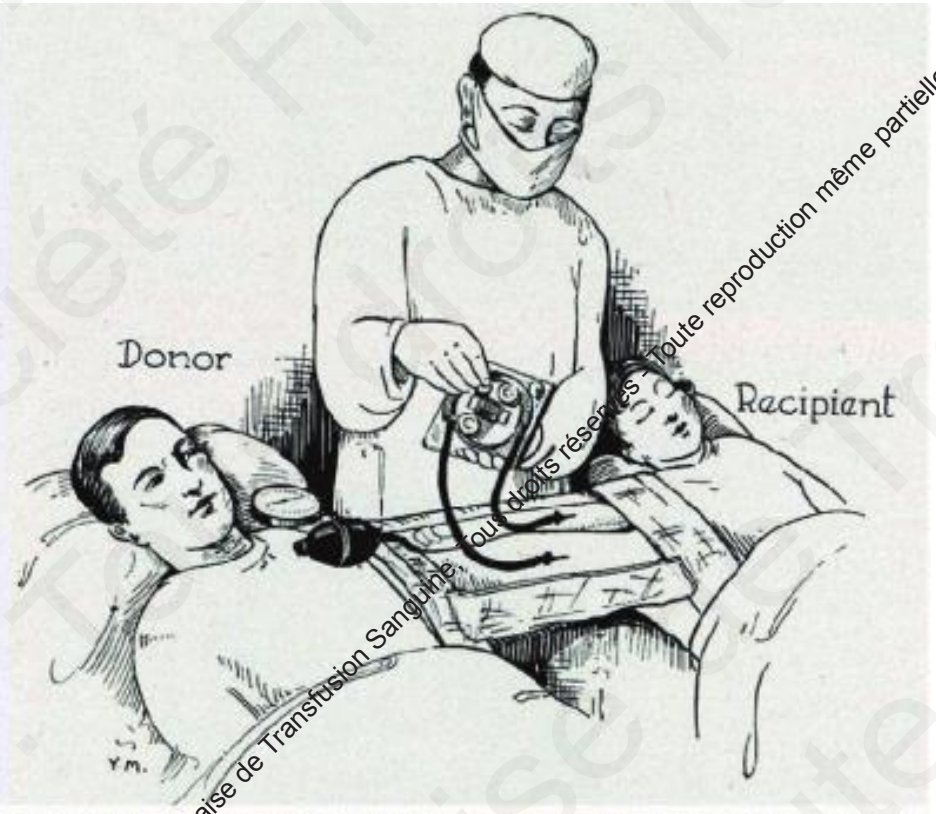
- 2017: au congrès d'une société savante, la transfusion sanguine est imaginée, pensée: des débats s'ouvrent:
  - Produits sanguins
  - Médicaments dérivés du sang
  - ANSM, PEI, Swissmedic.
  - Mise sur le marché (AMM)
  - Aspects médico-économiques
  - Etudes cliniques (designs, financements, comités d'éthique de la recherche)
  - Surveillance, vigilance
  - Conflits d'intérêts
  - Médecine de précision, médecine personnalisée

1922

1941

The DeBakey roller pump is the device that made possible the first successful open-heart surgery in 1953. Almost forgotten is that it was invented for use in direct donor-to-patient transfusion by **Michael DeBakey as a medical student** in the 1930s. Citrated stored blood became available soon thereafter and the roller pump became obsolete for transfusion.





| Site 5

Qui oserai ?

- Congeler du plasma destiné à la transfusion
- Garder des érythrocytes à 4°C
- Inventer des solutions de conservation
- Imaginer des techniques d'inactivation des pathogènes
- Mettre sur le marché des plastiques avec du DEHP
- Valider la durée de stockage permettant que 25% du produit soit inutile 24 heures après transfusion
- Séparer les plaquettes, les mélanger
- Quelle autorité autoriserai la mise sur le marché sans:
  - Validation des processus
  - Validation clinique
  - Validation des vigilances

Sans les acquis

- **Guerres**
- Pionniers
- Inconscience
- Science (ABO, anticoagulants, groupes sanguins)
- Opportunités (bons sens et mauvais sang)

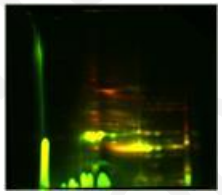
La transfusion sanguine n'existerai pas !

Mon propos

- Explorer les lésions de stockage : vision de notre laboratoire de recherche *(pour vous dégoûter définitivement)*
  - Globules rouges
  - Plaquettes
- *Je ne parlerai pas de clinique*
- Discuter des conséquences
- Proposer un futur, offrir des perspectives

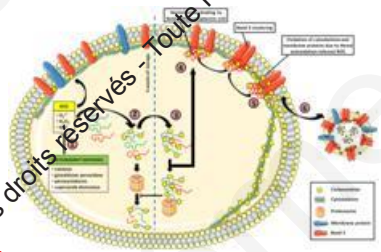
Red blood cells

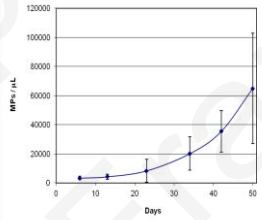
Storage of RBCs

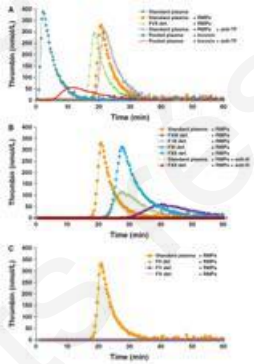


Protein carbonylation

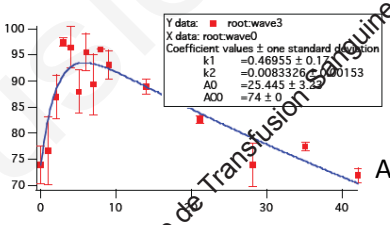
Cysteine oxidation







Microparticles and thrombin generation

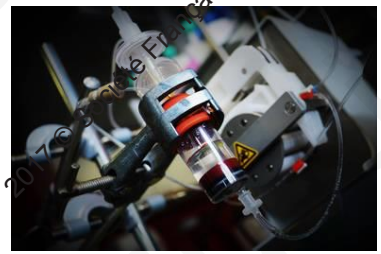


Antioxidant power

Protein oxidation

RBC aging

Aging and vesiculation

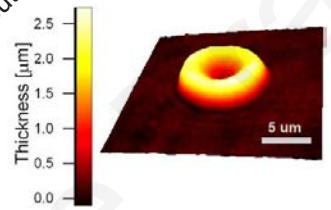


Perfusion bioreactor of RBCs

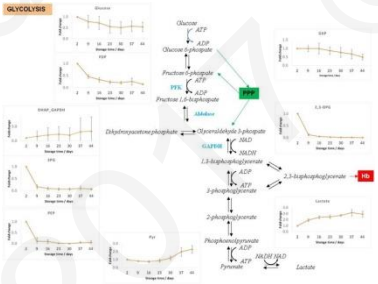
Metabolism and aerobic/anaerobic conditions

Phosphorylation of membrane proteins

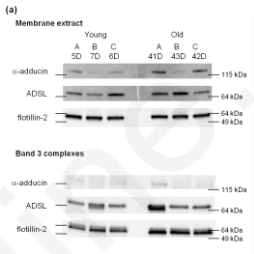
Membrane protein complexes

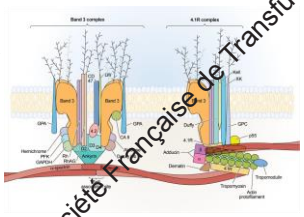


Digital Holographic Microscopy



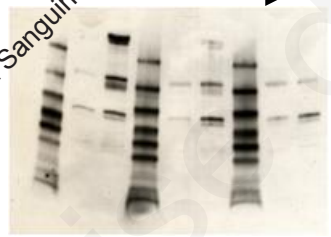
Metabolomics





Immunoprecipitation of band3 complexes

Storage time

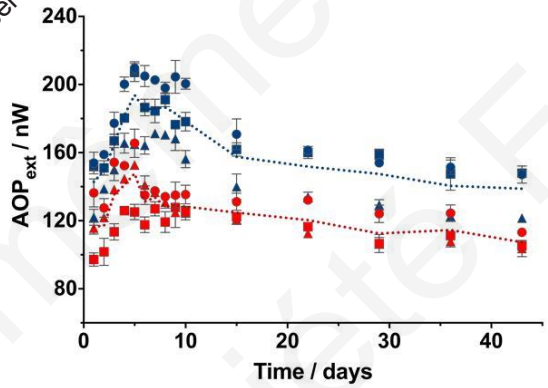


pY-proteins

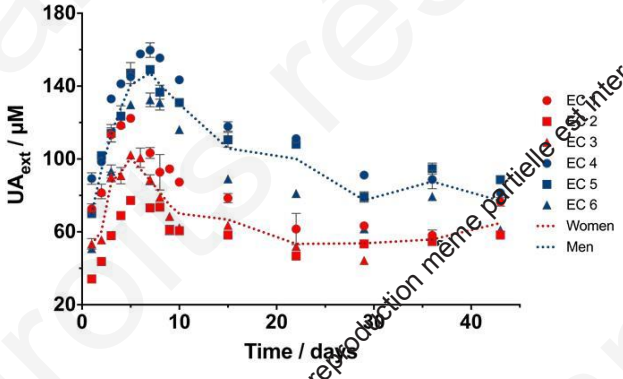
Uric acid efflux and antioxidant power

- Clear variation of the antioxidant power in RBC concentrates

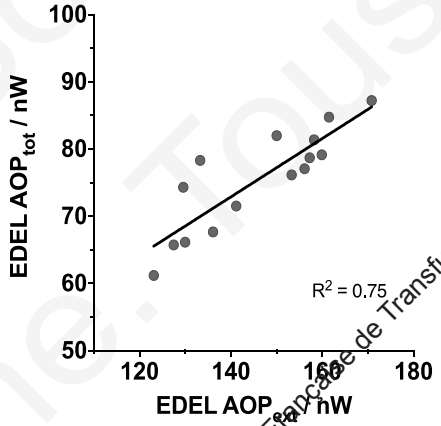
Edel potentiostat *and screen-printed disposable electrode strip in which the sample is loaded.*

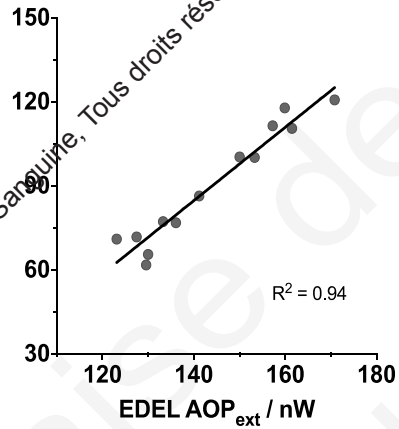


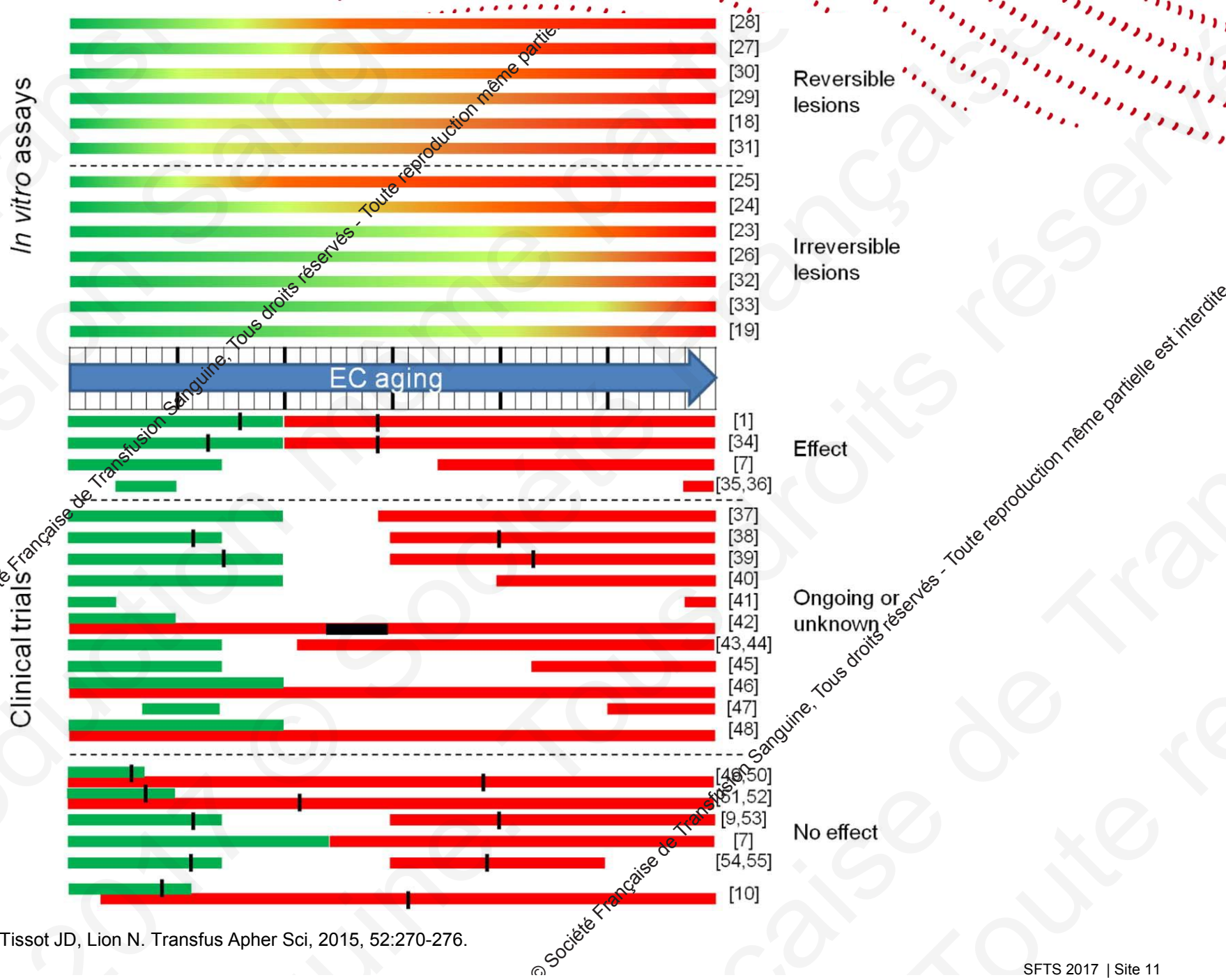
(a)



(b)



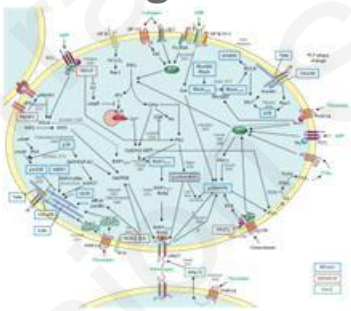




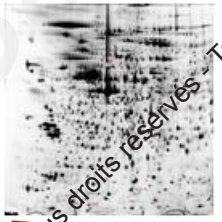
Prudent M, Tissot JD, Lion N. Transfus Apher Sci, 2015, 52:270-276.

Platelets

Pathogen inactivation and storage of platelet concentrates



Activation/aggregation pathways

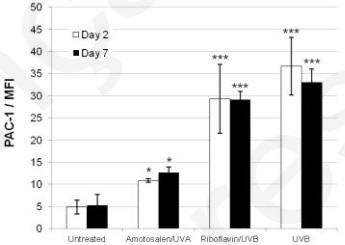


2DE

Proteomics

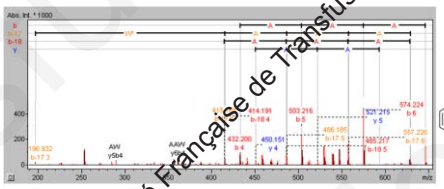
Protein oxidation

Pathogen-reduced PC

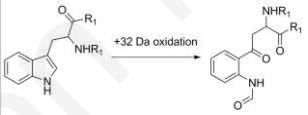


PLT activation

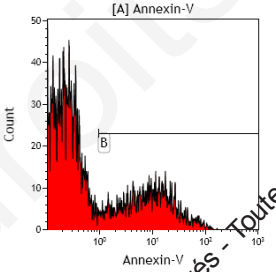
Functional analyses



LC-MS/MS analysis

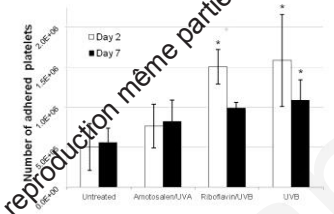


Metabolism / Antioxidant

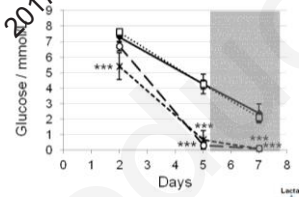


COAT PLT

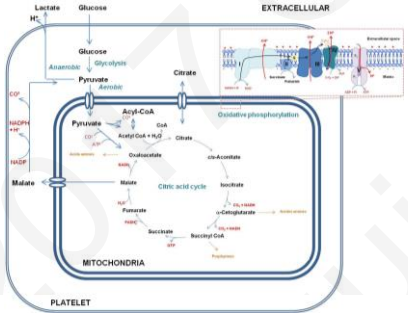
Platelet /process quality



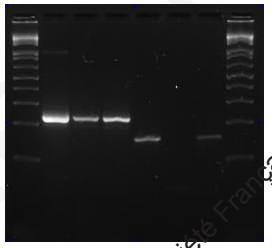
Adhesion assays



Metabolomics

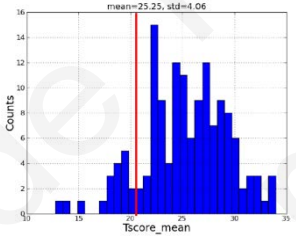


mRNA and mtDNA



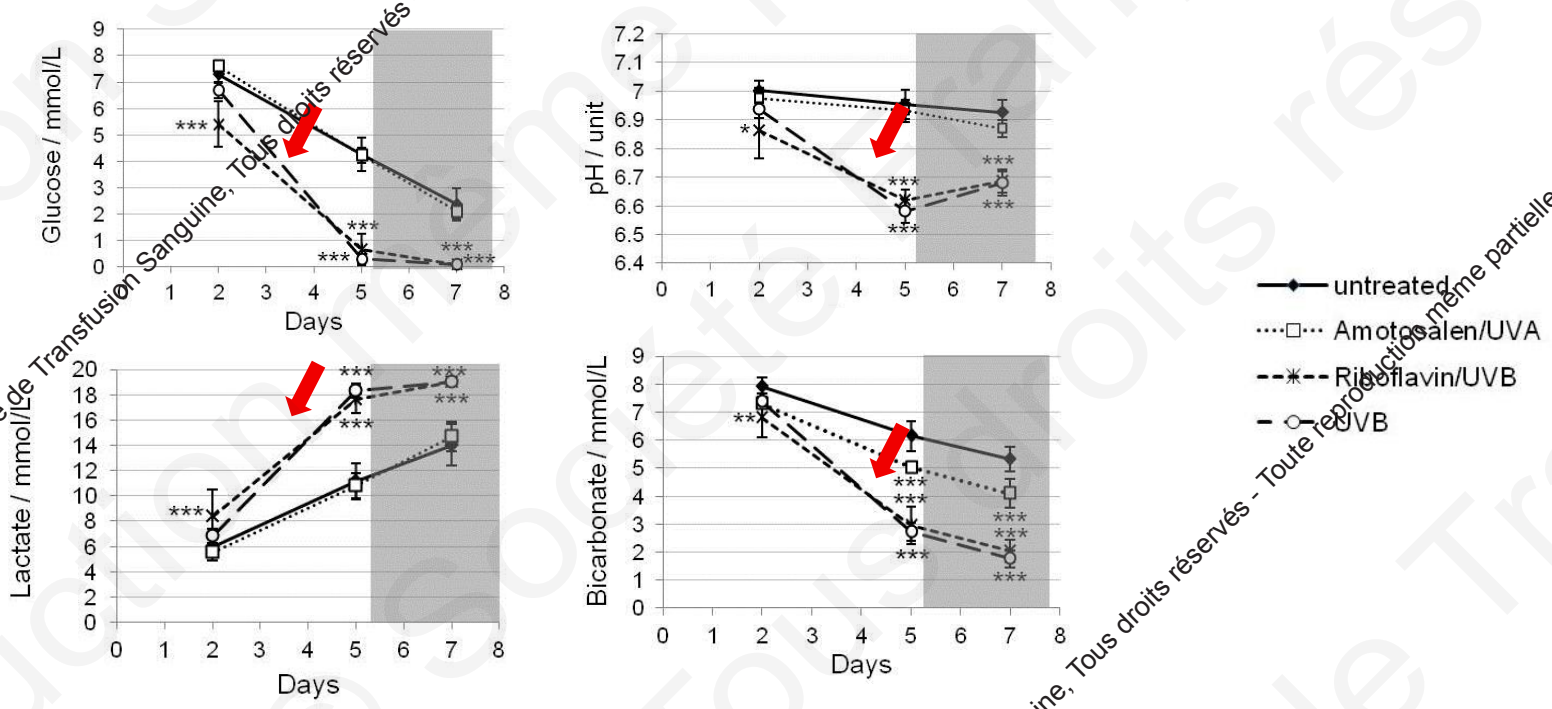
mtDNA in platelets

AOP and PR treatment completeness



ThromboLUX analysis

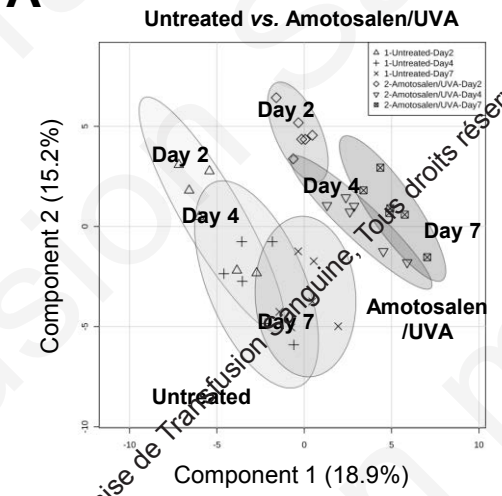
Metabolism

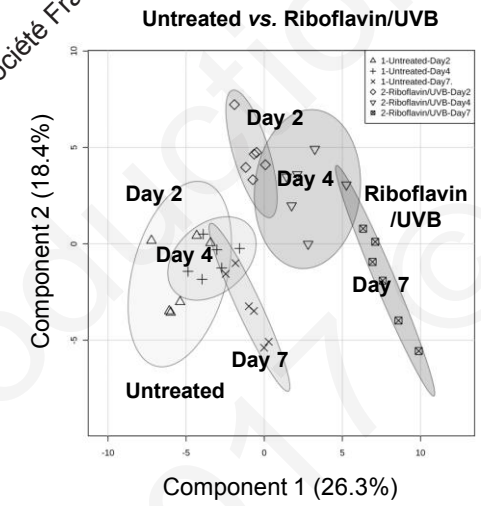


- Glucose consumption during storage with an increase glycolysis in riboflavin/UVB and UVB alone.

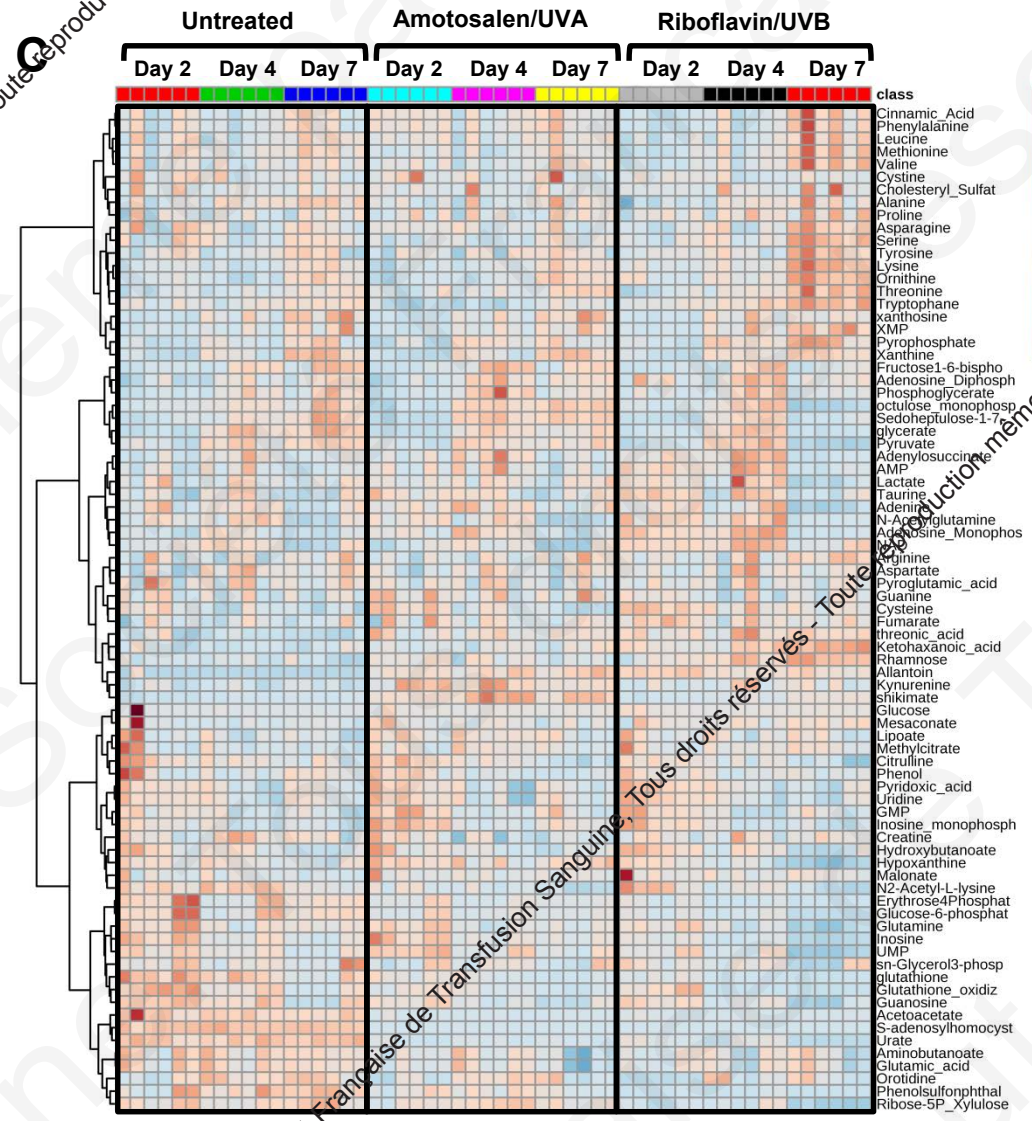
Abonnenc *et al.*, *Transfusion*, 2015

Metabolism





PCA of PR-PC (n = 5)



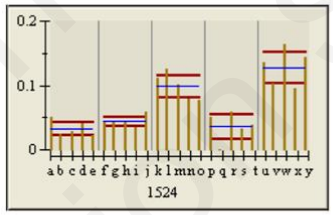
Metabolite expression

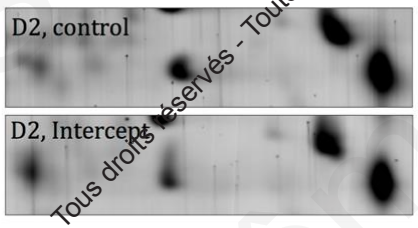
Abonnenc *et al.*, *Metabolomics*, 2016

Proteomics - Intercept

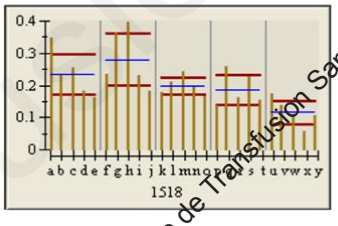
Gel analysis

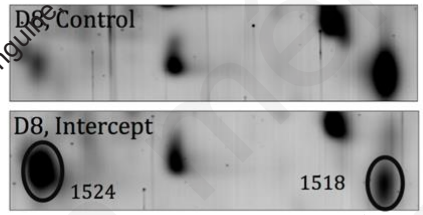
D1 D2C D2I D8C D8I





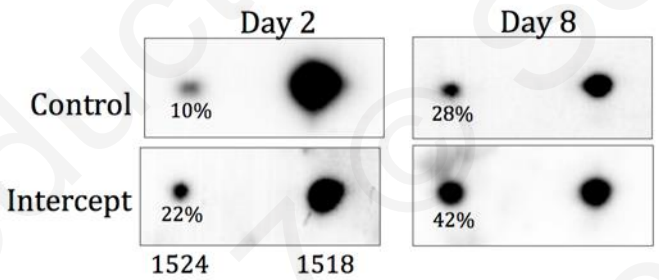
p = 0.003





p < 0.001

oxidized DJ-1 native DJ-1

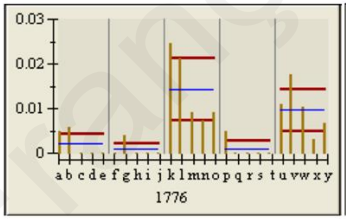


Western blot against DJ-1

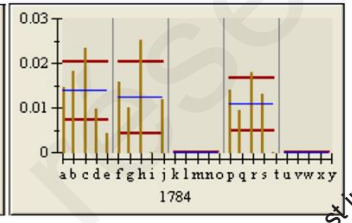
→ Increase of oxidized DJ-1 upon treatment

Gel analysis

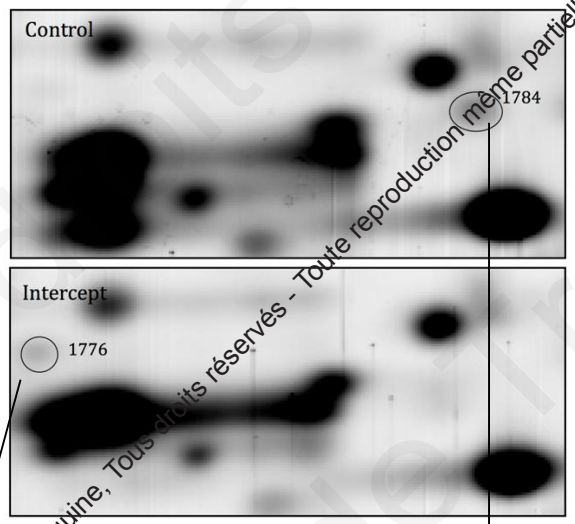
D1 D2C D2I D8C D8I



D1 D2C D2I D8C D8I



p = 0.002



G(i)α2 (C-ter fragment)

Glutaredoxin5 (possibly glutathionylated)

Galectin-1 (stable in WB)

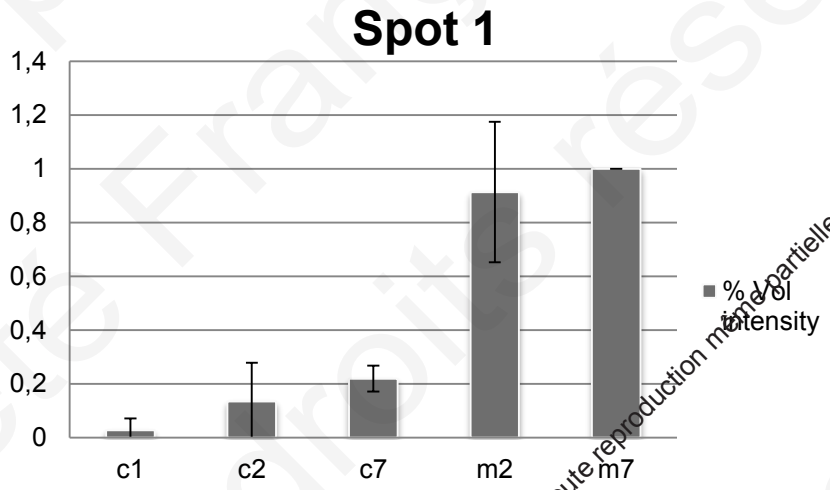
Glutaredoxin5

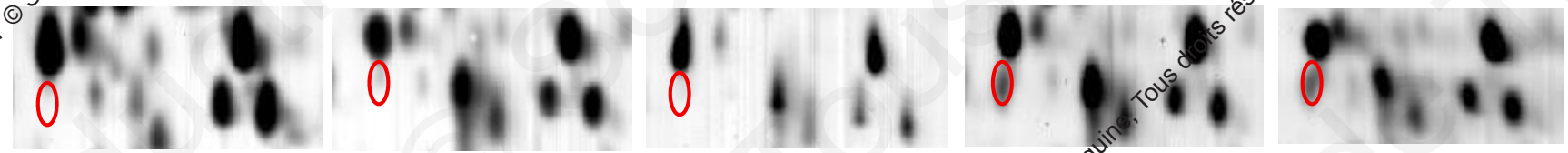
→ Degradation of G(i)α2 and modification of GRX-5

Prudent *et al.*, *J Proteomics*, 2012

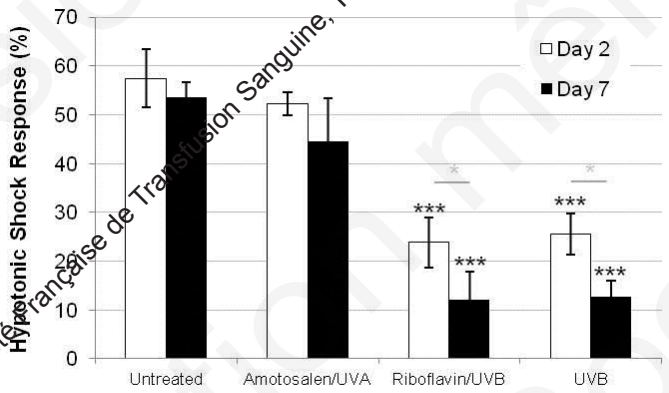
Proteomics – Riboflavin/UVB

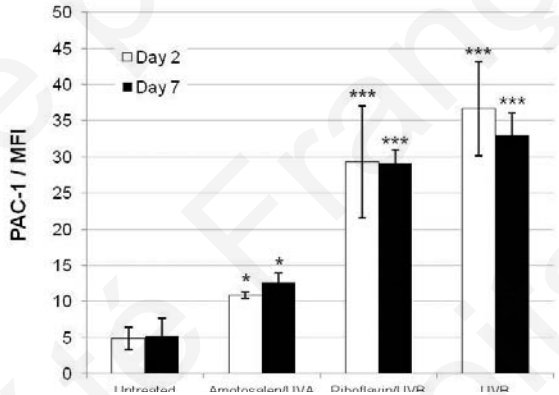
- 2D gel analyses
- Only three spots were significantly affected
  - Spot 1 : GLCCD4
  - Spot 4 : 1433Z
  - Spot 5 : CAPZB1

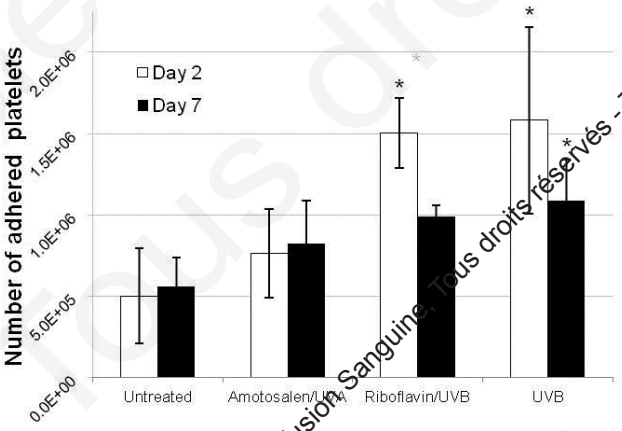




Platelet functions







- Decrease of the HSR in treated platelets with a marked effect for riboflavin/UVB and UVB alone.
- Increase of PAC-1 activation (fibrinogen receptor) expression after treatment and adhesion of platelets to fibrinogen-coated plates.

Abonnenc *et al.*, *Transfusion*, 2015

Proteomics

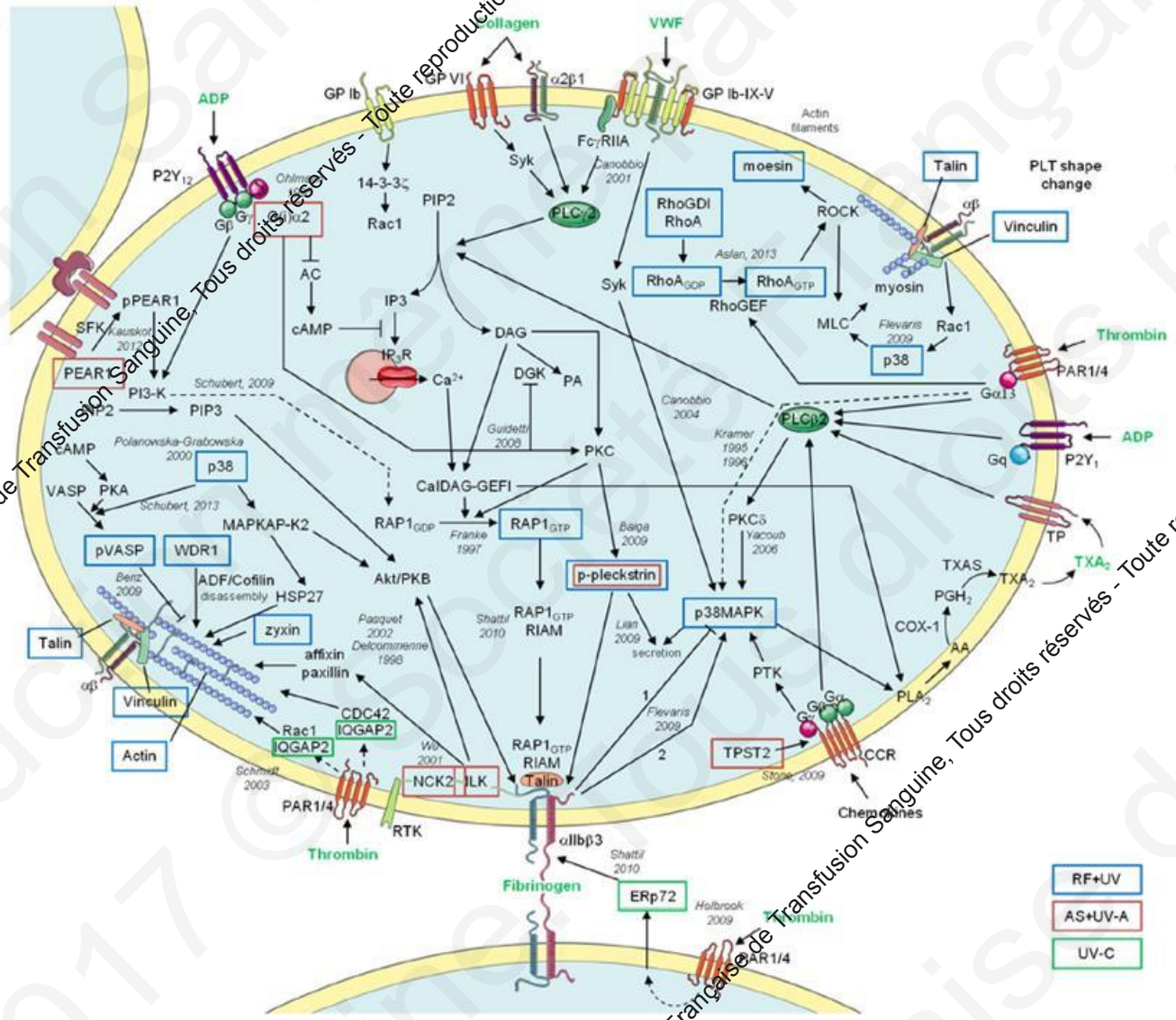
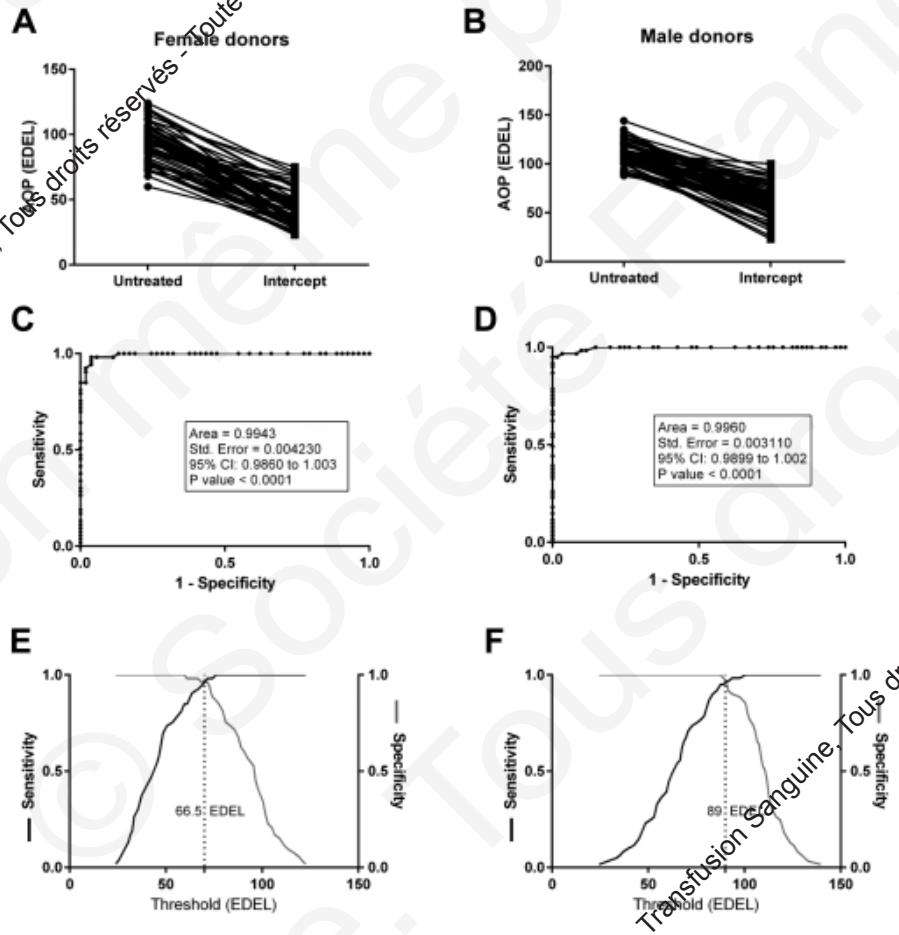


Fig 3. Platelet activation/aggregation pathways and proteins potentially affected by PI. Only potentially affected pathways are pictured. Part of the mechanisms was based on Ref [101,111], and cited references in italic. Illustrations used elements from Servier Medical Art [112].

Prudent *et al.*, *Transfusion Med Rev*, 2014

Antioxidant power as a quality control marker for completeness of amotosalen and ultraviolet A photochemical treatments in platelet concentrates and plasma units



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Volume 56, Issue 7, pages 1819-1827, 17 MAY 2016 DOI: 10.1111/trf.13638
http://onlinelibrary.wiley.com/doi/10.1111/trf.13638/full#trf13638-fig-0003

Processing and storage lesions in banked BCs.

	Lesions (including storage and processing such as pathogen inactivation)	Potential markers	
		Quality control	Clinic
RBC concentrates			
Metabolism	Loss of metabolic modulation (2,3-DPG, ATP, urate...)	+	+
	Accumulation of lactate and pH drop	++	++
	Ion leakage (K^+, Fe^{3+})	+	+++
	Decrease of antioxidants defences	+	+
Macromolecules	Loss of ATP-dependent protein function	−	+
	Protein oxidation (sulfenic acid, carbonylation)/degradation	+	+
	Membrane proteins (band3 dimerization, delocalisation such as preoxiredoxin-2)	+	+
	Haemolysis	+++	+++
	Lipid oxidation	−	+
Phenotype	Exposure of senescence markers (phosphatidylserine)	+	+++
	Shape change/spherocytic shift	++	+
	Number of RBCs	+++	+++
Function	Reduced deformability	+++	+++
	Microvesiculation, release of lipids	++	++
	Aggregation properties	++	++
Platelet concentrates			
Metabolism	Metabolic shifts	+	−
	pH drop	+	++
	Accumulation of lactate	+	+
	Antioxydant drop	+++	+
Macromolecules	Protein relocalization	+	−
	Protein oxidation (cysteine oxidation and carbonylation)	+	−
	Protein activity	−	+
	mRNA, miRNA, mtDNA	++	−
Phenotype	Activation markers	+	++
	Exposure of senescence markers (phosphatidylserine)	+	+
	Shape change/size	+	+
	Number of platelets	+++	+++
Function	Deformability (decrease HSR...)	++	+
	Increase adhesion properties	−	++
	Loss of COAT platelets	+	++
	Variations (agonist-dependent) in aggregation properties	−	++
	α-degranulation	+	+

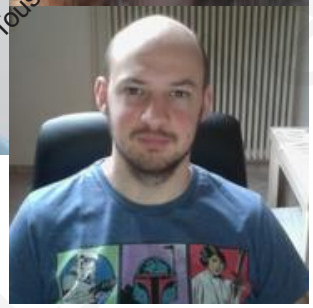
Que choisir ? Que faire ? Vous êtes perdus, moi aussi…

- Quels sont les tests qui doivent être proposés pour une mise sur le marché ?
- Quels sont les tests qui doivent être utilisés en tant que contrôle de la qualité ?
- Quelles sont les relevances pour la pratique clinique ?
- Oseriez-vous proposer la mise sur le marché des un produits sanguins labiles tels que nous les utilisons aujourd'hui ?

Propositions

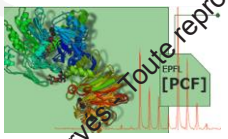
- Création d'un **groupe de travail** cordonnant:
  - Les projets de Recherche fondamentale - translationnelle
  - Création de Laboratoires de références nationaux – internationaux
  - Création de comités de suivi
- Nous devons assurer le(s) meilleur(s) produit(s)
- La **sécurité** passe par:
  - La sécurité «infectieuse»
  - La sécurité «biologique» et biochimique
  - La sécurité éthique (du don, du produit, du receveur, de la population)
  - La sécurité institutionnelle / politique / organisationnelle
  - La sécurité financière
  - La sécurité académique (création et transmission des savoirs)

«*Throm*bobinoscope»



Collaborations

Proteomic Core Facility, EPFL
Dr Marc Moniatte, PhD
Romain Hamelin, MSc



Biomolecular screening facility, EPFL
Dr Gerardo Turcatti, PhD
Dr Benjamin Rappaz, PhD
Dr Pierre Marquet, MD, PhD

LEPA, EPFL
Prof Hubert H Girault, PhD
Dr Andreas Lesch, PhD
Sunny Maye, PhD student

Edel for Life, Switzerland
Dr Philippe Tacchini, PhD

Service of Hematology and Central Hematology Laboratory, CHUV
Prof Lorenzo Alberio, MD
Dr Debora Bertaggia Calderara, PhD

Neuroproteomics and clinical anatomy UNIL/CHUV
Dr Beat Riederer, PhD

Protein analysis facility, UNIL
Dr Manfredo Quadroni, PhD
Dr Bertrand Rochat, PhD

Chaire de recherche du Canada en génie métabolique appliqué, Polytechnique Montréal, Canada
Prof Mario Jolicoeur, PhD

Chosun University, South Korea
Prof Inkyu Moon, PhD
Keyvan Jaferzadeh, PhD srtudent