EDQM Blood Conference
Innovation in Blood Establishment Processes

14-15 January 2025
Strasbourg, France

Session B3:
Novel component development & clinical outcome monitoring
(10:30 – 12:00)

Moderators: **Tor Audun Hervig**, Irish Blood Transfusion Service, Ireland
Vanja Nikolac-Markić, Head of SoHO Quality Section, EDQM

Speakers: **Vanessa Agostini**, San Martino Hospital, Italy
Richard Benjamin, Cerus Corporation, USA
Xavier Delabranche, University of Strasbourg, Établissement Français du Sang & Department of Anesthesia and Intensive Care, Strasbourg University Hospital, France

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Hypoxic Red Blood Cells: An Innovative Blood Product

Dr. Vanessa Agostini

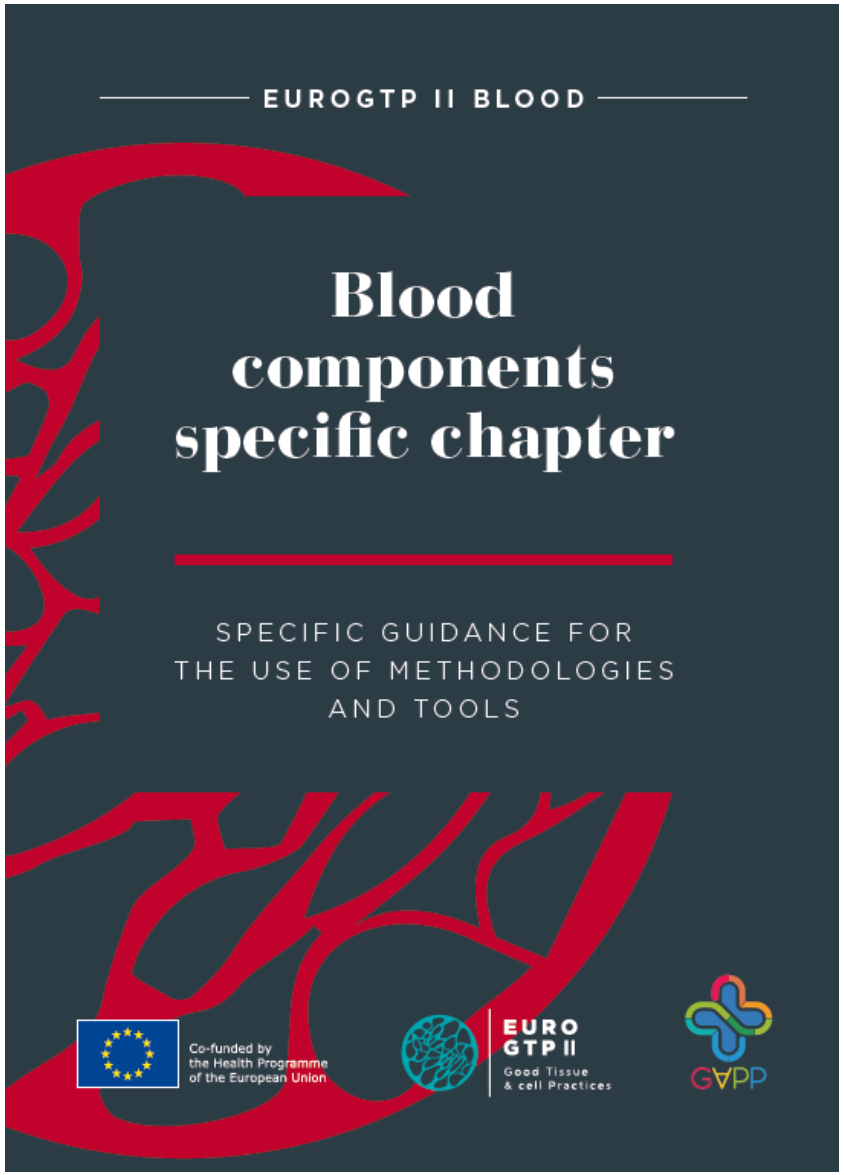
Transfusion Medicine Department

IRCCS Ospedale Policlinico San Martino, Genoa-Italy

Regional Blood Center, Liguria-Italy

Disclosures

- Baxter
- Werfen
- BioVIIIX
- Vifor
- CSL Behring



The *Good Practices for demonstrating safety and quality through recipient follow-up Project* (hereinafter referred to as 'EuroGTP II project'), and the *facilitating the Authorisation of Preparation Process for blood and tissues and cells Action* (hereinafter referred to as 'GAPP Joint Action'), developed this methodology and Interactive assessment tool, to provide recommendations and to improve the quality of healthcare delivery within the field of human tissues, cells and blood components. This tool represents the views of the EuroGTP II project and GAPP Joint Action, which were achieved after careful consideration of the scientific evidence available at the time of preparation. In the absence of scientific evidence on certain aspects, a consensus between the EuroGTP II and GAPP partners has been obtained.

The aim of the methodologies and tools is to aid tissue and blood bankers and healthcare professionals in the evaluation of safety, quality and efficacy of Blood, Tissue and Cells (BTC) and BTC therapies, therefore providing for effective care of their patients.

However, adherence to guidance does not guarantee a successful or specific outcome, nor does it establish a standard of care.

The Euro GTP II Methodologies (Annex I. Methodologies Wall Chart) and Interactive Assessment Tool (IAT) have been developed to assist professionals involved in the provision of BC to:

- Determine if a BC or preparation process has any novelty **(Step 1)**
- Assess the risks associated with the BC or preparation process **(Step 2)**
- Determine the extent of any studies and/or follow up required to assure the safety and efficacy of BC **(Step 3)**

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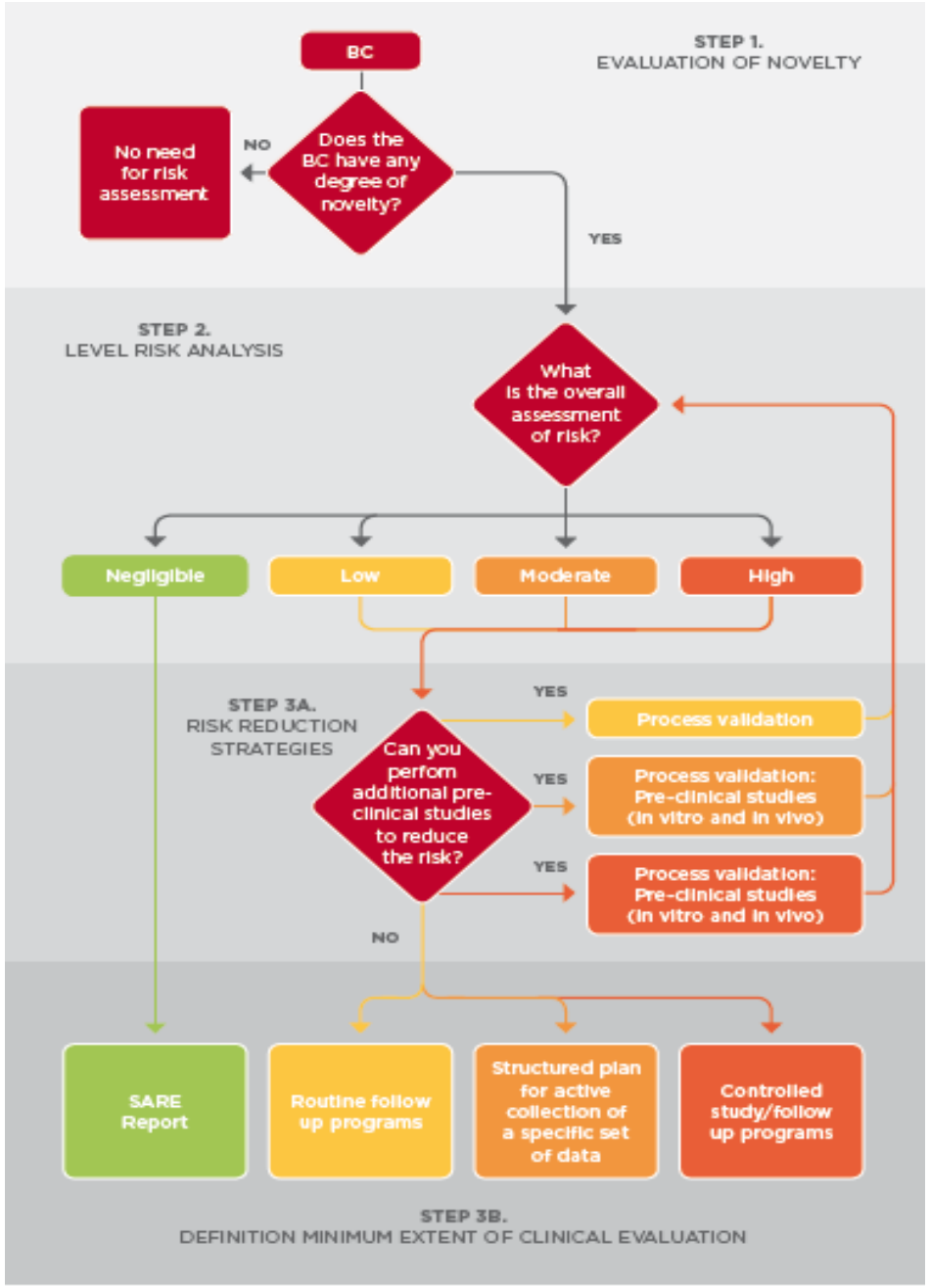

Novelty: Any change to an established/consolidated blood, tissue or cell preparation process that may or may not result in a new BTC or to the mode of application of this BTC[11].

Level of Risk*	Extent of Studies needed
Moderate	**Step 3A. Risk reduction strategies** Novel procedures or treatments that exert a moderate risk and are considered innovative. The treatment has shown proof of principle and there is reassuring data in literature in terms of both safety and efficacy at least in animal studies and pre-clinical data shows normal incremental response. The studies that have published this data should have a sound methodology and published in peer-reviewed journals. In order to implement an innovative treatment, an **enhanced validation** is necessary including and a range of **additional quality controls** performed to monitor CPPs, CQAs, and the impact of the implemented Blood therapy should be carefully monitored. Since reassuring data of this innovative treatment is already available, a more specific monitoring of the published critical parameters can be performed instead of a registration of all critical parameters. **Step 3B. Extent of clinical investigation** Use might either be considered a change in clinical practice or as part of an approved research study, to be determined based on clinical usage/data to date. Use might be restricted in first instance to small scale pilot studies. A CFUpP to monitor safety through haemovigilance may be enhanced above standard based on risk. **Clinical Investigation Plan (CIP)**, where implemented, should assess reassuring mid-term safety including data on patients' wellbeing.
High	**Step 3A. Risk reduction strategies** A new procedure can be offered to patients in an experimental design aiming at showing proof of principle, short-term safety and/or efficacy. Likely to have to further define some critical variables in BC quality. An **extensive validation including** (where relevant) animal models, and including a range of **additional quality controls** performed to monitor CPPs, CQAs, and the impact of the implemented changes is required. This extensive validation should include: **Non clinical studies:** preferably there should be studies showing the experimental procedure is safe in animals. **Pre-clinical Studies:** when experimental treatments encompass a laboratory phase, then at least the viability of cells should be looked at in detail, monitored and registered. **Step 3B: Extent of clinical investigation** The BC should only be used clinically in the context of an Clinical Investigation approved by an independent Ethics Committee and compared to standard therapy (where applicable) until the residual risks have been adequately mitigated. The good practices of clinical setting for BTC[4] (adapted from Good Clinical Practices[5] principles) must be adhered to. The clinical use of novelties is likely to require a CIP and CA approval. It cannot to be used outside of an approved study. **Follow up program:** experimental treatments should only be offered to a selected and limited patient cohort and these patients should be clearly informed on the experimental status and should receive information about possible risks, alternative treatments etc. HBB should only offer experimental treatments or treatments based on experimental procedures after approval by a commission of medical ethics.

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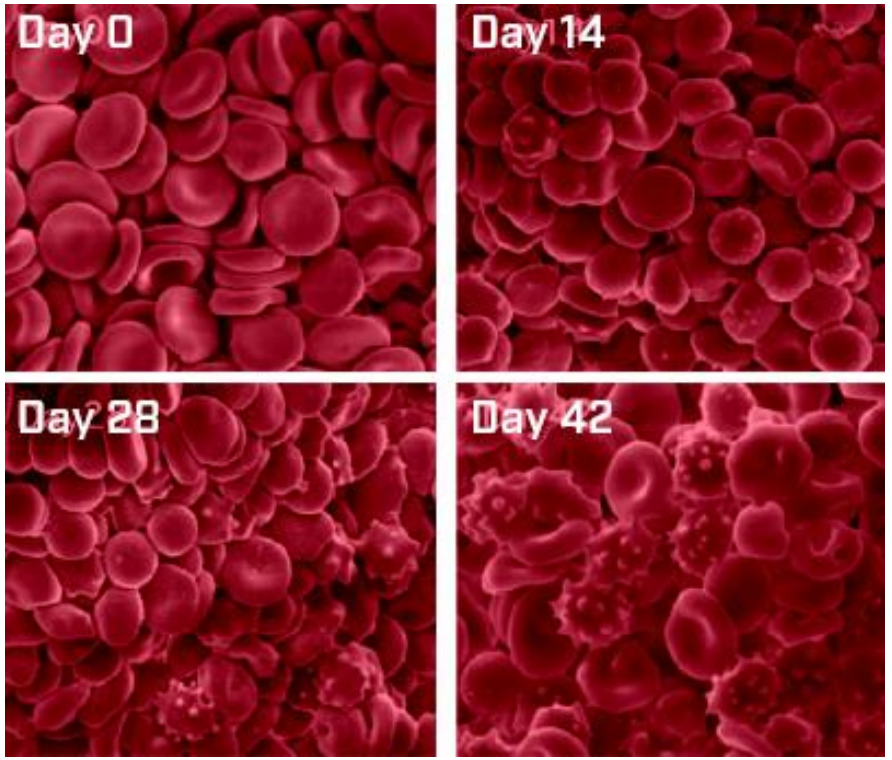
Background: RBC Storage Events

- RBCs are subject to metabolic and oxidative impairments accumulating during storage[1]
- RBC deformability progressively diminishes over time, affecting microcirculation perfusion[2]
- Hypoxic storage reduces the oxygen content of RBC units from day of donation and throughout storage[3]
- Hypoxic storage reduces oxidative impairments that occur during normal storage, providing more viable cells at transfusion[1]
- Hypoxic RBCs unload oxygen better than conventional RBCs[5]



Degradation of conventional RBCs over the storage period[4]

Potential to improve the clinical outcomes of patients that receive blood transfusions in a variety of therapeutic realms

RBC, red blood cell.

1. Yoshida T, et al. Blood Transfus, 2019;17(1):27-52; 2. Burns JM, et al. Blood Transfus. 2016;14(1):80-8; 3. Landry P, et al. Poster presented at: The International Society of Blood Transfusion; 2018; 4. Mustafa I, et al. Biomed Res Int. 2016:4529434; 5. Rabcuka J, Swietach and Coker; Blood Advances; Vol. 6, Issue 18; 2022.

How is Red Blood Cell Quality Evaluated?

Evaluation is based on RBC recovery….

- ≥75% recovery of transfused RBCs at 24 hours
- <1% hemolysis

NOT RBC Functionality

Unexplored concepts in RBC Transfusion

Age (biological) is not always an indicator of Red Blood Cell Quality

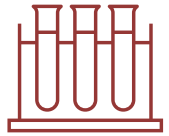
Specific vulnerable populations that receive RBCs:

trauma and chronically transfused patients

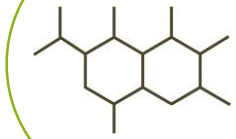
Classical strategies to reduce the rate of development of storage lesions

1. Manipulation of pH
2. Supplementation of metabolic precursors
3. Manipulation of osmotic balance and increase of the volume of the suspending medium
4. Reduction of oxidative stress by adding protective molecules or removing oxidants from RBC suspension

Evidence to support hypoxic storage



In vitro, hypoxic storage reduces oxidative impairments that occur during normal storage, providing more viable cells at transfusion1



Metabolomic analyses of hypoxic RBCs have shown increased ATP synthesis and a decrease in oxidative stress biomarkers2

In animal models, hypoxic RBCs facilitated more effective resuscitation from hemorrhagic shock than conventionally stored RBCs[3]

ATP, adenosine triphosphate; RBC, red blood cell.

1. Yoshida T, et al. *Blood Transfus* 2019;17:27–52. 2. D'Alessandro A, et al. *Transfus* 2020;60:786–98 3. Williams AT, et al. *Shock* 2020;53:352–62.

TRANSFUSION OF ANAEROBICALLY OR CONVENTIONALLY STORED BLOOD AFTER HEMORRHAGIC SHOCK

Alexander T. Williams,* Vivek P. Jani,* Travis Nemkov,† Alfredo Lucas,* Tatsuro Yoshida,‡ Andrew Dunham,‡ Angelo D'Alessandro,† and Pedro Cabrales*

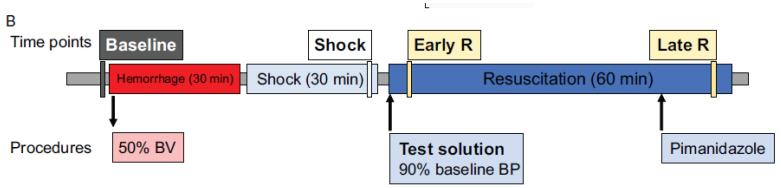
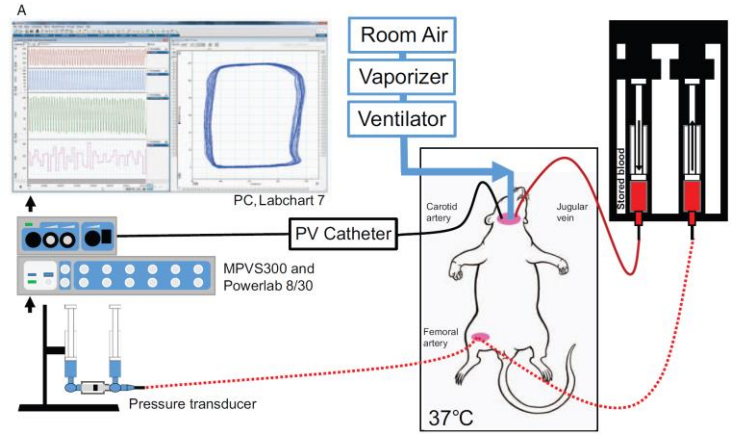


FIG. 1. **Experimental setup.** (A) Rats were instrumented with catheters in the left femoral artery and left jugular vein, a tracheal cannula, and a miniaturized pressure–volume catheter introduced through the right carotid artery. (B) Animals were hemorrhaged for half their blood volume, held in shock for 30 min, and then transfused to a goal blood pressure. Resuscitation phase was 1 h long, and pimonidazo le (stain for hypoxic tissues) was injected 15 min before the end of the resuscitation period.



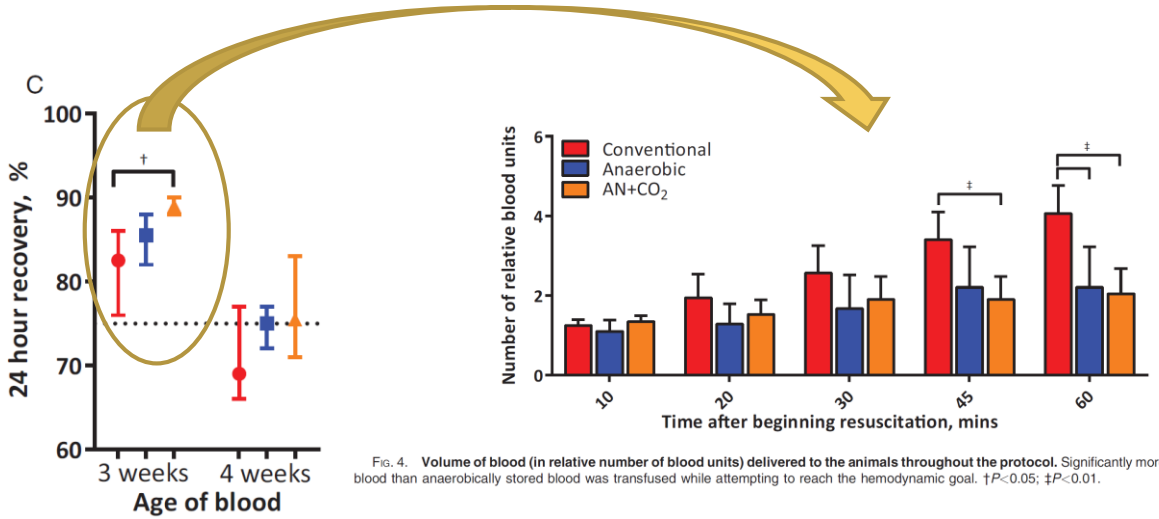
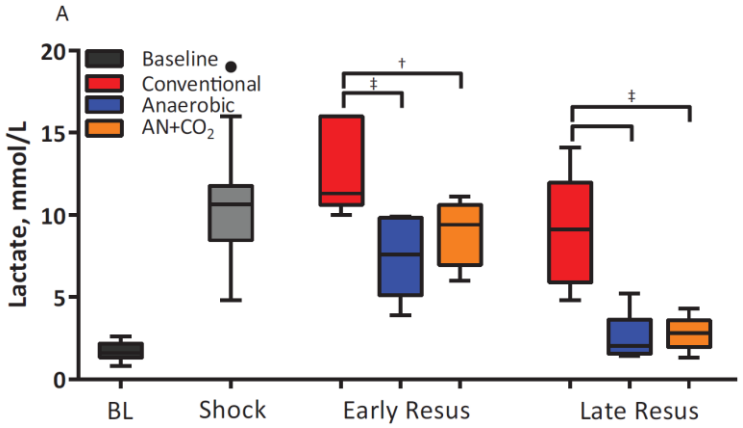


FIG. 4. **Volume of blood (in relative number of blood units) delivered to the animals throughout the protocol.** Significantly more conventionally stored blood than anaerobically stored blood was transfused while attempting to reach the hemodynamic goal. †$P<0.05$; ‡$P<0.01$.



Anaerobic Storage Reduced Transfusion Requirement for Preclinical Hemorrhagic Shock Resuscitation

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Hypoxic RBC Processing System

- Hemanext Inc. has developed a CE mark-certified device to process and store RBCs hypoxically – CPD/PAGGSM Leukocytes-Reduced (LR), O_2/CO_2 Reduced

Hypoxic RBCs may reduce transfusion burden in transfusion-dependent patients and attenuate the oxidative stress associated with acute major bleeding

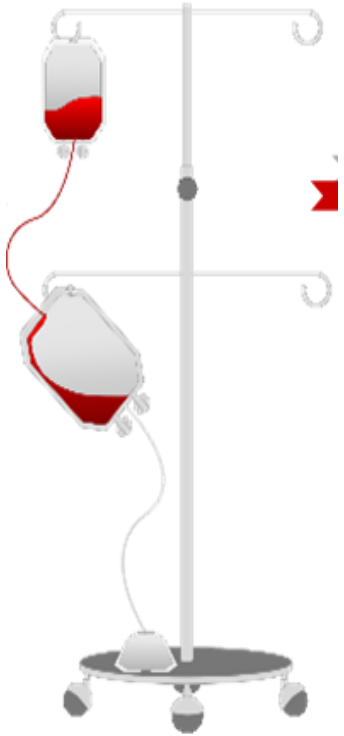
CPD, citrate-phosphate-dextrose; HSB, Hemanext storage bag; ORB, oxygen reduction bag; PAGGSM, phosphate-adenine-glucose-guanosin-saline-mannitol; RBC, red blood cell.

Hypoxic RBC Processing and Storage System

STEP 1 – Sterile Transfer LR RBC to ORB

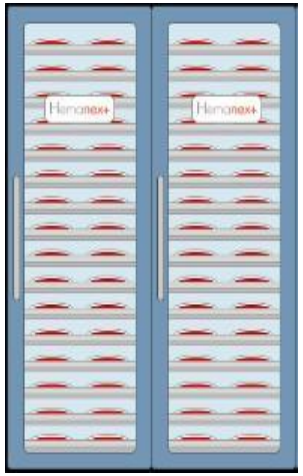
LR RBC

Oxygen Reduction Bag (ORB)



STEP 2 – Agitate in commercial shaker

Agitator



3 HOURS

STEP 3 – Transfer to HSB and store for use

Hemanext Storage Bag (HSB)

Upon completion of the ORB treatment, RBCs are transferred to an oxygen-impermeable storage bag, the HSB – HEMANEXT ONE® Storage Bag.

RBCs are then transferred to a double layered bag, the HEMANEXT ONE® ORB – Oxygen Reduction Bag, which reduces blood oxygen saturation to below 20% in three hours.

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European Experience: Validations and Safety Study

Process validations of the *in vitro* performance of the hypoxic storage system - CPD/PAGGSM Leukocytes-Reduced, O_2/CO_2 Reduced – were executed at blood banks in 4 countries, followed by a safety study in Norway.

Validated center for hypoxic RBC production

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Methods

Process Validations:

- Whole blood (WB) was collected and processed within 24 hours at ambient hold (20–24°C).
- Each unit generated 1 unit of HRBC that was stored for 42 days at 2–6 °C.
- Study acceptance criteria at 42 days were total hematocrit (HCT) >50% and hemolysis <0.8%.[1]

1. Agostini et al. Poster (ISBT); June 2024;

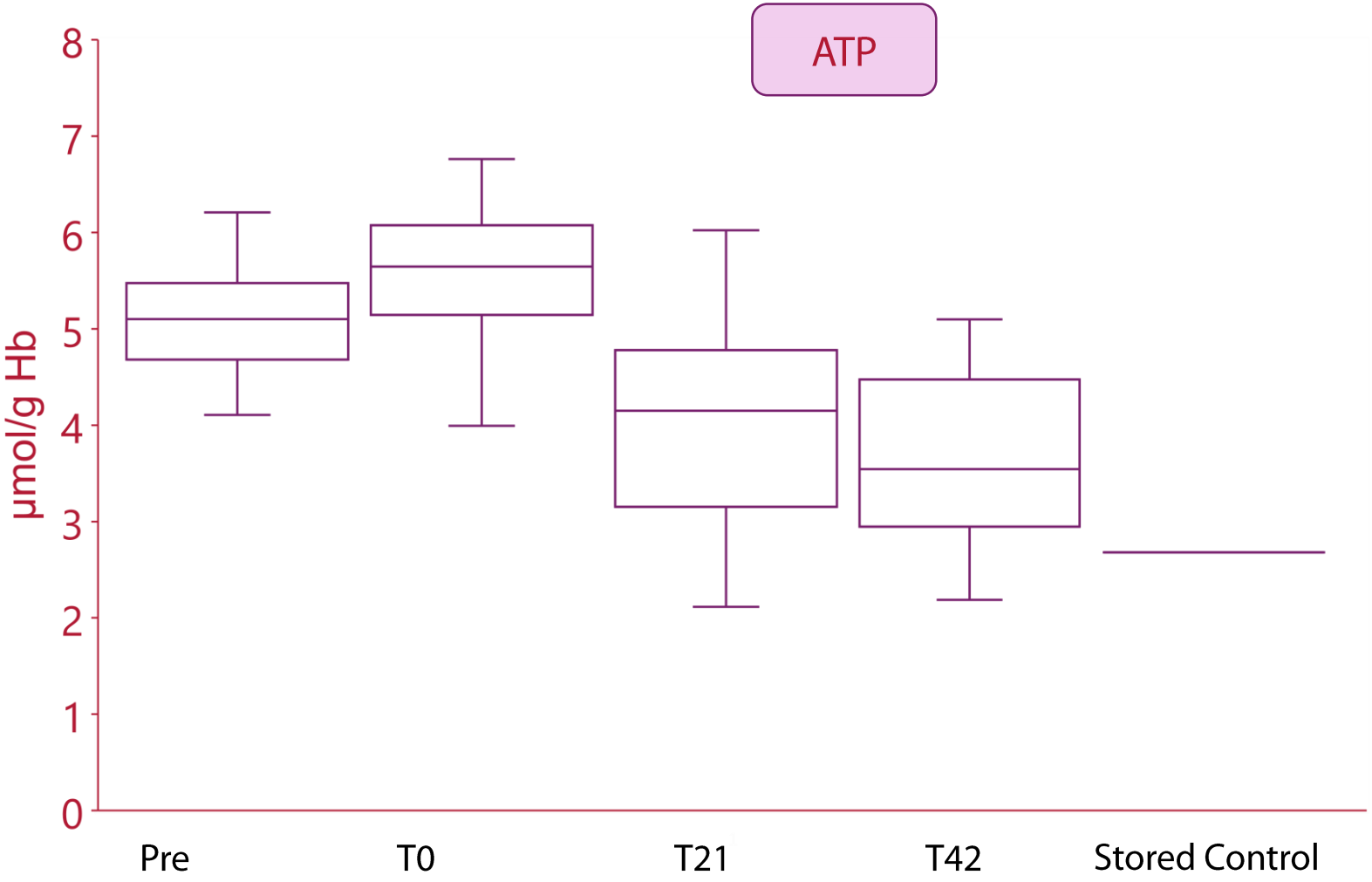
Results: Validations
Hematocrit and Hemolysis

	Baseline	Day 21	Day 42
HCT, mean (SD), %			
Germany	61 (2.4)	62 (2.8)*	63 (2.3)
Italy	61 (2.6)	66 (8.1)	63 (5.6)
Norway[a]	57 (1.7)	–	58 (2.6)
Switzerland	60 (2.2)	–	64 (3.4)
Hemolysis, mean (SD), %			
Germany	0.13 (0.02)	0.19 (0.04)*	0.25 (0.07)
Italy	0.10 (0.03)	0.20 (0.09)	0.25 (0.09)
Norway[a]	0.10 (0.03)	–	0.20 (0.05)
Switzerland	0.10 (0.02)	–	0.41 (0.19)[b]

Germany, N=26; Italy, N=30; Norway, N=33 (Bergen) and N=21 (Oslo); Switzerland, N=31.
*Day 23
[a]Average results from two blood banks: Oslo and Bergen
[b]One unit had hemolysis higher than 0.8% (0.81%) at end of storage but this result remains within 90% acceptance criteria

Italian Validation Results

ATP levels were measured as a key energy biomarker



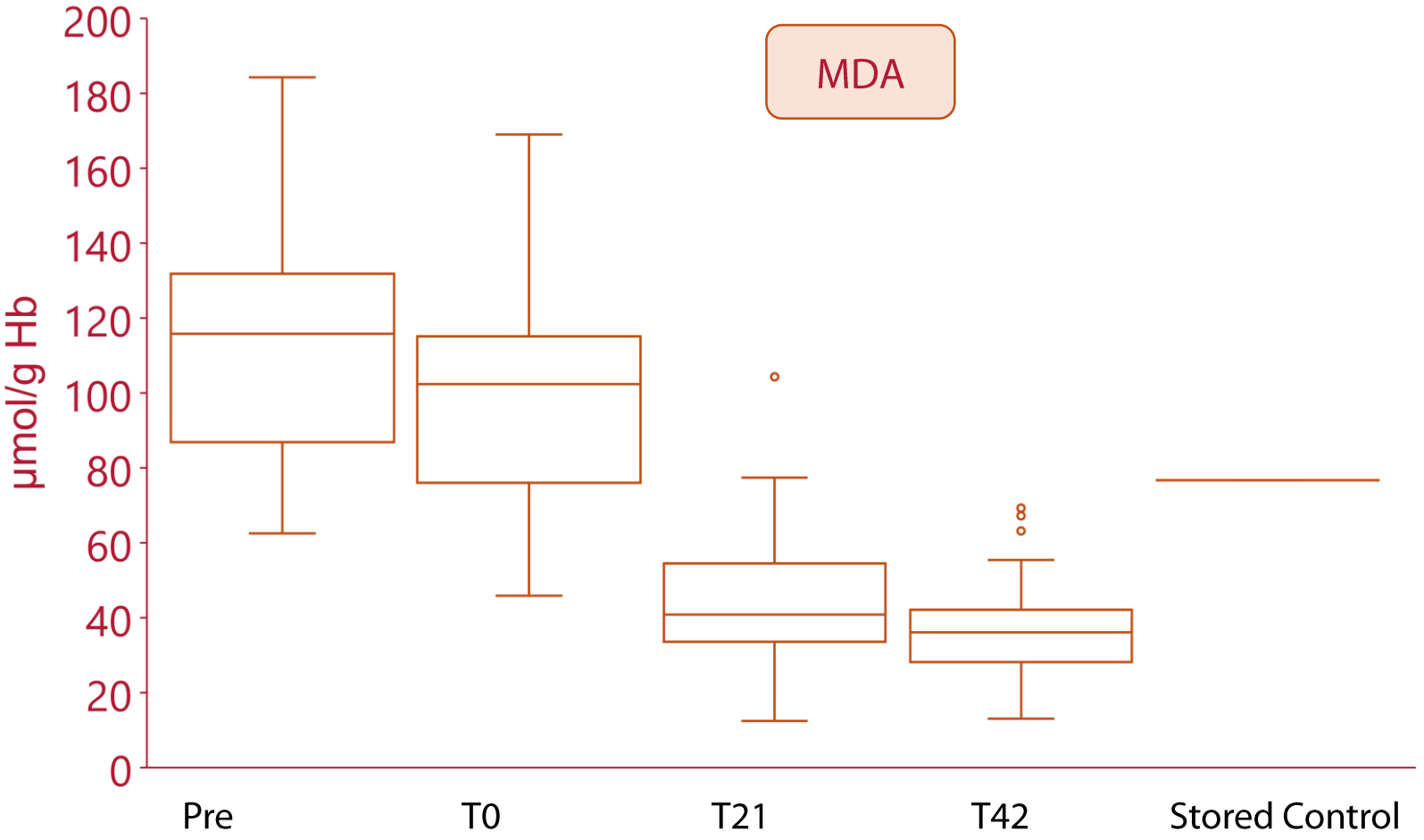
Higher ATP levels in hypoxic blood[1] vs. conventionally stored blood

ATP, adenosine triphosphate; Hb, hemoglobin.

1. D'Alessandro A, et al. *Transfus* 2020;60:786–98
2. DOF.Abstract.Preparation of Hypoxic RBC for Transfusion of Thal Study Patients.2022.

Italian Validation Results

MDA levels were measured as a key lipid peroxidation/oxidative stress biomarker



Lower MDA levels in hypoxic blood[1] vs. conventionally stored blood

MDA, malondialdehyde; Hb, hemoglobin.

1. D'Alessandro A, et al. *Transfus* 2020;60:786–98
2. DOF.Abstract.Preparation of Hypoxic RBC for Transfusion of Thal Study Patients.2022.

Methods

Safety Study:

- Transfusion-dependent cancer patients
  - Hemoglobin (Hgb) transfusion trigger < 9 g/dL,
  - Received > 2 units RBCs in 1 transfusion event and
  - Diagnosis of MDS/hematologic malignancy
- Acutely bleeding burn patients
  - Total Body Surface Area (TBSA%) burn ≥ 10% and ≤ 50%
  - Expected to require > 2 units of RBCs in 1 transfusion event.

- Adverse events (AEs) up to the subsequent transfusion or 28 days (± 1 day) post-transfusion were assessed.[2]

2. Reikvam et al. Poster (AABB); October 2024

Results: Safety Study
Patient Characteristics and Adverse Events

	Cancer Patients n=10	Burn Patients n=10
Male	8	9
Female	2	1
Age, years	72 ± 16	46 ± 19
Pre-Transfusion Hgb, g/dL	8.0 ± 0.7	10.6 ± 2.3
Post-Transfusion Hgb (1 hour), g/dL	9.2 ± 0.8	10.9 ± 2.0
Adverse Events / Related to HRBC	9 / 0	13 / 0
Serious Adverse Events / Related to HRBC	0 / 0	2 / 0

- A single center in Norway enrolled 10 CA and 10 AB patients after approval by the Ethics Committee.
- All CA and AB patients received one 2-hour transfusion of 2 units HRBC.
- In the Cancer cohort, Hgb levels increased 15% after HRBC administration.
- Burn patients had a 2% Hgb increase due to intra-operative blood loss replacement.
- One patient experienced 2 SAEs, wound infection and oliguria.
- No AE was deemed related to the blood product or device.

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Conclusions

These are the first reports validating hypoxic RBCs for transfusion, in preparation for a clinical study of chronically transfused patients with hematologic malignancy and thalassemia

Compared with conventionally stored blood, hypoxically stored RBCs:

- met acceptance criteria for transfusion
- maintained high levels of ATP
- attenuated MDA accumulation, an indirect estimate of RBC membrane oxidation

- Validation studies demonstrate feasibility of manufacturing without altering the biological characteristics of the product as documented by the quality controls
- No safety issues were seen in a pilot study of cancer and acutely bleeding burn patients.

- **HRBC represent an innovation in the preparation of blood components.**

ATP, adenosine triphosphate; MDA, malondialdehyde; RBCs, red blood cells.
DOF.Abstract.Preparation of Hypoxic RBC for Transfusion of Thal Study Patients.2022.

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Thank you for listening

Transfusion Efficacy of Amustaline/Glutathione Pathogen-reduced Red Blood Cells: Results of a Randomised, Controlled Clinical Trial

Richard J Benjamin, MD PhD.
Professor of Laboratory Medicine
Georgetown University
Washington DC

Chief Medical Officer
Cerus Corporation

Conflicts of Interest and Funding

- Richard J. Benjamin is an employee and shareholders of Cerus Corporation, the sponsor of the ReCePI study.

- Studies are funded by the Biomedical Advanced Research and Development Authority (BARDA), DHHS.

Pathogen Reduction for Red Blood Cells

Potential direct patient benefits.

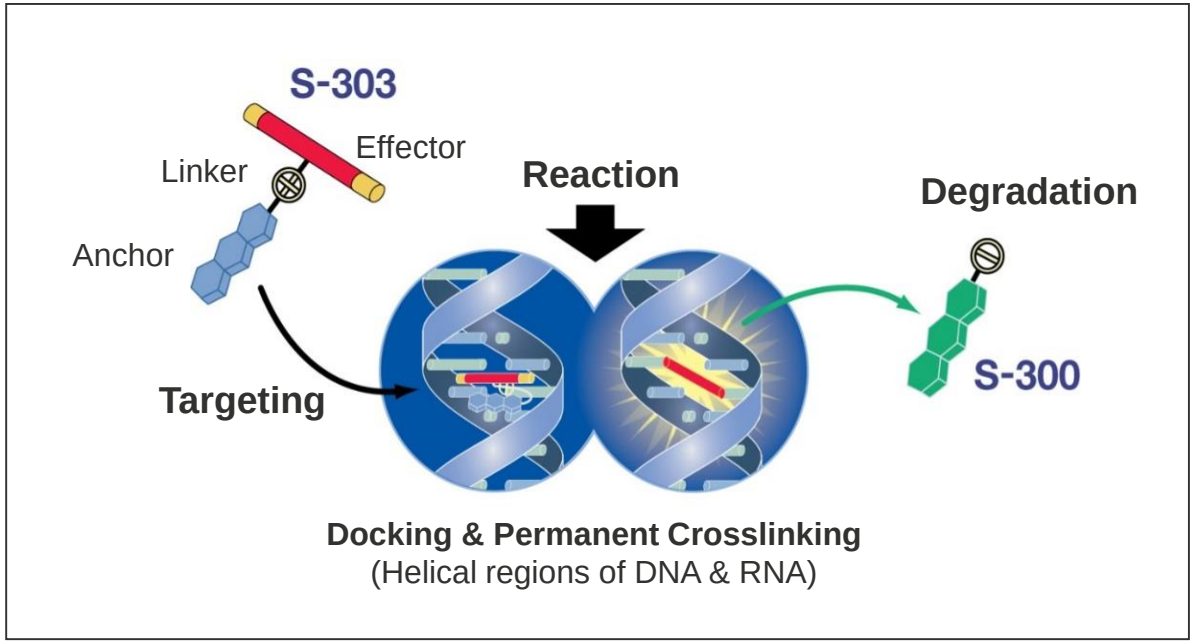
- Protection for emerging pathogens (Emergency Preparedness).
- Protection for known residual risks due to inadequate or selective interventions, *e.g., Bacteria, HIV* PREP risks, *Babesia, CMV, TA-GVHD, Malaria, Dengue, etc.*
- Improved product:
  - Replace irradiation of RBC: Reduced K^+ and hemolysis. Longer shelf-life/single inventory.
  - Reduced plasma exposure - “washed”: Reduce allergic reactions and TRALI risk.

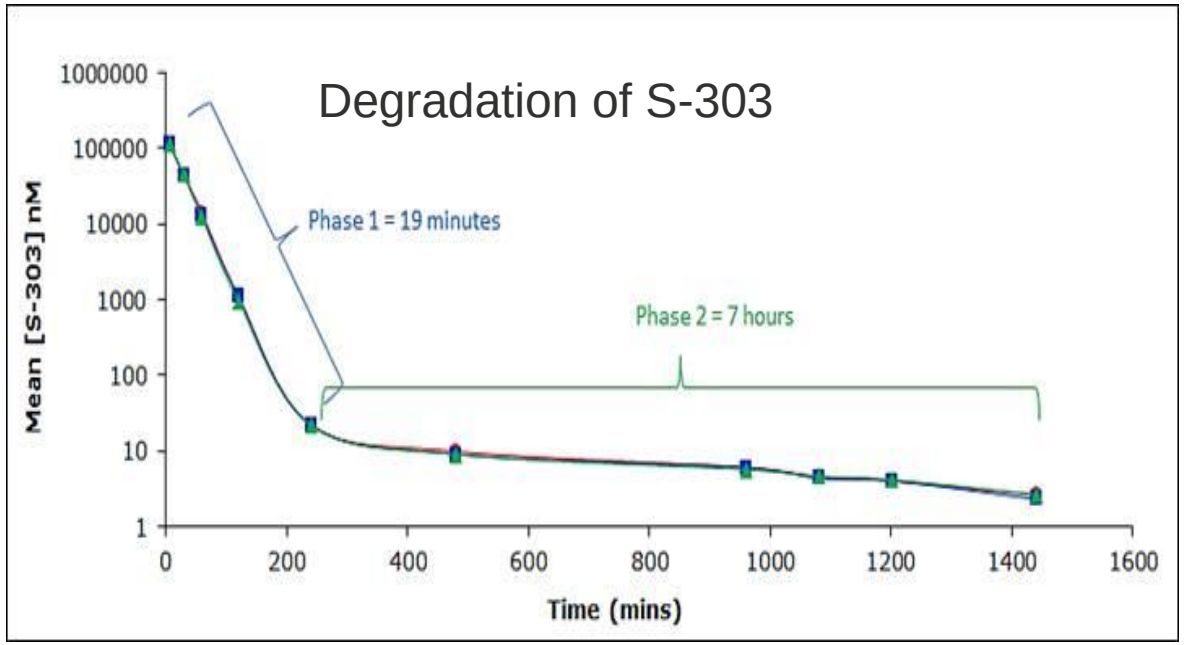
Potential benefits of Pathogen Reduction for all components.

- Avoid future additional viral marker tests, reassess current tests.
- Relaxed donor deferrals (e.g., malaria) → enlarge donor pool.

Pathogen Reduction: Mechanism of Action of Amustaline/Glutathione

- Amustaline (S-303) is a nucleic acid-targeted alkylator that quickly diffuses into viruses, bacteria, parasites and blood cells and is designed to react quickly and decompose.
- Glutathione (GSH) is used to quench side reactions with other biological materials.





- Rapid decomposition kinetics
- Single volume supernatant replacement after 24 hours
- Below the limit of quantitation (0.75 nM) after exchange

INTERCEPT Blood System for Red Blood Cells Clinical Program

Successfully Completed

In Vitro Characterization (SAG-M Sets)(n=65)
Report in Progress

Recovery & Lifespan (n=26) (SAG-M Sets)
Completed

STARS
Acute Transfusion (CV Surgery) (n=51)

SPARC
Chronic Transfusion (Thalassemia) (n=81)

ReCePI
Acute Tx (CV Surgery, n=321)

Ongoing

In Vitro Characterization (AS-1 Sets)(n=65)
Report in Progress

Recovery and Survival (AS-1 Sets)(n=24)
Protocol Approved

RedeS (n=500-800)

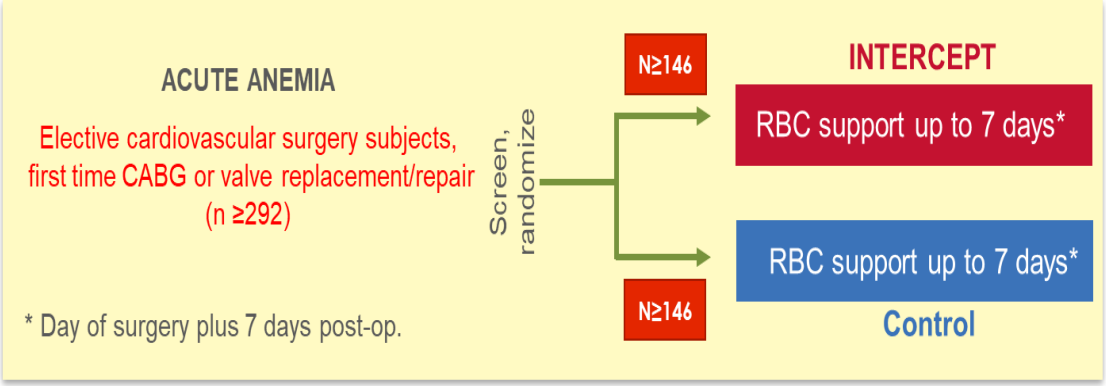
Acute (28d)	Chronic (28d +6m) (n=140)	RCE (n=20)
Complete	Enrolling	Enrolling

5 sites in the US and 1 site in Turkey (Ege University).

Cancelas et al. Vox Sanguinis (2017) 112, 210–218.
Brixner et al. Transfusion (2018) 58:905-16.
Aydinok et al. Br J Haematol. 2019 Aug;186(4):625-636.
Snyder et al. Trials 2023; 24:799.

ReCePI and RedeS trials are supported by BARDA contract number HHSO100201600009C.

Red Cell Pathogen Inactivation Study



Key Endpoints

- Incidence of Acute Kidney Injury.
- Related Treatment Emergent Adverse Events.
- Incidence of Antibodies to INTERCEPT RBCs.

Primary Endpoint

The proportion of patients who have received at least one study transfusion with a diagnosis of renal impairment (acute kidney injury: AKI) defined as:

Any raised serum creatinine (sCr) level, occurring after study RBC transfusion, of ≥0.3 mg/dL (or 26.5 µmol/L) from baseline within 48±4 hours of the end of surgery.

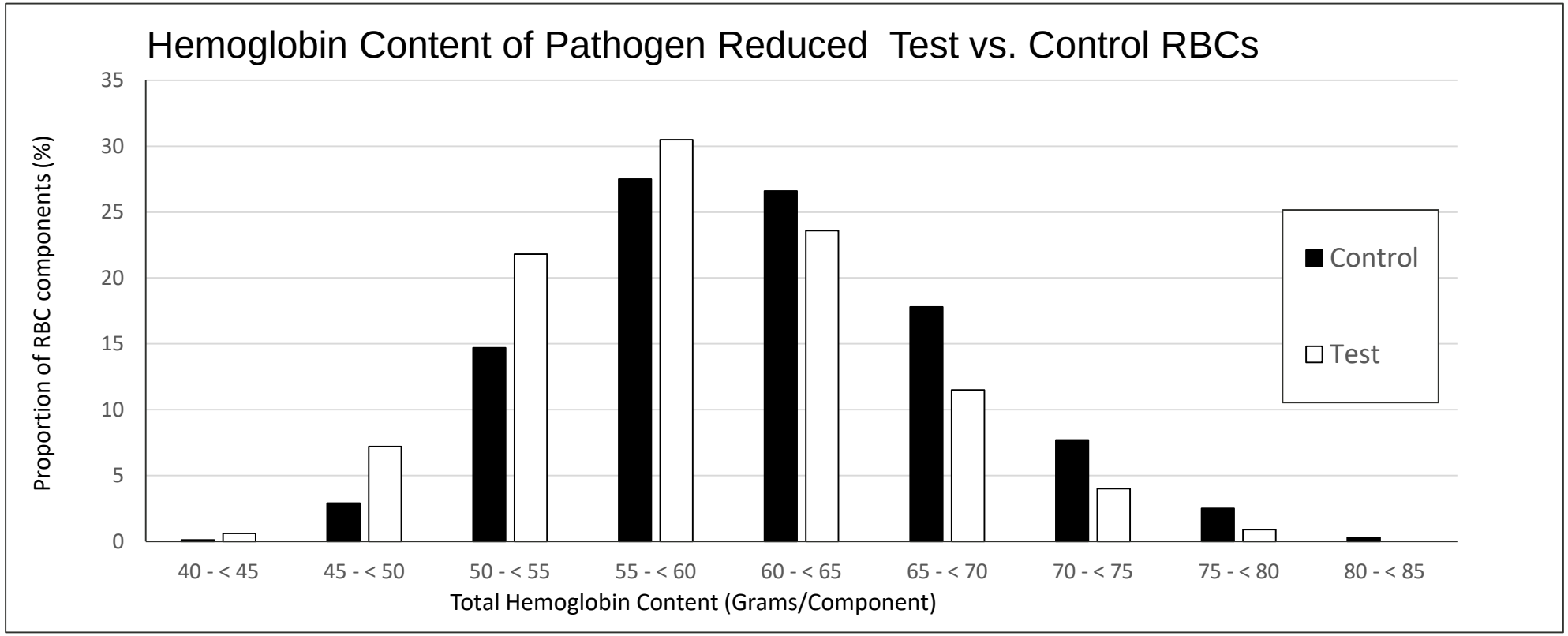
Non-Inferiority Test

The upper bound of the two-sided 95% CI was compared with 50% of the observed Control rate:

$$H_0: P_{Test} - P_{Control} \geq 50\% \times \hat{P}_{Control}$$
$$H_1: P_{Test} - P_{Control} < 50\% \times \hat{P}_{Control}$$

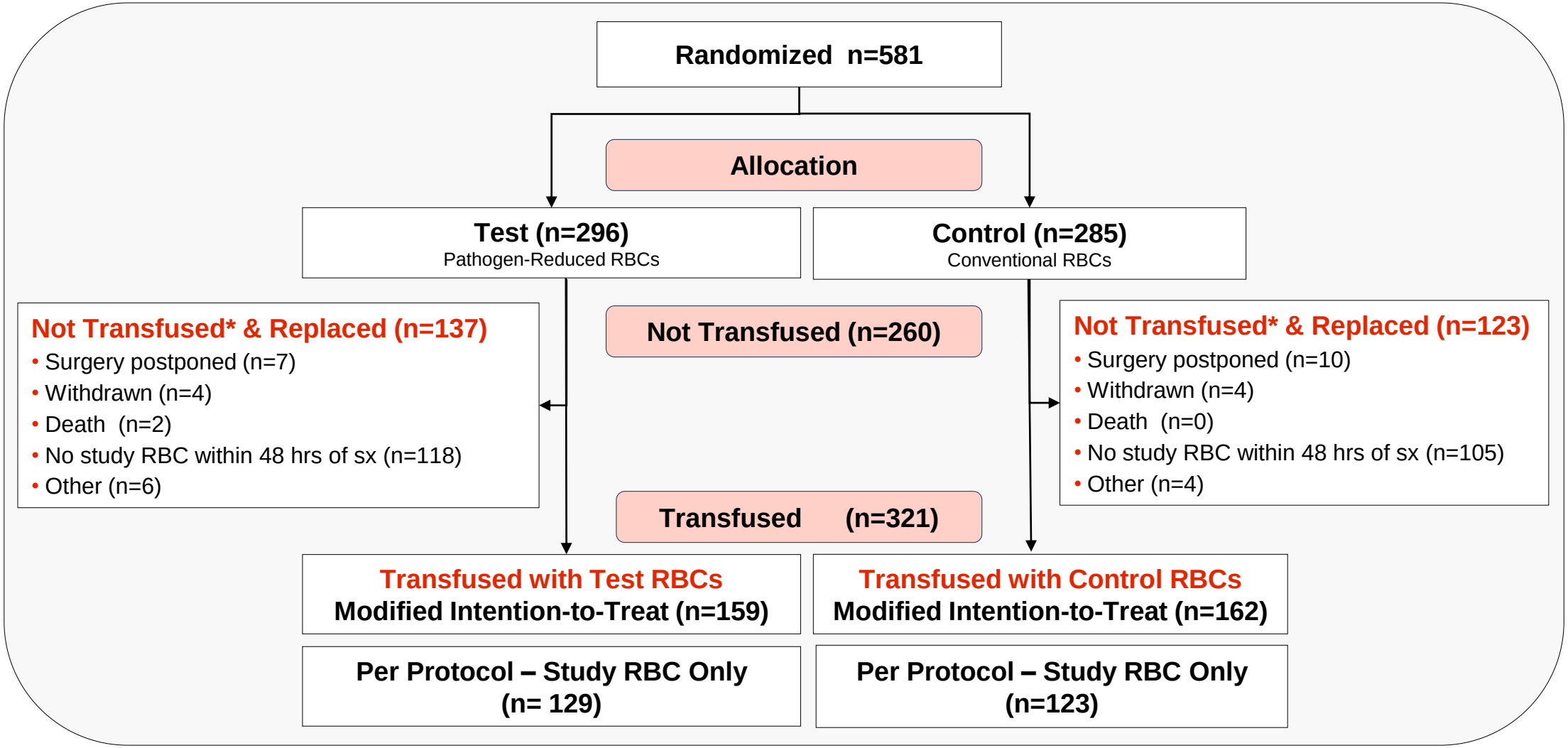
Non-inferiority achieved if the upper bound of a two-sided 95% CI of the treatment difference is less than 50% of the observed Control rate.

Hemoglobin Content of Test and Control RBCs Transfused



Study RBC Characteristics	Pathogen Reduced RBCs	Conventional RBCs	p-value
Total Study RBC units Transfused	456	524	0.049
Median Study RBC age (Days) (IQR)	23.8 (16.9-29.4)	21.8 (15.0-28.3)	<0.001
Median Hemoglobin (g) (IQR)	58.0 (53.0-62.0)	61.0 (57.0-66.0)	<0.001

Consort Diagram



* Subjects who received no study RBCs during or within 48 hours of the end of surgery were withdrawn from the study and replaced by newly randomized subjects.

ReCePI

Baseline Characteristics: mITT Population

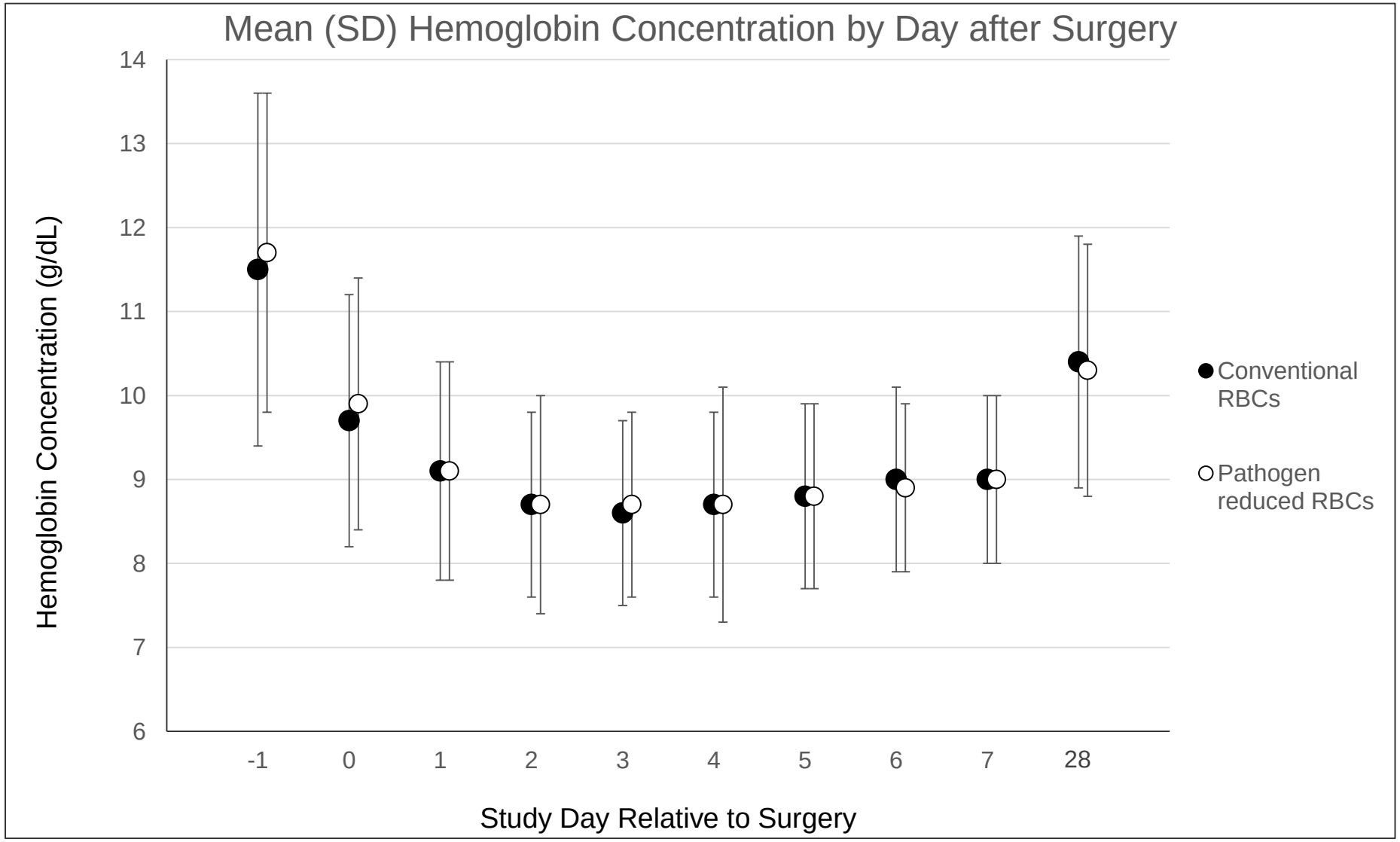
- Test and Control groups were well balanced with regards to Medical History and Surgery performed.

	Pathogen Reduced RBCs	Conventional RBCs
Age (Years)	65.8 (11.7)	63.8 (14.0)
Sex (n [%])		
Female	80 (50.3%)	82 (50.6%)
Race (n [%])		
White	134 (84.3%)	133 (82.1%)
Black or African American	22 (13.8%)	22 (13.6%)
Asian	2 (1.3%)	2 (1.2%)
Other	1 (0.6%)	5 (3.1%)
Ethnicity (n [%])		
Hispanic or Latino	6 (3.8%)	11 (6.8%)
Not Hispanic or Latino	153 (96.2%)	151 (93.2%)
Height (cm)	168.5 (11.36)	168.7 (10.40)
Weight (kg)	80.6 (18.18)	81.7 (20.86)
BMI (kg/m2)	28.1 (5.60)	28.6 (6.55)
TRUST Score	3.5 (1.25)	3.4 (1.28)
Serum Creatinine (mg/dL)	1.06 (0.30)	1.04 (0.32)
Hemoglobin (g/dL)	11.67 (1.89)	11.54 (2.09)

Surgical Outcomes were Well Balanced

Surgical Factors	Transfused	
	Pathogen Reduced RBC (n=159)	Conventional RBC (n=162)
Surgery Duration (Hours) (Mean ±SD)	8.3 ± 2.4	8.6 ± 2.5
Volume of Salvaged Blood (mL) (Mean ±SD)	514 ± 496	519 ± 524
Surgical Day 0 Blood Loss (mL) (Mean ±SD)	501.7 ± 936.7	513.6 ± 645.3
Total Blood Loss over 7 days (mL) (Mean ±SD)	2005 ± 1708	2054 ± 1421
Aortic Cross-Clamp Use Duration (Hrs) (Mean ±SD)	2.1 ± 1.1	2.2 ± 1.2
Use of Deep Hypothermic Circulatory Arrest (%)	15.1	17.9
Hemoglobin Day 0 post surgery (g/dL) (Mean ±SD)	9.9 ± 1.5	9.7 ± 1.5

Patient Hemoglobin (g/dL) after Surgery (mITT Population)



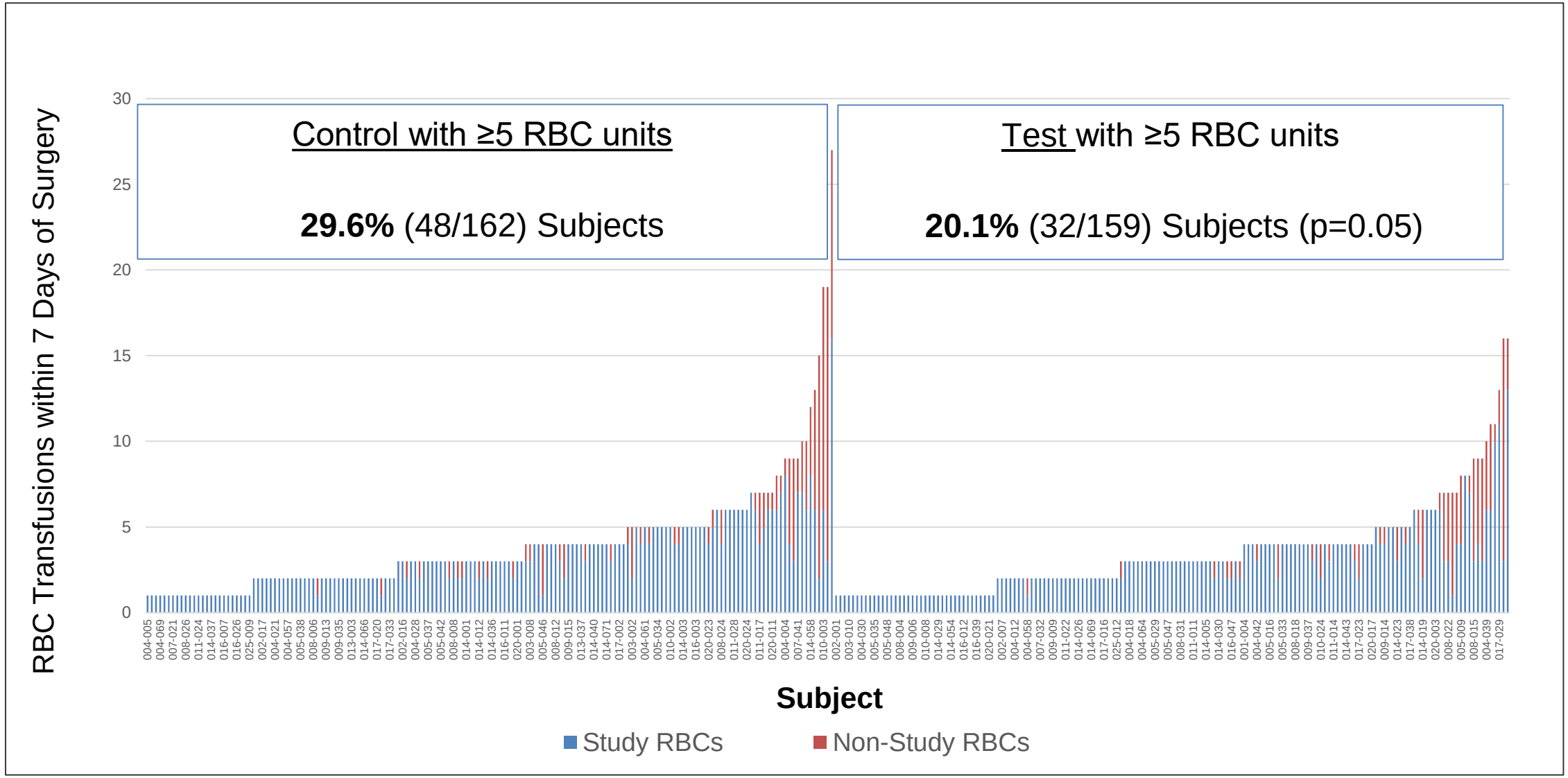
- No difference in post-surgery hemoglobin levels.

Transfusion within 7 Days of Surgery (mITT)

Blood Components (units) - Median (IQR)	Pathogen Reduced RBC (n=159)	Conventional RBC (n=162)	p-value
Total Study + Non-study RBCs	3 (2 to 4)	3 (2 to 5)	0.086
Total Study + Non-study RBC Hemoglobin	**169 (102-240)**	**188 (126-295)**	**0.008***
Plasma	**2 (1 to 3)**	**2 (1 to 4)**	**0.021***
Platelets	2 (1 to 2)	2 (1 to 2)	0.653
Cryoprecipitate[#]	2 (1 to 2)	2 (1 to 2)	0.375

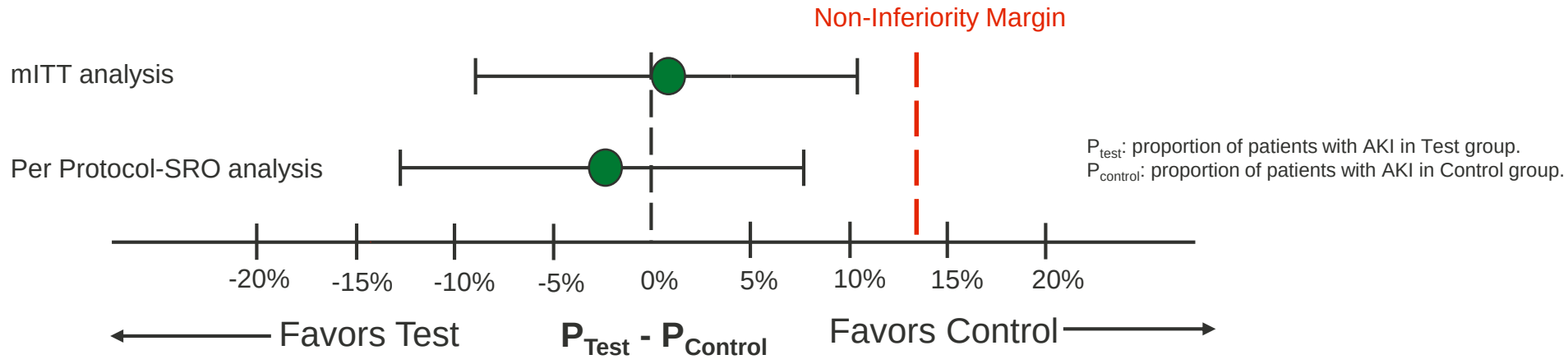
*p <0.05; [#] pools of 5

RBC Transfusions During the Acute Transfusion Period



Primary Efficacy Endpoint of Acute Kidney Injury

Acute Kidney Injury (AKI) Endpoint	Pathogen Reduced RBCs (N=159)	Conventional RBCs (N=162)	Adjusted Test – Control [95% C.I.][1]	Non-Inferiority Margin	p-value for Non Inferiority
Modified Intention to Treat	29.3% (46/157)	28.0% (45/161)	0.7% [-8.9%, **10.4%**]	**14.0%**	**0.001**
Per Protocol - Study RBCs Only	24.0% 31/129	26.8% 33/123	-2.6% [-13.0%, **7.8%**]	**13.4%**	**0.03**

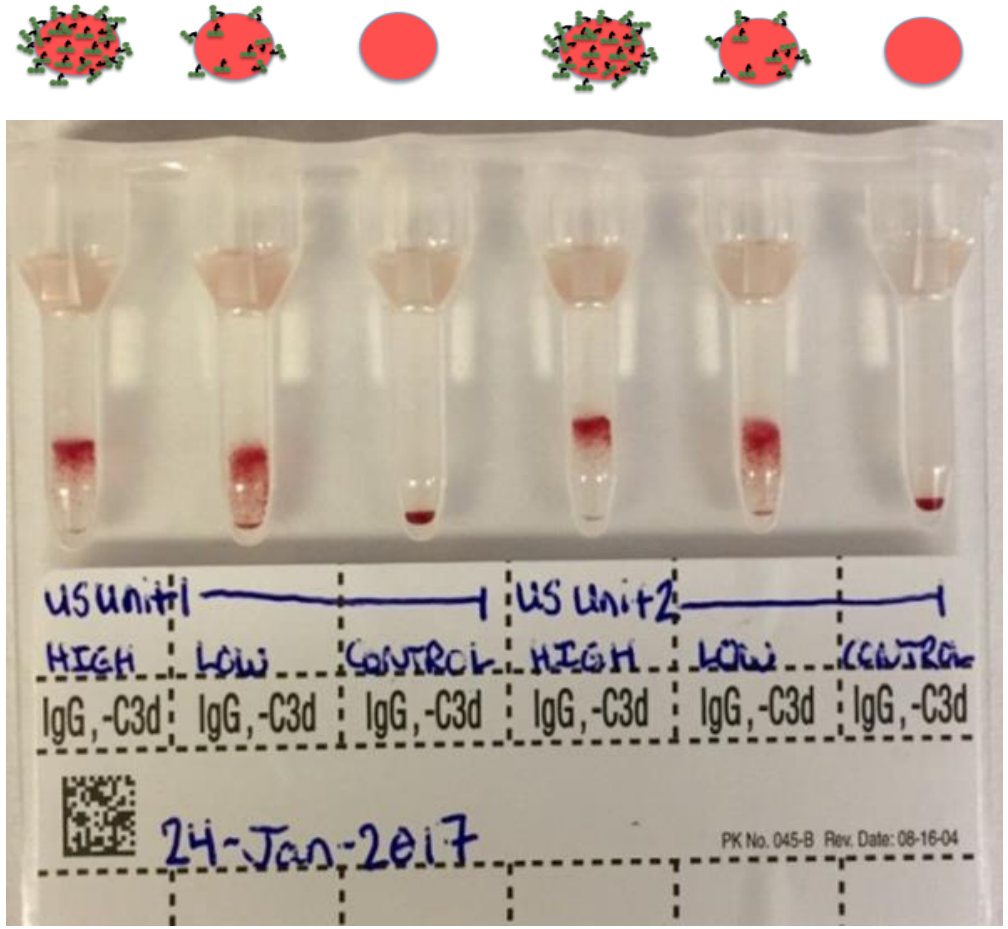


Safety Endpoints Summary Related to Cardiac Surgery

Adverse Events ≤ 28 Days of Last Study RBC	Pathogen Reduced RBCs		Conventional RBCs		p-value
Acute Kidney Injury (clinical report)	41/159	(25.9%)	36/162	(22.2%)	0.472
Stroke	2/159	(1.3%)	7/162	(4.3%)	0.105
Myocardial Infarction	0/159	(0.0)	0/159	(0.0%)	-
Cardiac Arrythmia or Conduction Defect	79/159	(49.7%)	83/162	(51.2%)	0.779
Thrombotic Event, excluding Stroke	12/159	(7.5%)	10/162	(6.2%)	0.612
Infection, excluding Pneumonia	23/159	(14.5%)	23/162	(14.2%)	0.895
Pneumonia	7/159	(4.4%)	13/162	(8.0%)	0.163
Seizure/Delirium/Encephalopathy	16/159	(10.1%)	14/162	(8.6%)	0.684
Adverse Events	153/159	(96.2%)	147/162	(90.7%)	0.055
Serious Adverse Events	66/159	(41.5%)	57/162	(35.2%)	0.219
Deaths within 30 Days	9/159	(5.6%)	7/162	(4.3%)	0.628
Deaths on Study (75 Days)	13/159	(8.2%)	10/162	(6.2%)	0.498

Screening for Antibodies with Specificity for Pathogen Reduced RBCs

- Validated assay for Pathogen Reduced RBC antibodies.
- RBCs labelled with different Acridine density:
  - High, Low and Zero (Control).
- Performed at baseline, and when a routine antibody screen was performed, and at Day 28 and Day 75.
- On recognition of an antibody to PR RBCs:
  - Subject no longer received PR RBCs.
  - Investigated for hemolysis every ~ 2 weeks (3-5 x).
  - RBCs frozen for later flow cytometry analysis.
  - Samples sent to reference lab for confirmation.



Antibodies with Pathogen Reduced RBC Specificity

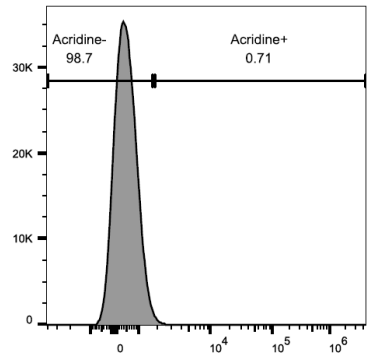
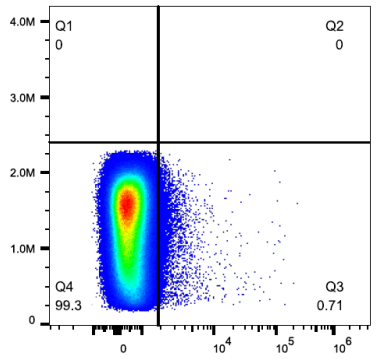
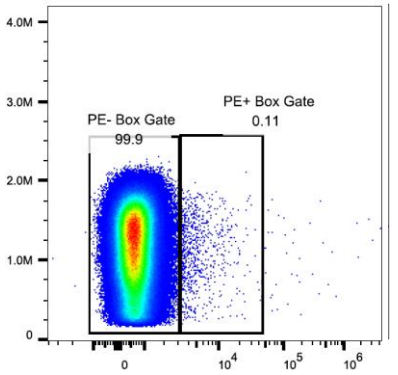
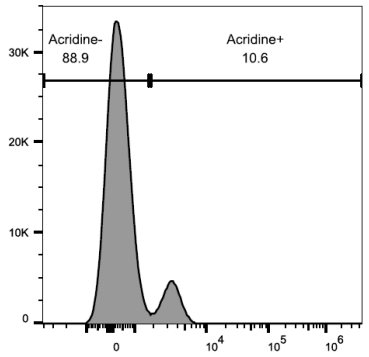
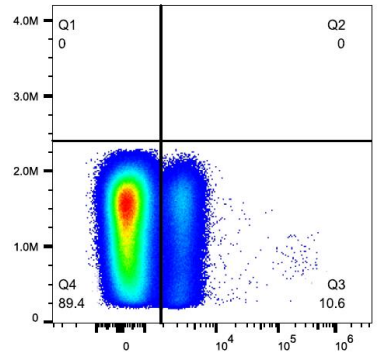
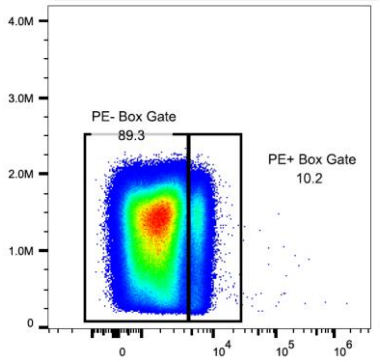
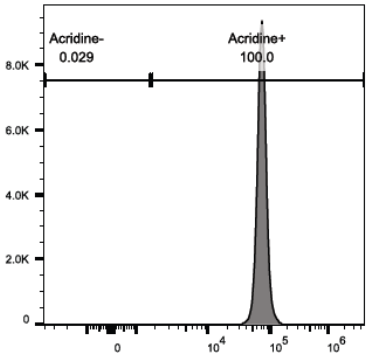
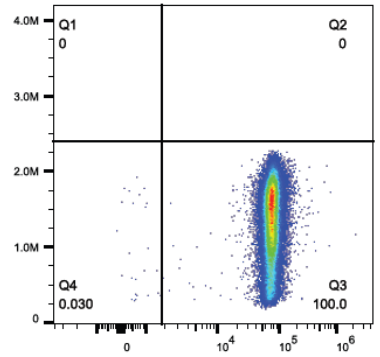
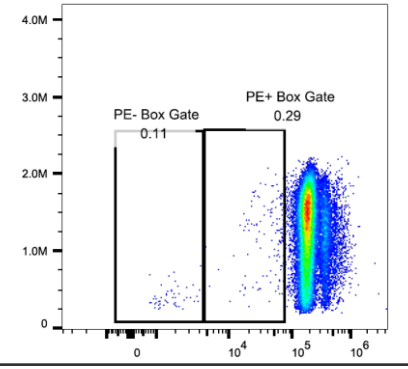
Study Subject*	Study RBC Exposure	Study Day of Discovery	Maximum Reactivity & Titer	DAT	Monocyte Mononuclear Assay	Evidence of Hemolysis	Antibody
008-014	1 Test unit	43	1:8	Neg.	Non-reactive	No hemolysis	Inhibited by acridine
016-012	1 Test unit	80	Neat	Neg.	Indeterminate	No hemolysis	Inhibited by acridine
011-011	3 Test units	32	S-303H 1+ S-303L 2+ Titer not performed	Pos.	Non-reactive	No hemolysis Eluate performed	Inhibition by acridine unknown
002-029	1 Test unit	26	S-303H 2+ S-303L 0+ Titer not performed	Neg.	Indeterminate	No hemolysis	Not inhibited by acridine
010-049	3 Test units	30	Titer 1:2	Neg.	Non-reactive	No hemolysis	Inhibited by acridine

Data were reviewed by the DSMB and FDA at the time of occurrence and the study was allowed to proceed.

Flow Cytometry Analysis with Acridine-specific mAb

- Subject 011-011 received 3 units of PR RBCs.
- Antibody first detected on Day 32 post-surgery.
- Reactivity 1+
- DAT & Eluate reactive.

- *Karim C. et al. Transfusion (in press)*

	Anti Acridine -PE		Anti Human IgG-PE
Negative Control (Control RBCs)			
011-011 Day 39 Mean Acridine 9.7% Acridine 301 mol./cell IgG 10.1%			
Positive Control (Fresh PR RBCs ± human anti acridine) Acridine 7,500 mol./cell			

Overall Summary

- ReCePI met the Primary Efficacy endpoint in all subgroups.
- Subjects in the Test arm tended to use fewer RBCs and less Hemoglobin.
- Overall Incidence of AEs, SAEs, and Deaths did not differ between groups.
- Five treatment-emergent antibodies were detected with specificity for Pathogen Reduced RBCs - all in the Test Arm.
- No antibodies showed any evidence of clinical significance.
- Flow cytometry showed persistent circulating Pathogen Reduced RBCs with antigen loss in all five cases.

- <u>Conclusion:</u> Pathogen Reduced Red Cells met study requirements for safety and efficacy

ReCePI Study Group:

- Michael E. Sekela, MD, Gill Heart Institute University of Kentucky, Lexington, KY (mese223@uky.edu)
- Edward L. Snyder, MD, Yale University School of Medicine, New Haven, CT (edward.snyder@yale.edu)
- Ian J. Welsby, BSc, MBBS, FRCA, Duke University Medical Center, Durham, NC (ian.welsby@duke.edu)
- Yoshiya Toyoda, MD, Temple University Hospital, Philadelphia, PA (yoshiya.toyoda@tuhs.temple.edu)
- Mohamed Alsammak, MD, Temple University Health System, Philadelphia, PA (mohamed.alsammak@tuhs.temple.edu)
- Neel R. Sodha, MD, Rhode Island Hospital, Providence, RI (nsodha@lifespan.org)
- Thomas M. Beaver, MD, University of Florida, Gainesville, FL (thomas.beaver@surgery.ufl.edu)
- J. Peter R. Pelletier, MD, University of Florida, Gainesville, FL (pelletierp@ufl.edu)
- Alesia Kaplan, MD, University of Pittsburgh Medical Center, Pittsburgh, PA; Vitalant, Pittsburgh, PA (akaplan@vitalant.org)
- Roman M. Sniecinski, MD, MSc Emory University, Atlanta, GA (rsnieci@emory.edu)
- T. Brett Reece, MD, University of Colorado Hospital, Denver, CO (brett.reece@cuanschutz.edu)
- Ronald G. Pearl, MD, Stanford University, Stanford, CA (rgp@stanford.edu)
- Robertson D. Davenport, MD, University of Michigan, Ann Arbor, MI (rddvnprt@med.umich.edu)
- James D. Gorham, MD PhD, University of Virginia Health System, Charlottesville, VA (JDG8Z@uvahealth.org)
- John S. McNeil, MD, University of Virginia Health System, Charlottesville, VA (JSM6J@uvahealth.org)
- Ravi Sarode, MD, University of Texas Southwestern Medical Center, Southwestern, Dallas, TX (ravi.sarode@utsouthwestern.edu)
- Tina S. Ipe, MD, Our Blood Institute, Oklahoma City, OK (tina.ipe@obi.org) and University of Arkansas for Medical Sciences, Little Rock, AR
- Gregory A. Nuttall, MD, Mayo Clinic, Rochester, MN (nuttall.gregory@mayo.edu)
- Peyman Benharash, MD, UCLA, Los Angeles, CA (pbenharash@mednet.ucla.edu)
- Ileana Lopez-Plaza, MD, Henry Ford Hospital, Detroit, MI (iplaza@hfhs.org)
- Patrick Sadler, MD, Central California Blood Center, Fresno, CA (psadler@donateblood.org)
- Rita Reik, MD, OneBlood, Orlando, Florida (rita.reik@oneblood.org)
- Richard R. Gammon, MD, OneBlood, Orlando, Florida (richard.gammon@oneblood.org)
- John P. Pitman, PhD, Cerus Corporation, Concord, CA (jpitman@cerus.com)
- Kathy Liu, PhD, Cerus Corporation, Concord, CA (kliu@cerus.com)
- Stanley Bentow, PhD, Cerus Corporation, Concord, CA (sbentow@cerus.com)
- Laurence Corash, MD, Cerus Corporation, Concord, CA (lcorash@cerus.com)
- Nina Mufti, PhD, Cerus Corporation, Concord, CA (nmufti@cerus.com)
- Jeanne Varrone, MD, Cerus Corporation, Concord, CA (jvarrone@cerus.com)
- Richard J. Benjamin, MD PhD FRCPath, Cerus Corporation, Concord, CA (rbenjamin@cerus.com)

Free amotosalen does not induce non-specific degranulation of basophils from healthy volunteers in-vitro

Xavier Delabranche (MD, PhD)

Inserm UMR_S1255 Biologie et pharmacologie des plaquettes sanguines : hémostase, thrombose, transfusion – Établissement Français du Sang, Strasbourg

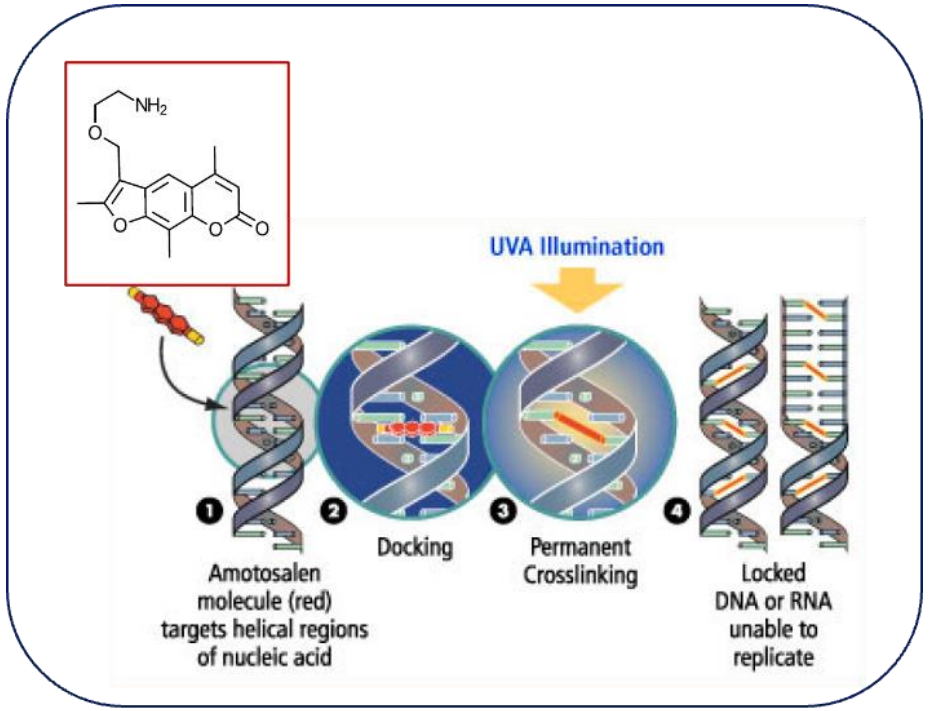
Anaesthesia, Critical Care and Perioperative Medicine, Les Hôpitaux Universitaires de Strasbourg

Disclosures for Xavier DELABRANCHE

Research Support/P.I.	No relevant conflicts of interest to declare
Employee	No relevant conflicts of interest to declare
Consultant	No relevant conflicts of interest to declare
Major Stockholder	No relevant conflicts of interest to declare
Speakers Bureau	No relevant conflicts of interest to declare
Honoraria	No relevant conflicts of interest to declare
Scientific Advisory Board	No relevant conflicts of interest to declare

INTERCEPT™ Blood System (IBS)

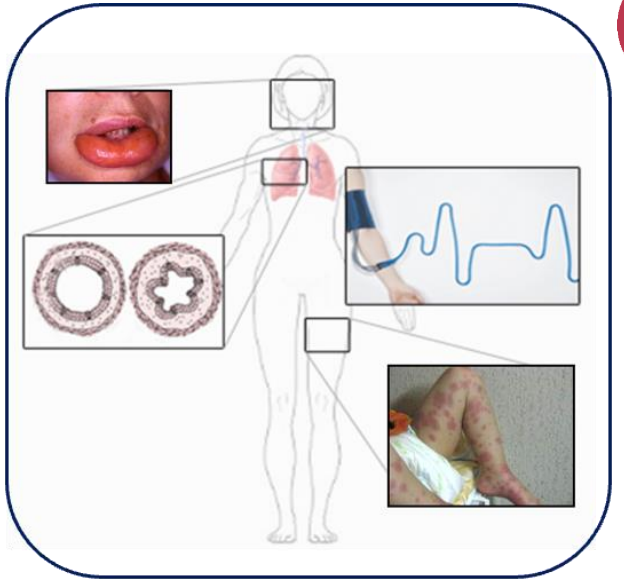
- **Amotosalen + UV-A (IBS, Cerus Corp., Concord, CA)**
  - Transfusion safety: pathogen reduction
  - Covalent bond with nucleic acids (DNA and RNAs)
- **Implementation in France**
  - FFPs: 2008, ≈ 10%
  - PCs: November 2017, 100%
- **Residual amotosalen and by-products**
  - After treatment (amotosalen + UV-A) and adsorption (CAD)
    - Free residual amotosalen < 7.5 µM (PCs) or < 2.0 µM (FFPs)
    - Photo-induced by-products
  - No specific antibody in phase III clinical trials



Lin L, Transfusion 2005;45:1610-20

Immediate hypersensitivity and transfusion

- **Immediate hypersensitivity**
  - Clinical definition (urticaria, bronchospasm, hypotension, shock)
  - During the four hours after transfusion initiation
  - Independently of involved mechanism (immunologic or not)
  - Four grades: 1 (urticaria) to 4 (death)
- **Incidence in France (2022)**

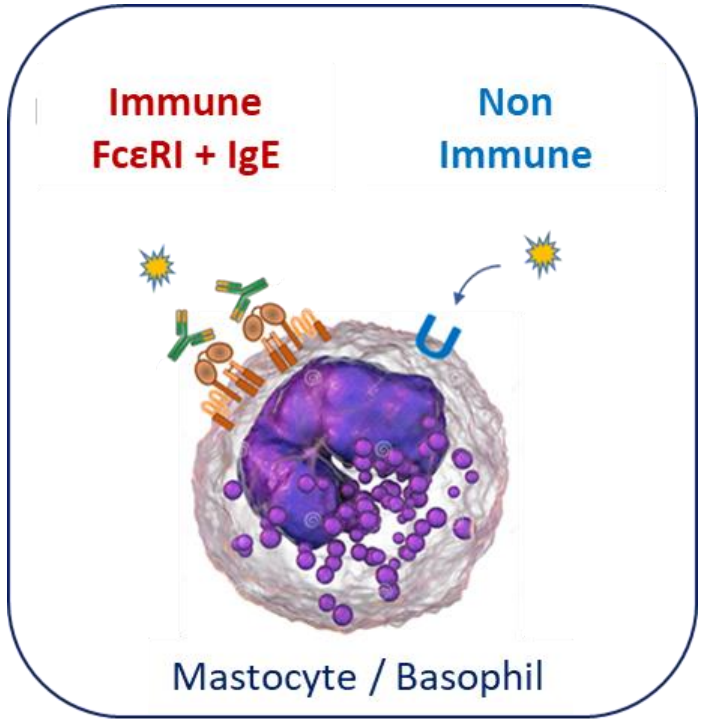


	Total	RBCPs	PCs	FFPs
Blood products (x 100,000)	29.7	24.0	3.3	2.4
Hypersensitivity reactions (/1,000)	742 (0.25)	173 (0.70)	359 (1.08)	210 (0.88)
Grade 3 with imputability 2 or 3 (/1,000)	47 (0.02)	4 (0.00)	18 (0.05)	25 (0.10)

ANSM, 20th hemovigilance national report – November 2023

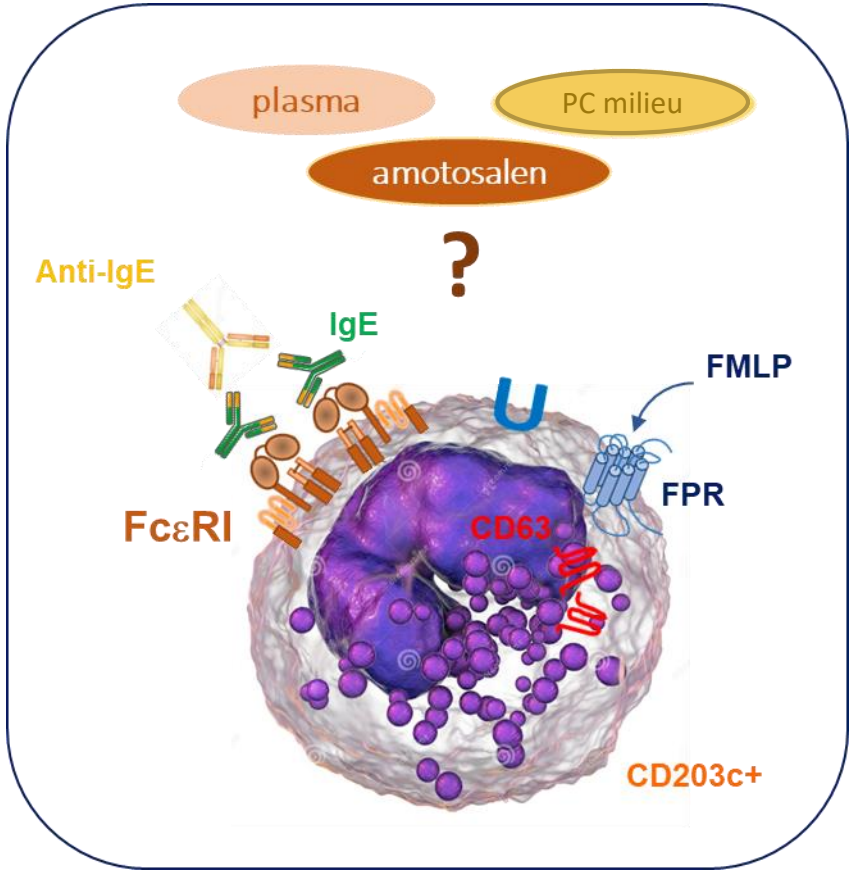
Can amotosalen induce hypersensitivity?

- **Epidemiology and imputability**
  - No increase in hypersensitivity side-effects since implementation
    - 17 millions blood products prepared with IBS over 22 years
    - Routinely available in 45 countries, including 15 for more than half PCs
  - No reaction after re-exposure in multiple transfused patients
  - Psoralens: 1 to 2 mg daily intake (citrus fruits, carrots, parsley…)
- **Hypersensitivity: mechanism?**
  - Non-specific histamine release (non-immune)
  - Allergy (IgE or IgG mediated)
- **If hypersensitivity: which consequences ?**
  - FFPs: substitution to quarantine-FFPs or SD-FFPs
  - PCs: non-IBS-PCs not available in some countries



How to confirm the diagnosis of hypersensitity?

- **Cutaneous testing**
  - Not available
  - Could be dangerous after sun exposure
- **Basophil activation test (BAT)**
  - Routinely used for allergy testing
  - Controls
    - Anti-IgE antibody: IgE pathway
    - fMLP (N-formyl-Met-Leu-Phe): non-immune pathway
  - Analyses
    - Free amotosalen
    - Plasma
    - Platelet storage milieu (45% Plasma + 55% PAS-III) at 22°C and 4°C



Basophil activation test

1. Sensitization

IL-3 [10 ng/mL], 10 min., 37°C

2. Stimulation

Free amotosalen, plasma, conservation milieu (30 min., 37°C)

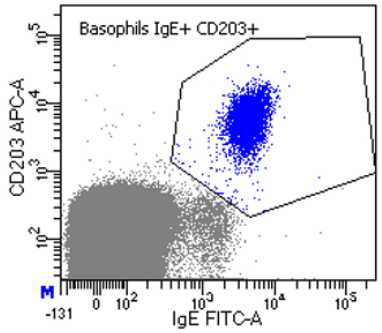
3. Immunolabelling

αIgE-FITC
αCD63-PE
αCD203-APC

Whole blood
(citrate 3.8%)

4. Basophil selection

$CD203^+/IgE^+$ (singlets)

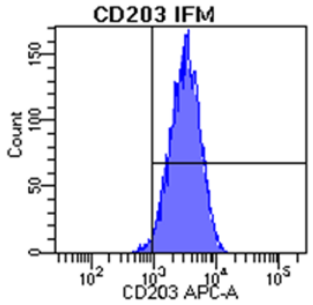


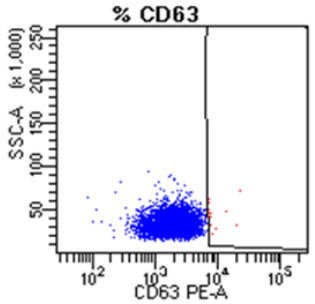
5. Activation/secretion markers

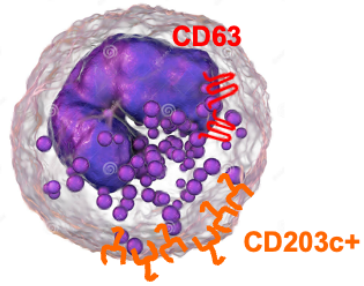
Stimulation index CD203c (MFI) = MFI_{Test} /$MFI_{Non\ activated}$

$CD63^+$ (%) correlated to histamine release

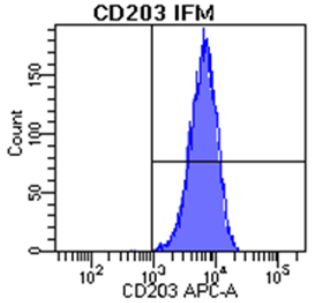
Non activated

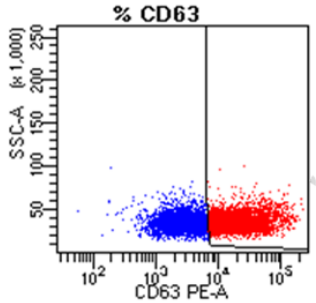


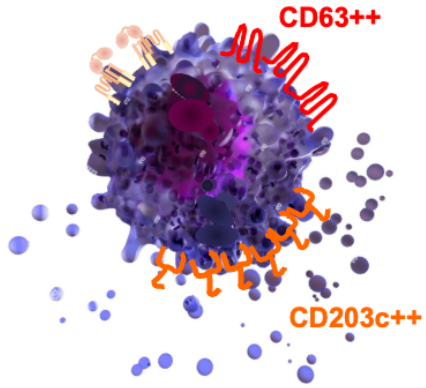




Activated



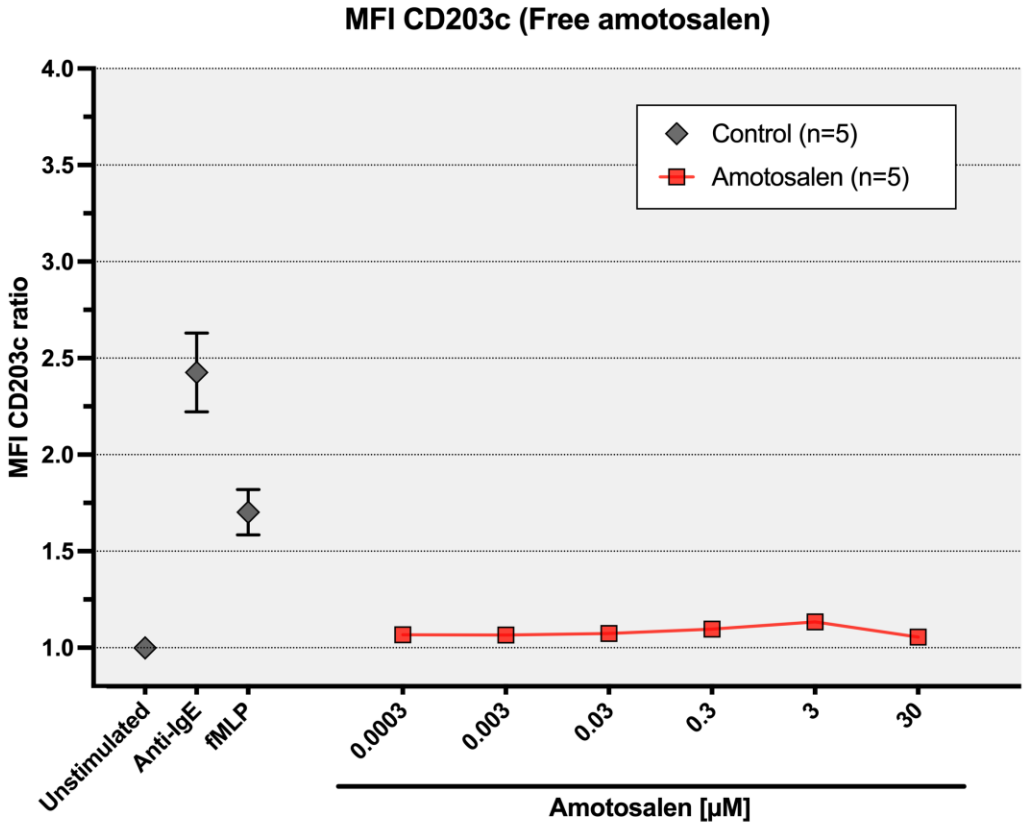


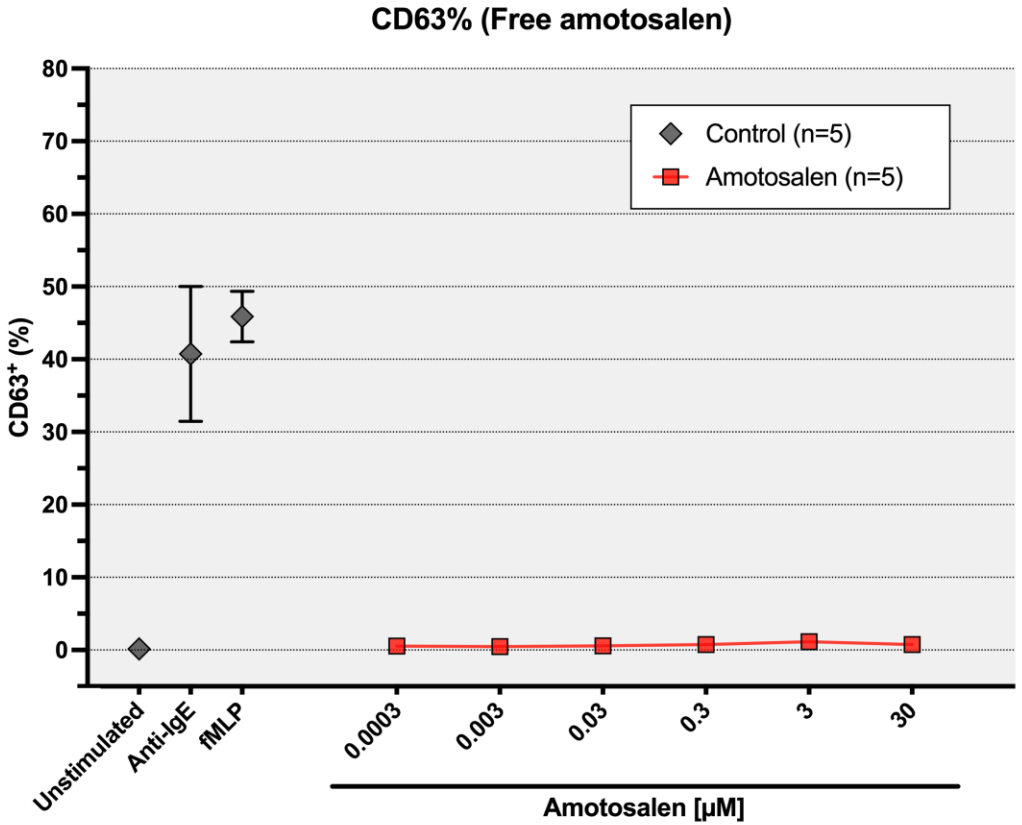


All healthy donors gave informed consent, and the trial is registered in the EFS data base

Free amotosalen

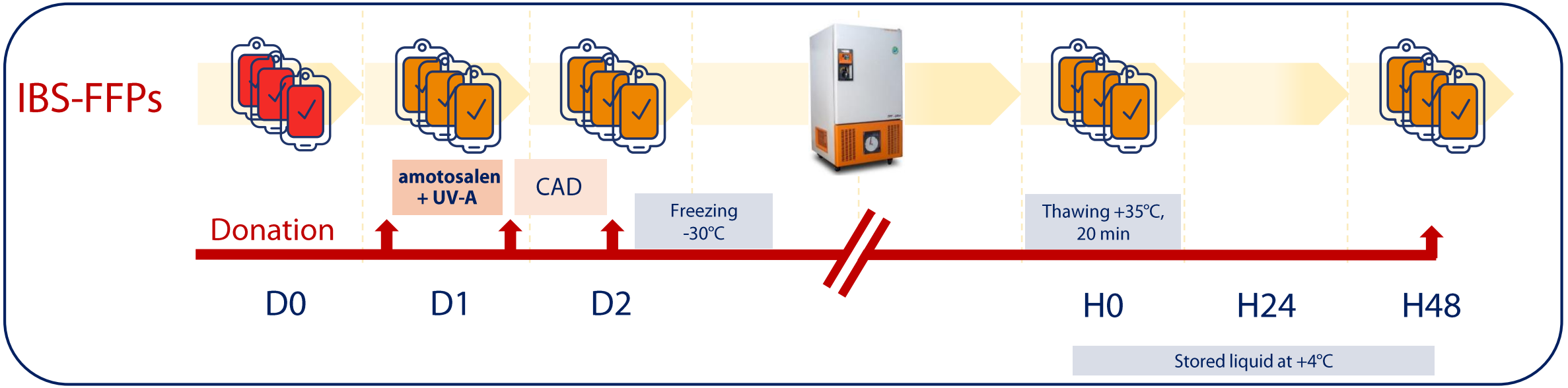
(3 µM = 2 APCs or 3 BC-PCs or 10 FFPs)



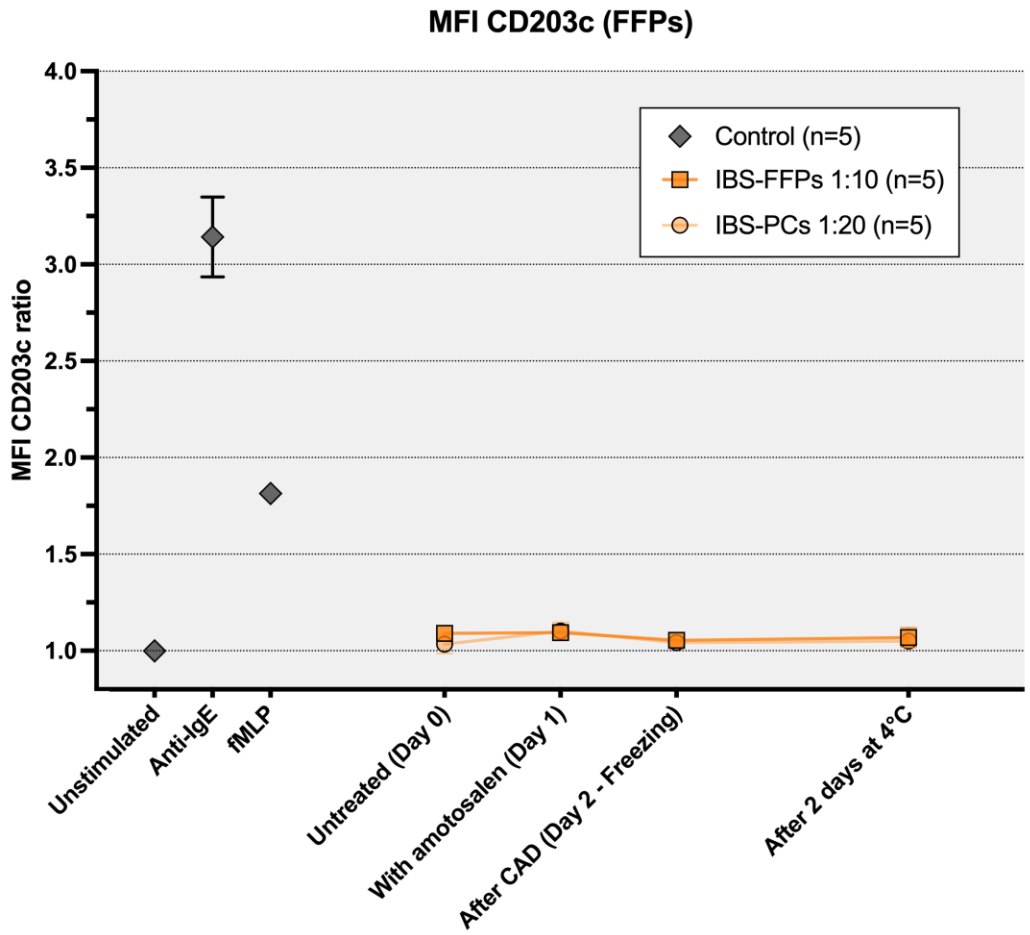


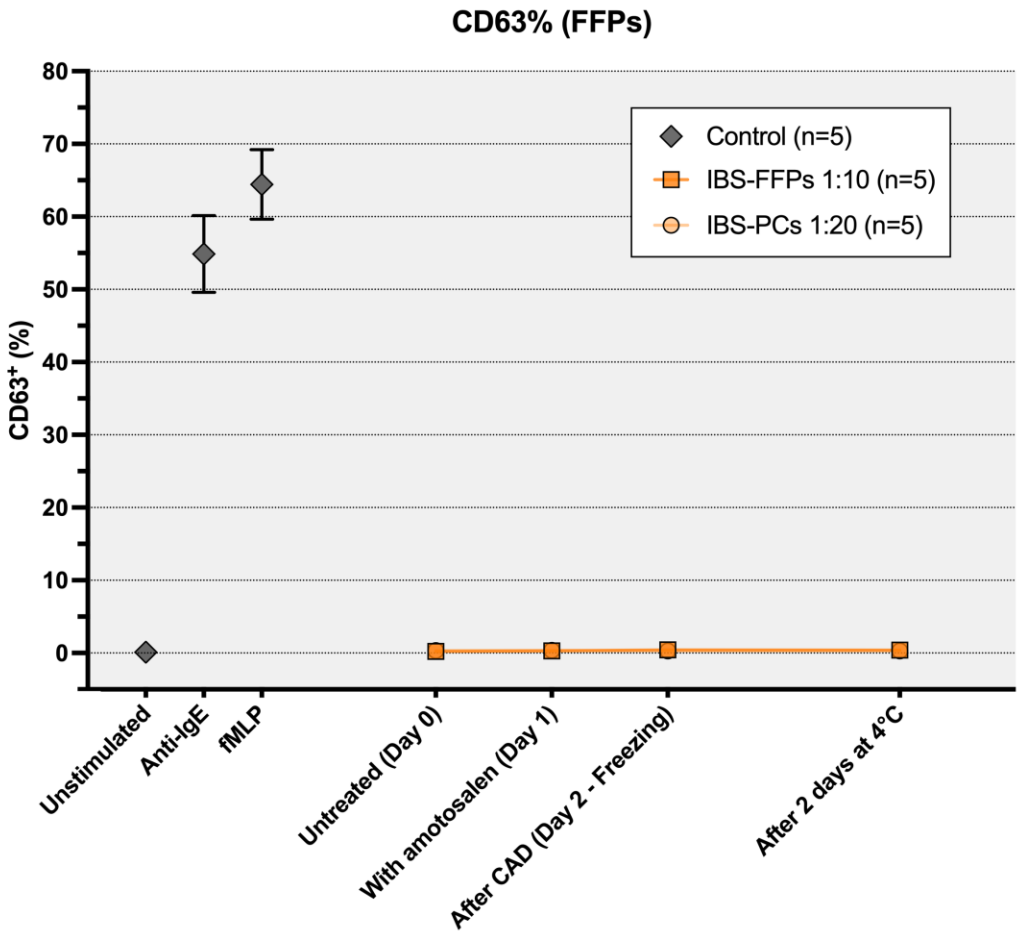
Free amotosalen has no effect on basophil activation

Fresh frozen plasma preparation



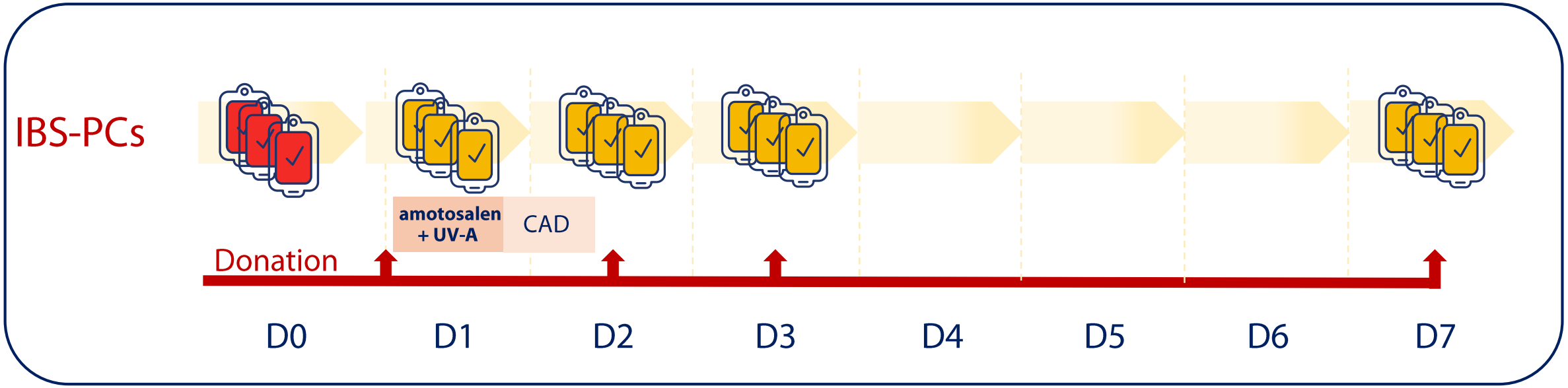
IBS-FFPs (1:10 = 10 FFPs)





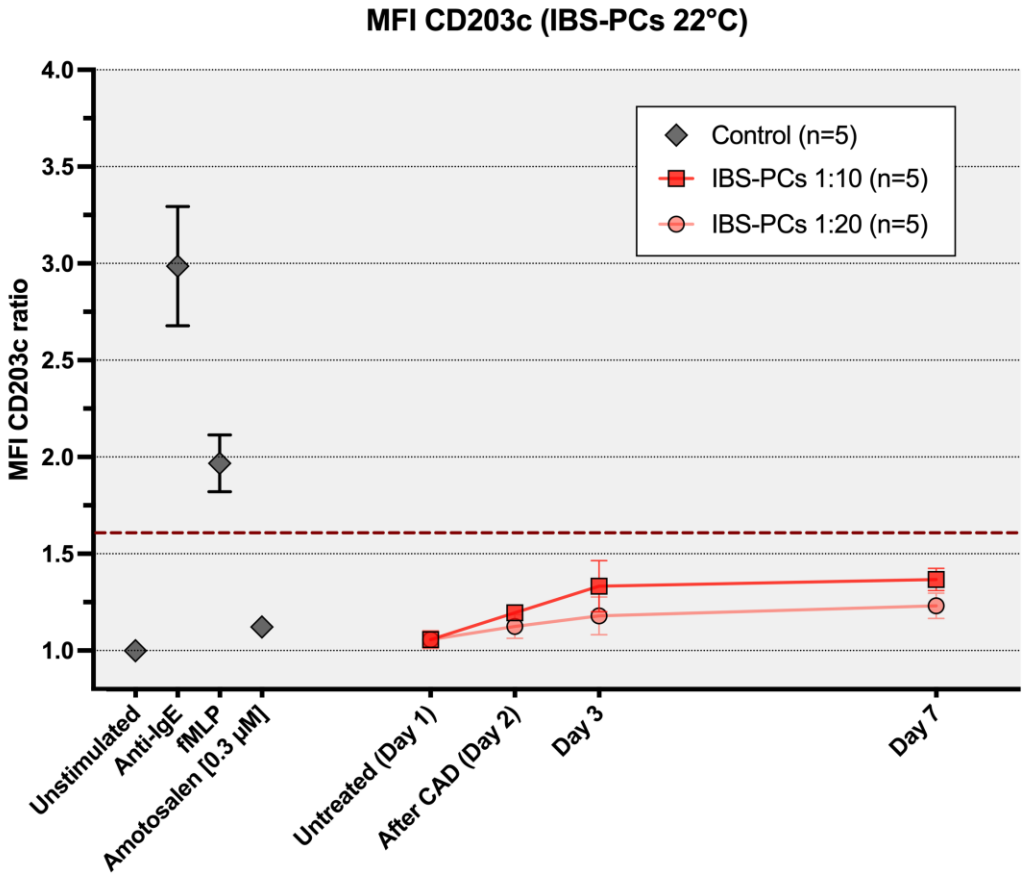
Amotosalen or photo-induced by-products in the plasma have no effect on basophil activation

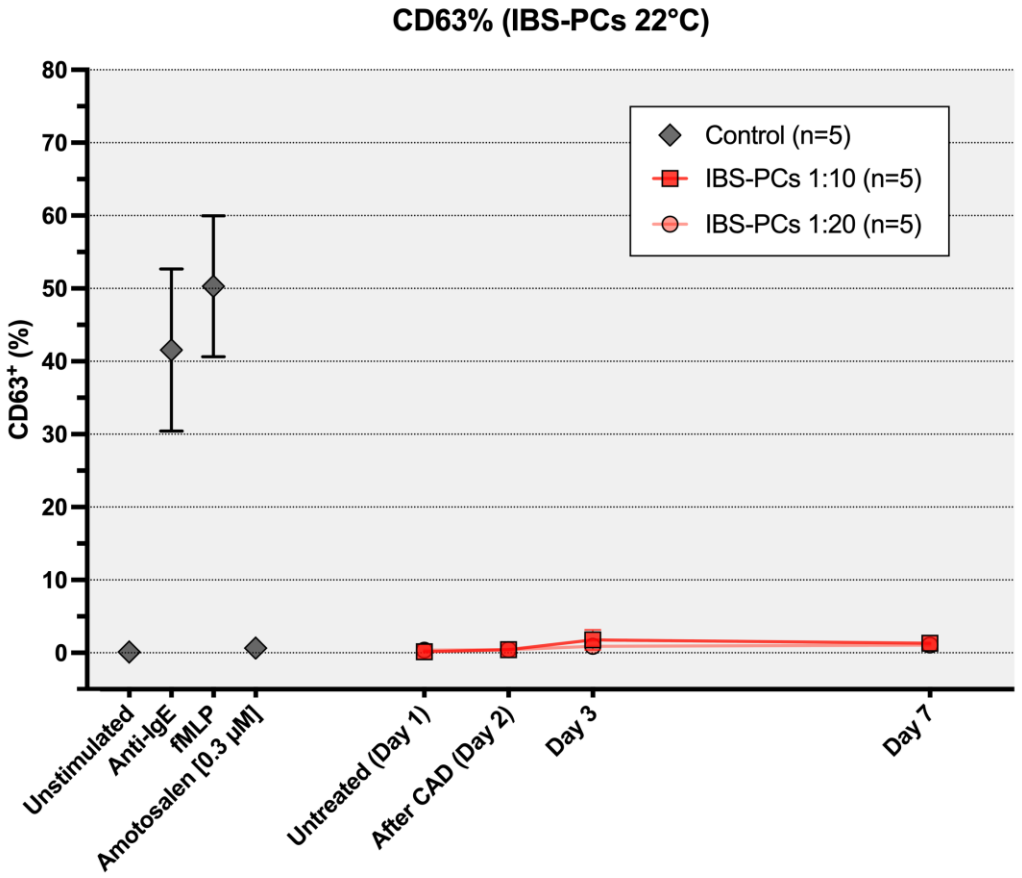
Buffy-coat platelet concentrates preparation



IBS-PCs at 22°C

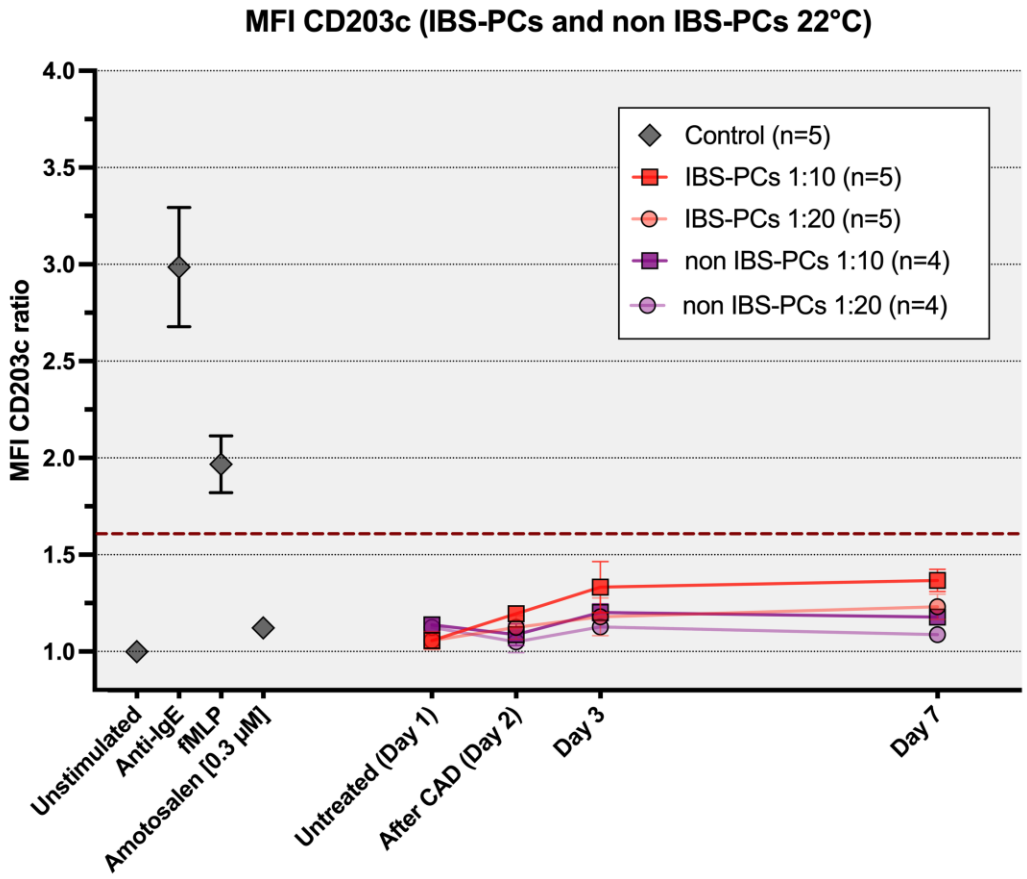
(1:10 = 2APCs or 3 BC-PCs)

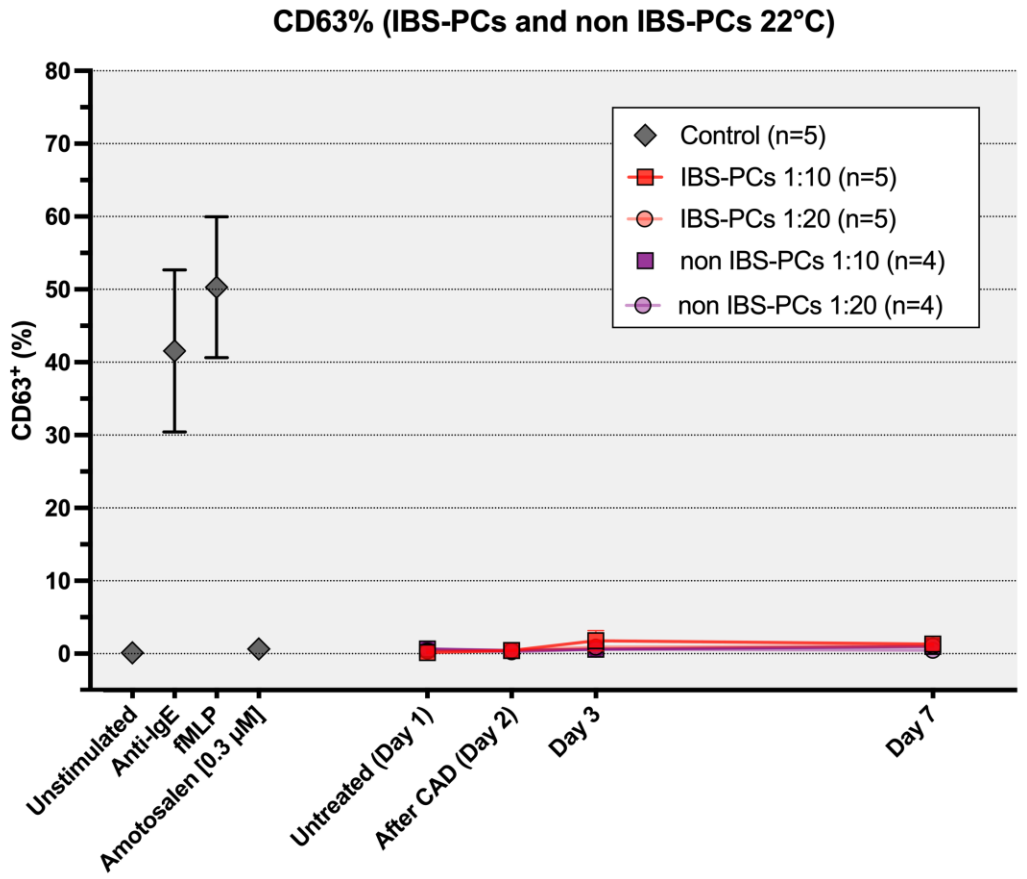




Non-significant increase in CD203c ratio over time

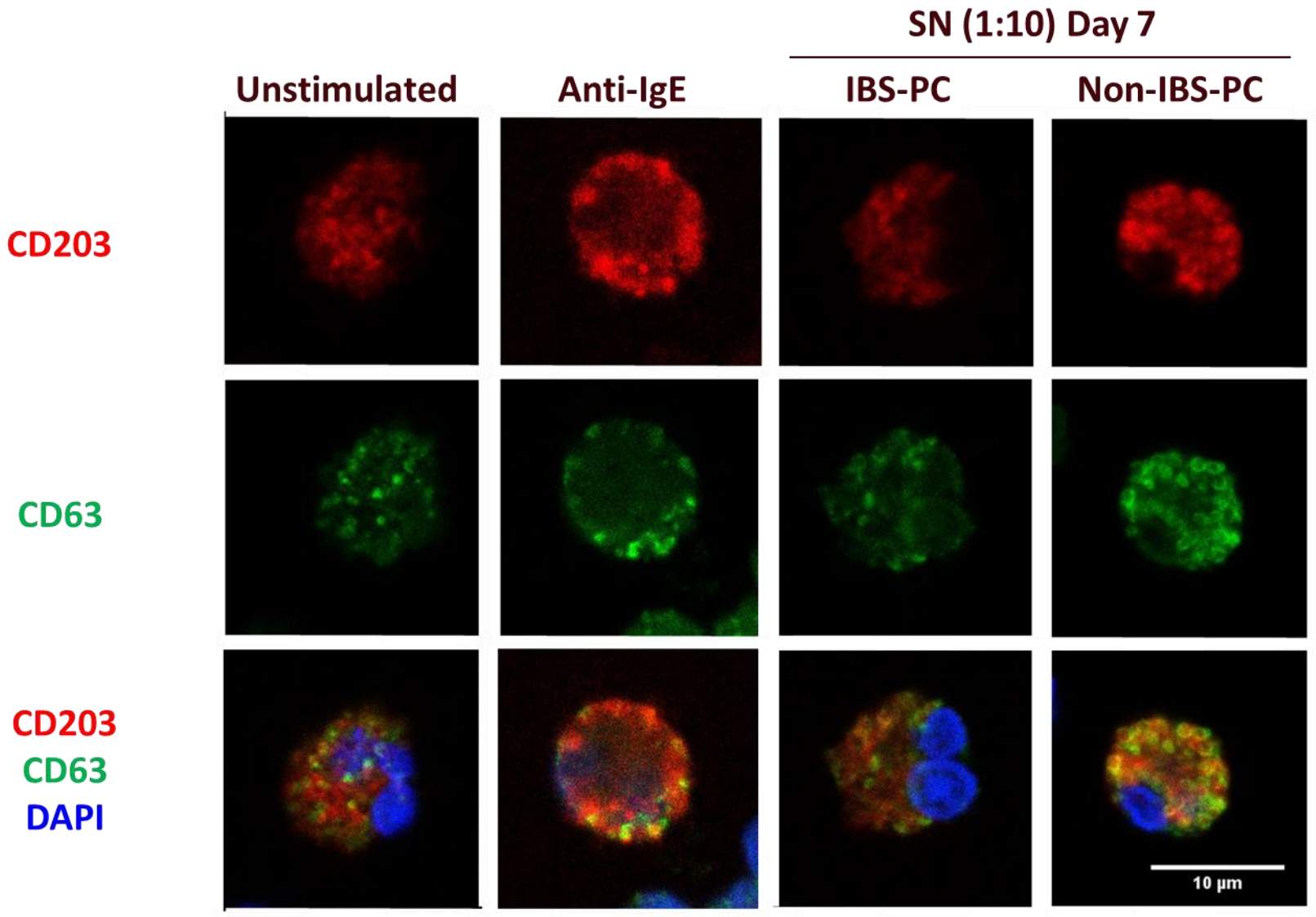
Combined IBS-PCs and non-IBS-PC at 22°C





Non-significant basophil activation (CD203c ratio) is also observed in non-IBS-PCs

Confocal images of basophils



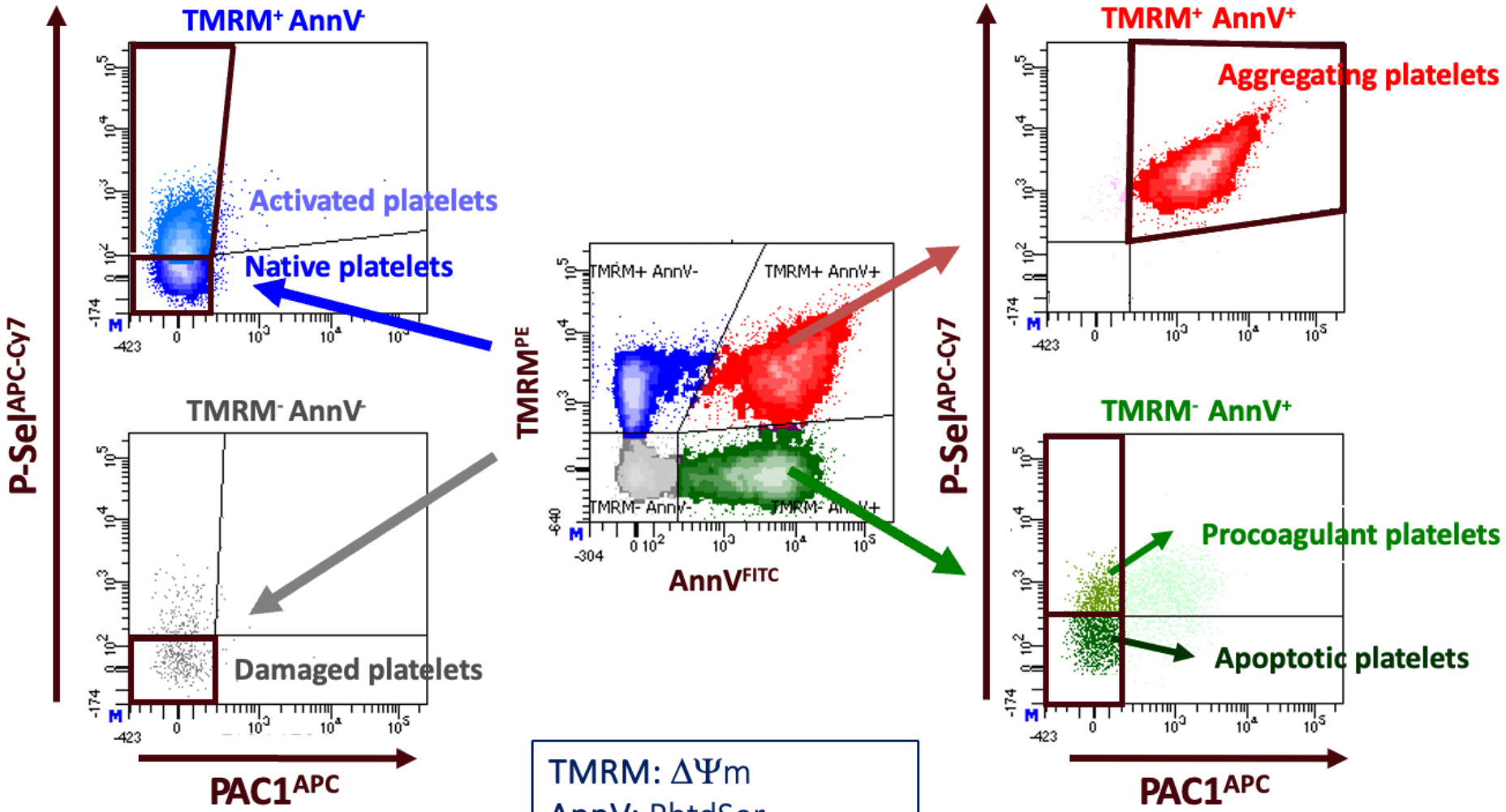
Summary for BC-PCs

- **Basophil CD63 is not exposed in response to BC-PC milieu**
- **CD203c ratio increases over time**
  - Only at the dilution 1:10 (equivalent to 3 BC-PCs or 2 APCs)
  - Also present in non-IBS-PCs
  - Below the threshold for allergy diagnosis (ratio 1.6-1.8)

➔ **Could storage lesions be involved?**

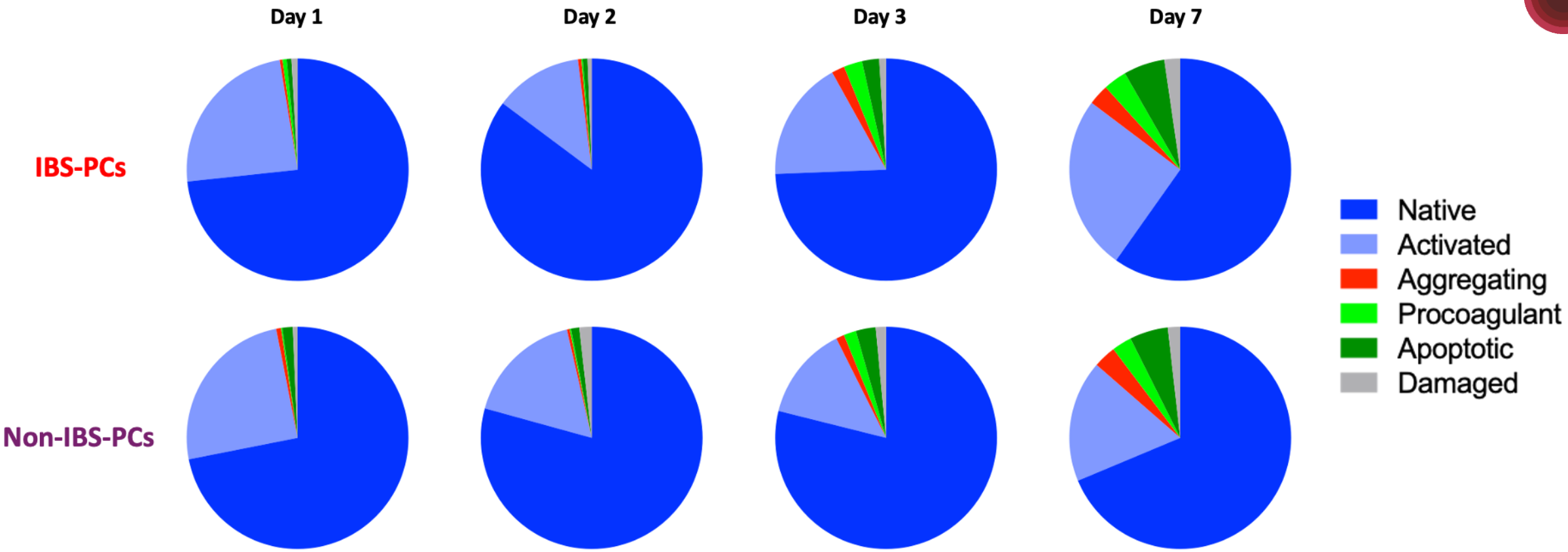
  - Change in platelet phenotype
  - Extracellular vesicles (EVs)
  - Soluble mediators

Platelet subpopulations in flow cytometry



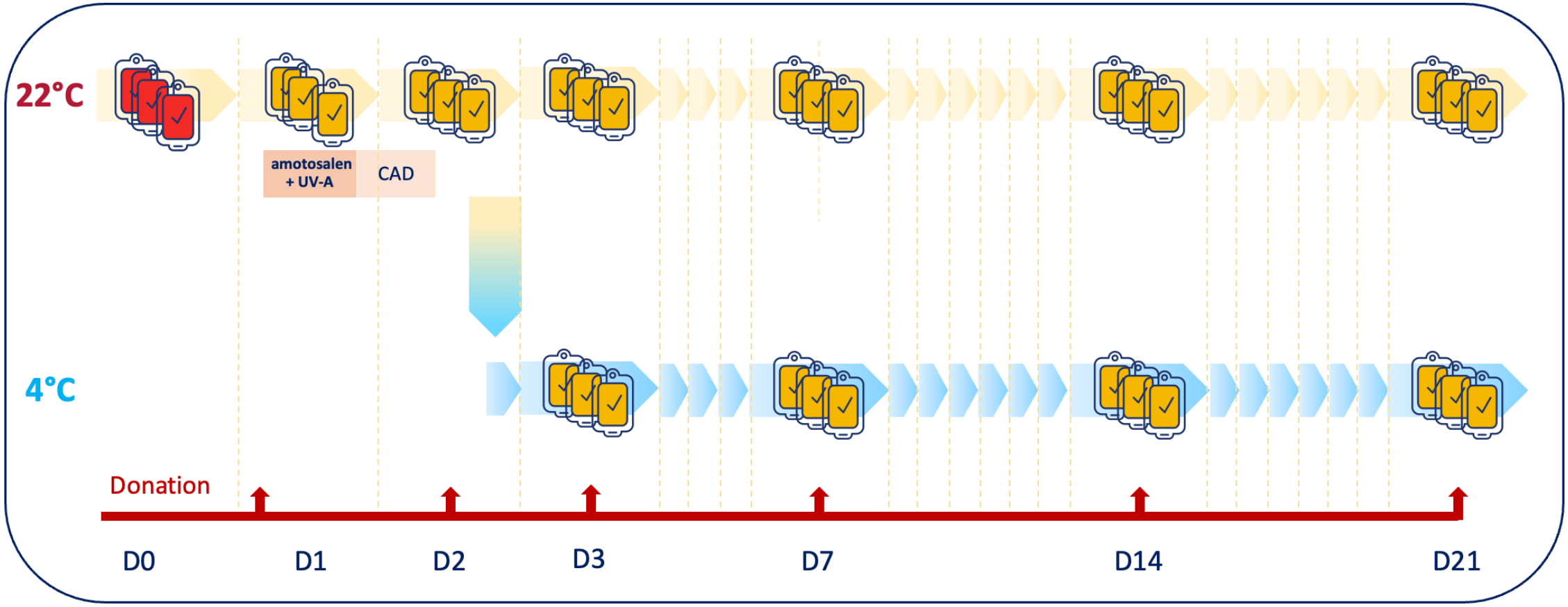
TMRM: $\Delta\Psi m$
AnnV: PhtdSer
Anti-CD62P: P-Sel
PAC1: activated αIIbβ3

Platelet subpopulations in IBS- and non-IBS-PCs

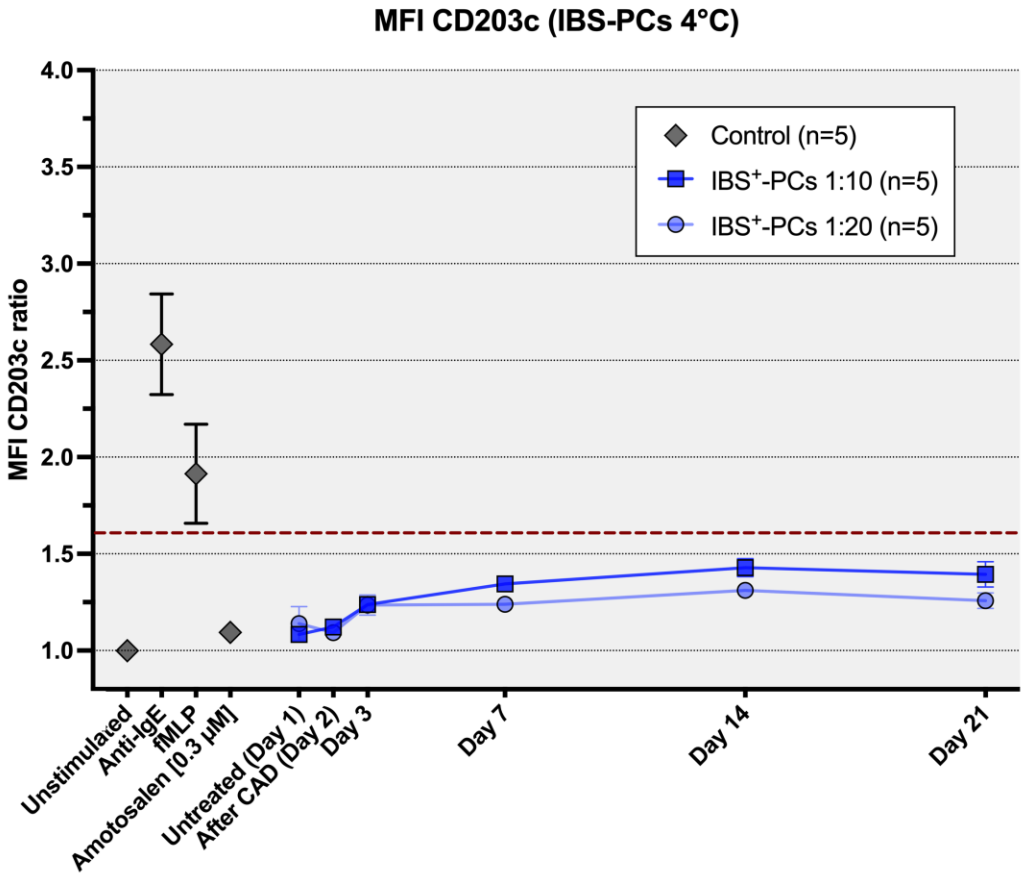


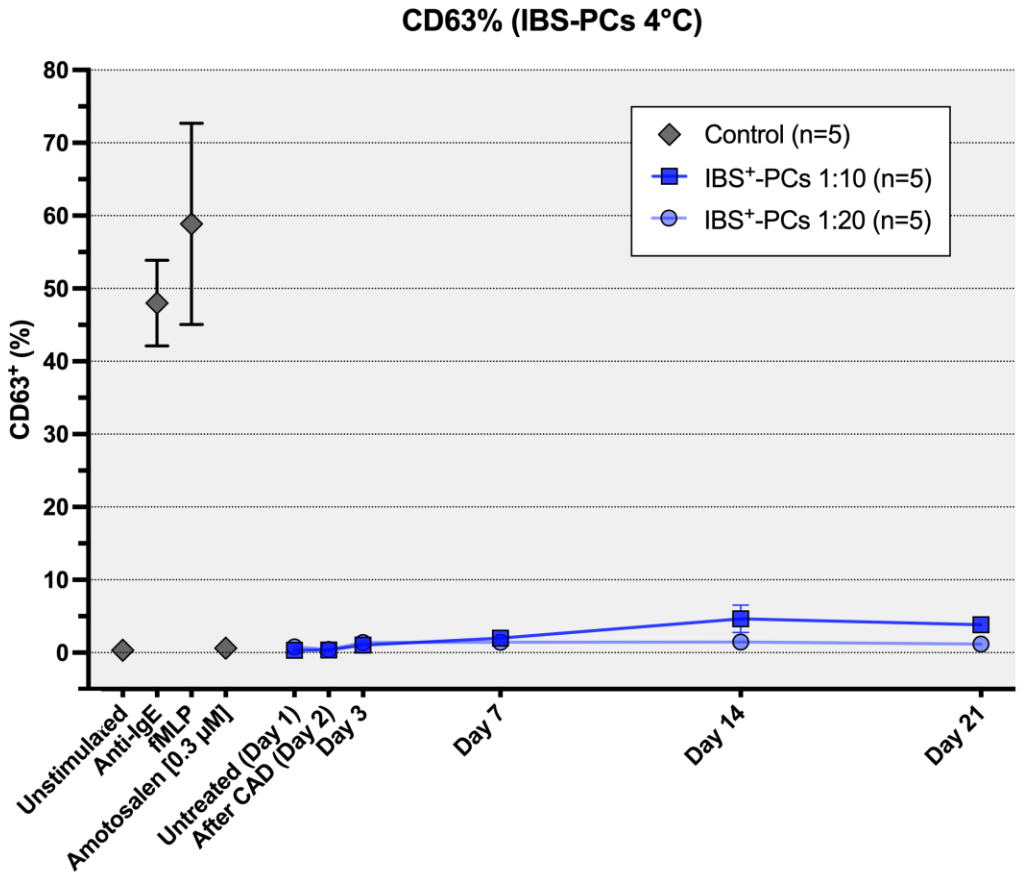
Amotosalen or photo-induced by-products do not modify platelet subpopulations

Cold-stored IBS-PC preparation (pool and split)



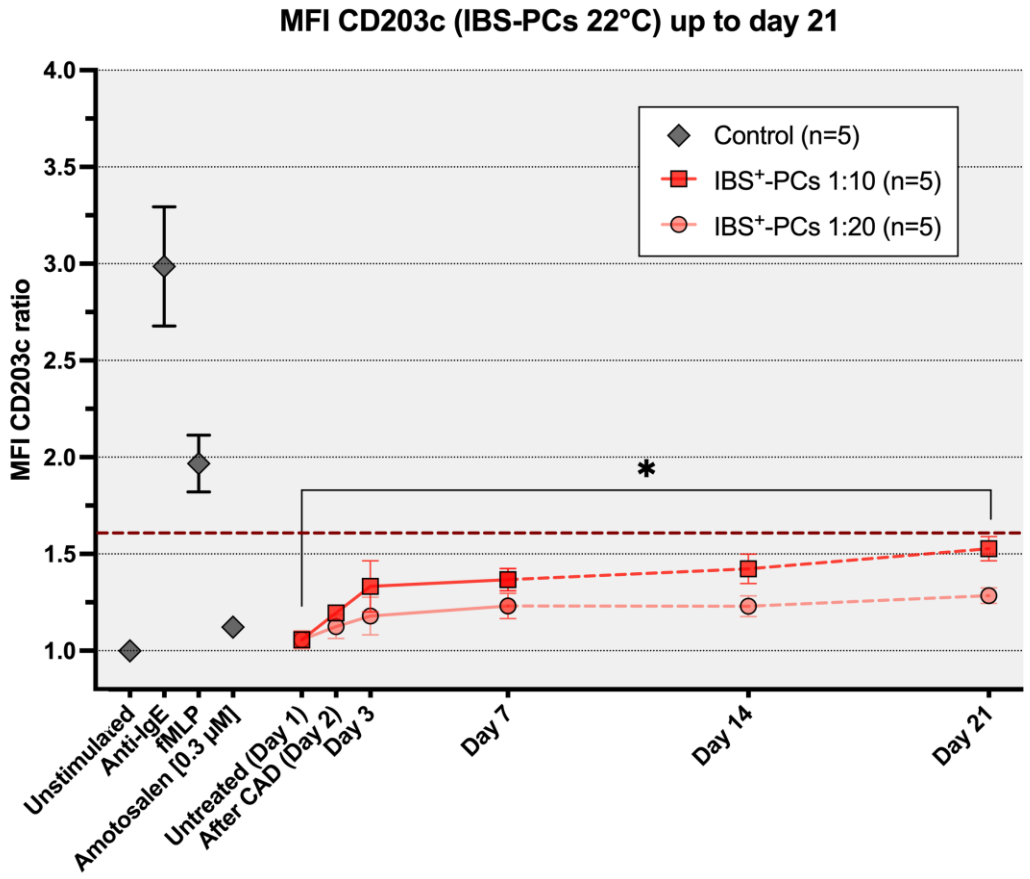
Cold-stored IBS-PCs up to day 21

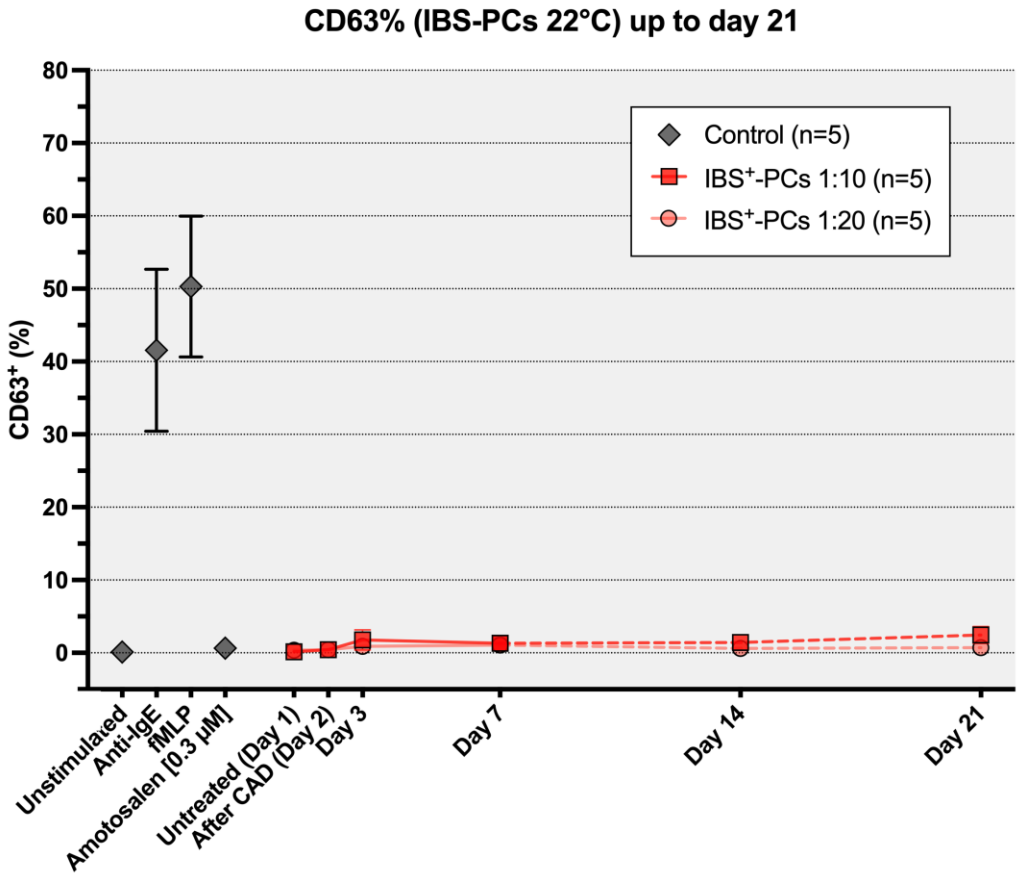




Non-significant increase of basophil activation (CD203c ratio)

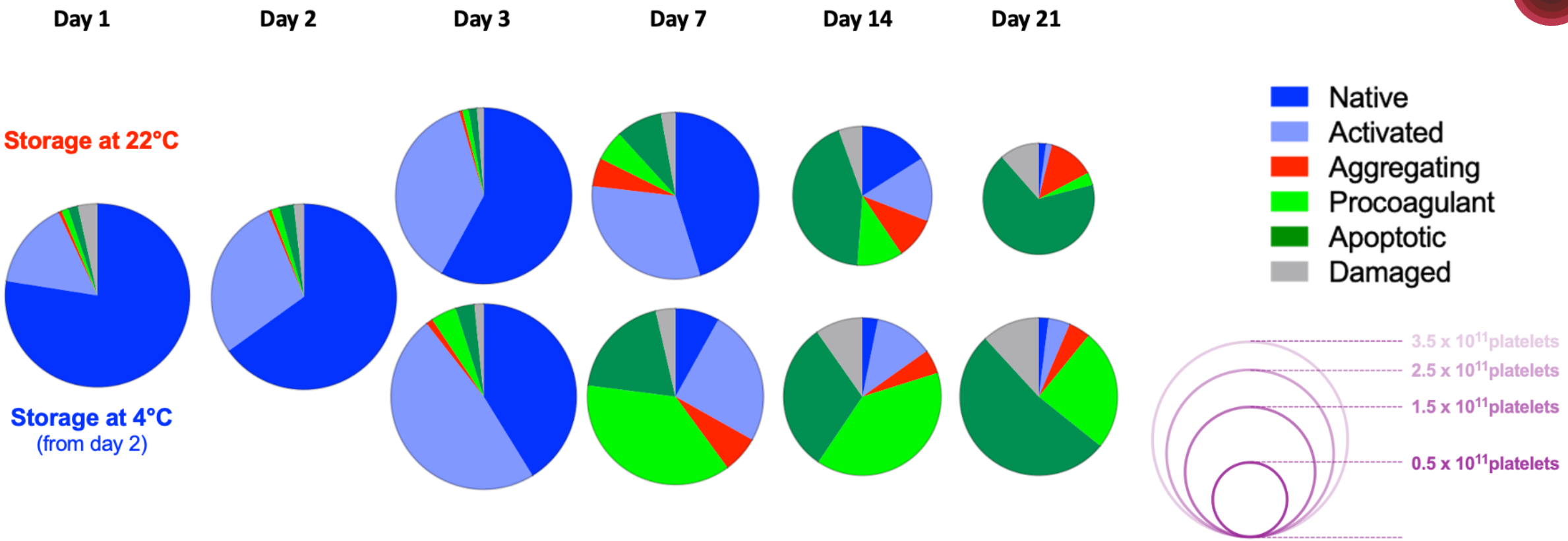
IBS-PCs up to day 21 at 22°C (as control)





Significant increase of basophil activation (CD203c ratio) only at D21
CD203c ratio remains below the threshold of hypersensitivity diagnosis

Cold stored IBS-PCs: platelet subpopulations



Phenotype changes are not responsible for basophil activation (CD203c)

Conclusion

- **Free amotosalen (up to 30 µM) does not induce basophil activation (non-immune pathway)**

- **Fresh frozen plasma (FFPs): free amotosalen or photo-induced by-products do not induce basophil activation**

Conclusion

- **Buffy-coat platelet concentrate (BC-PC) milieu:**
  - Does not induce CD63 exposure (correlated to histamine release)
  - Does not induce CD203c exposure up to day 7 at 22°C
  - Does not induce CD203c exposure up to day 21 at 4°C

➔ **BC-PC milieu tends to increase CD203c exposure at high concentration (1:10) independently of amotosalen or photo-induced by-products**

  - Independent of platelet subpopulations
  - May involve storage lesions

Clinical study (spring 2025)

- **Patient recruitment**
  - HS reaction in the four hours of IBS-PC transfusion
  - Two centres: Hôpitaux Universitaires de Strasbourg (HUS) and Institut de Cancérologie – Strasbourg Europe (ICANS)
  - One to three HS reactions per month (under-reported) ➔ 40 to 50 per year
- **Allergy clinic**
  - Past medical history including allergy and transfusion
- **Basophil activation test**
  - Controls: anti-IgE and fMPL
  - Tests:
    - Free amotosalen (0.0003 to 30 µM)
    - Three IBS-PCs (blood group A) milieu (1:10) at day 2, 5 and 7

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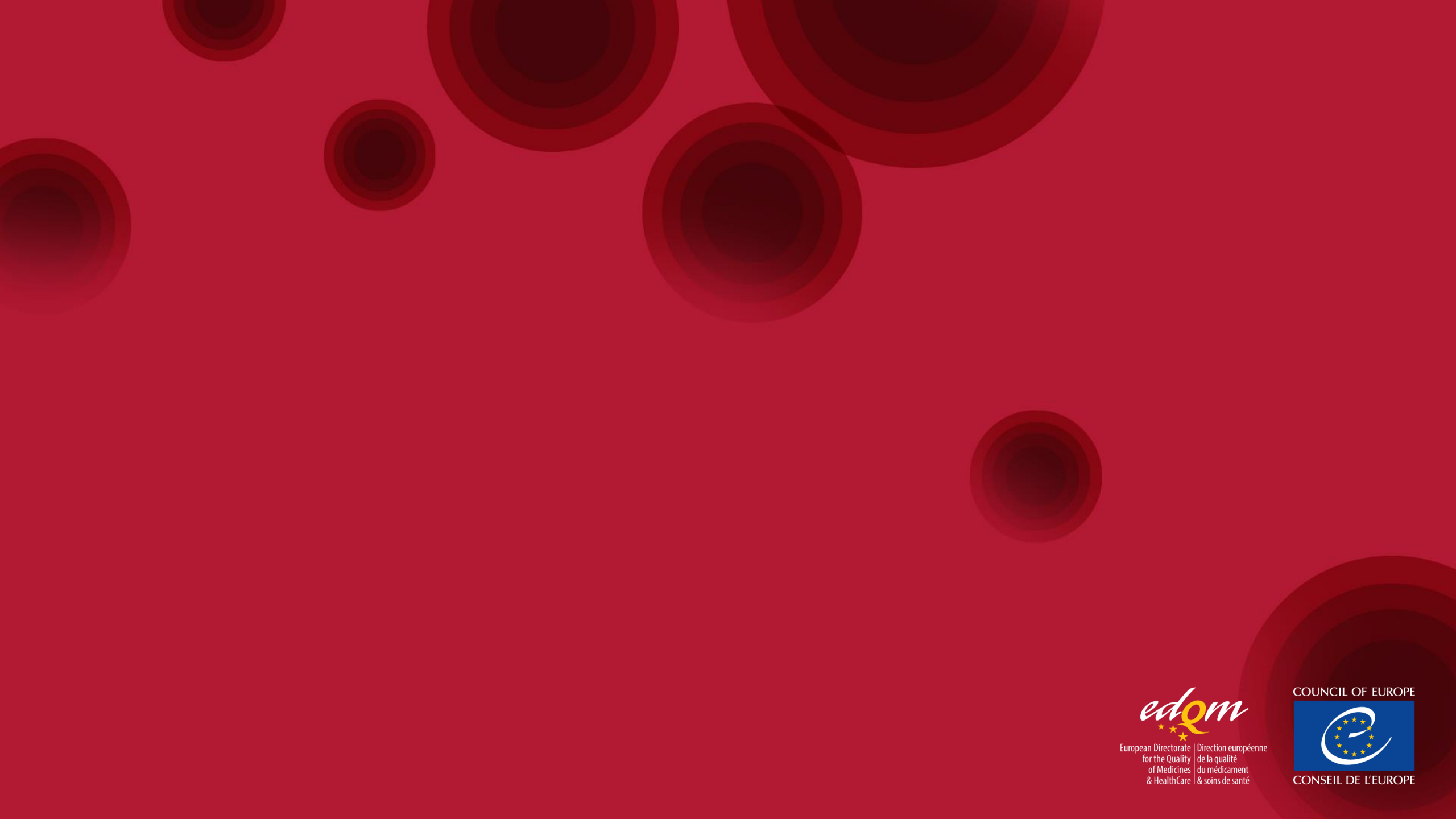
Adeline Galvanin

Hervé Isola

Paul-Michel Mertes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