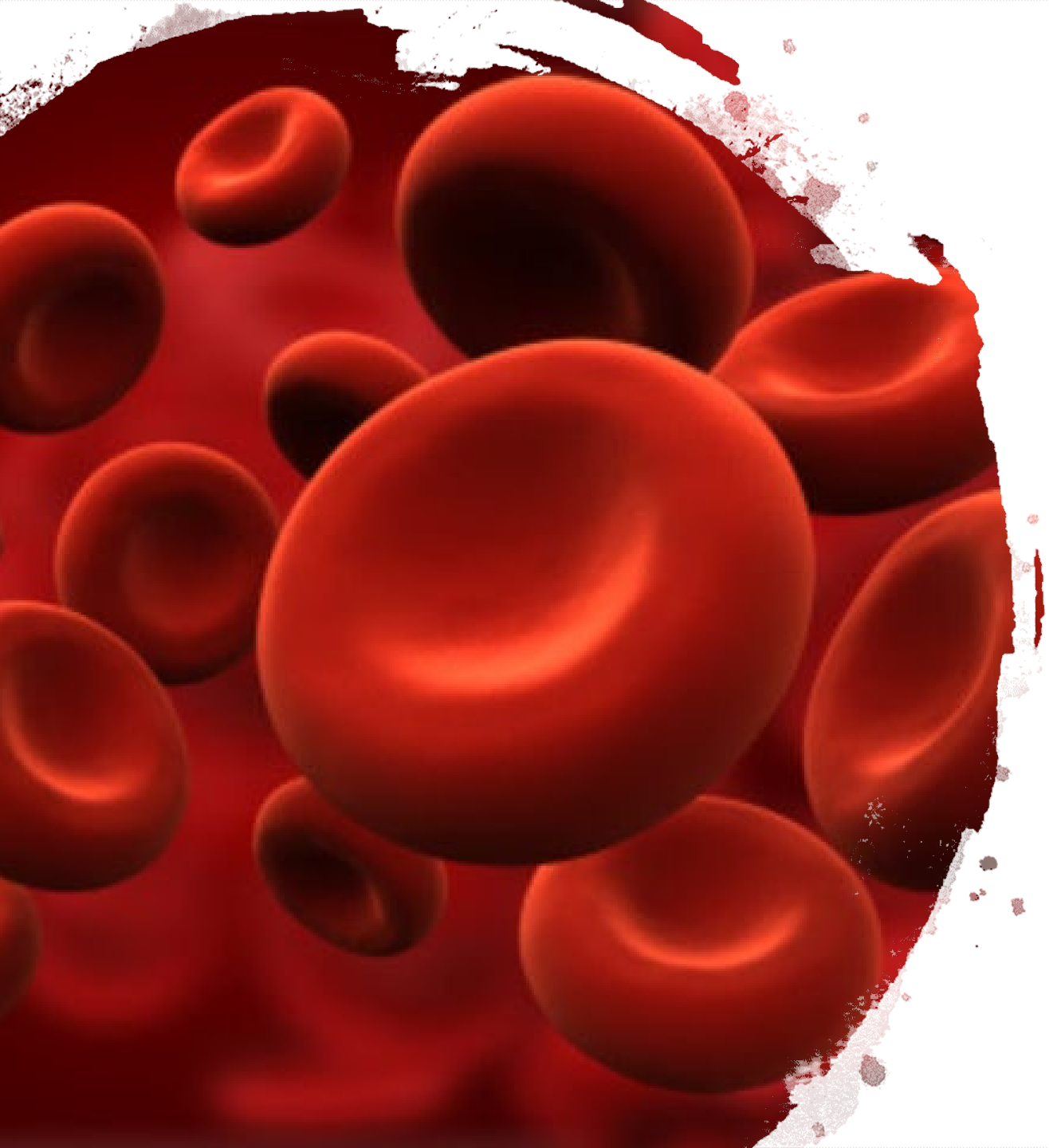


Dr Nicola Cogan

Bristol Institute for Transfusion Science
NHSBT Filton

Cultured Red Cells from R&D to GMP

BBTS 2019

Research – Cultured red cells

Blood transfusion challenges

- Alloimmunization
- Iron overload
- Infection

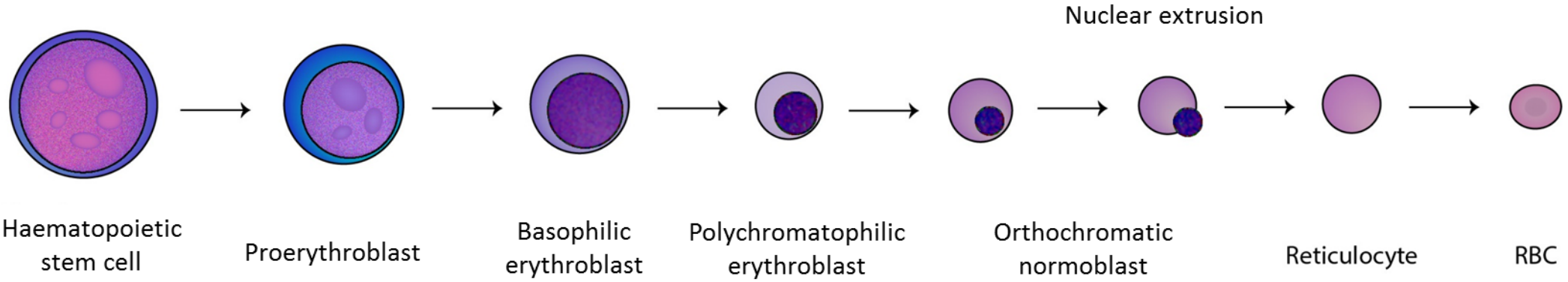
Cultured RBC therapeutic use

- Rare blood group phenotypes
- Haemoglobinopathies

RESTORE Clinical trial

- Compare recovery and survival
- Cultured vs standard RBC

Erythropoiesis – Making a red cell



Bone marrow

Maturation of erythroid precursors and iron uptake

Peripheral blood

RBC lifespan 120 days

Early studies of erythropoiesis

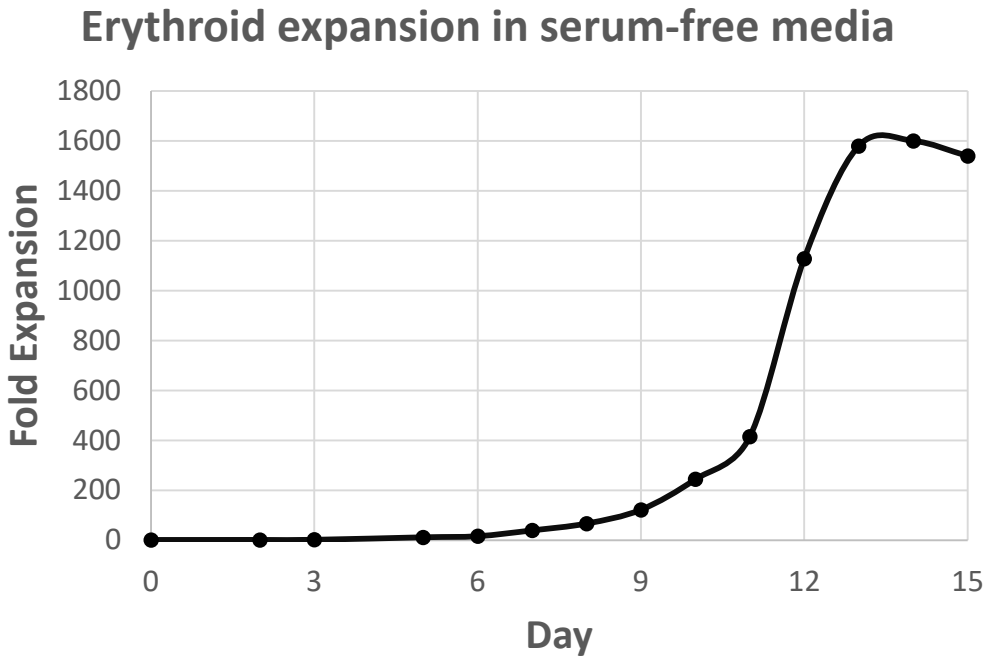
Separation of spleen colony forming units (CEU-S) from mouse bone marrow cells.
Pretlow *et al* Am. J. Pathol. 1973

Erythroid colony formation in cultures of mouse and human bone marrow: analysis of the requirement for erythropoietin.
Iscove *et al* J. Cell Physiol. 1974

Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture.
Iscove *et al* Exp. Hematol. 1975

Human cells in liquid culture described in 1980s. **Fibach *et al* Blood. 1989**

2-Stage culture process



x 1000

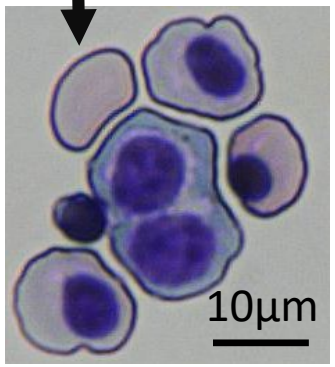
days

Stage 1: Serum-free media + SCF, IL3 and EPO

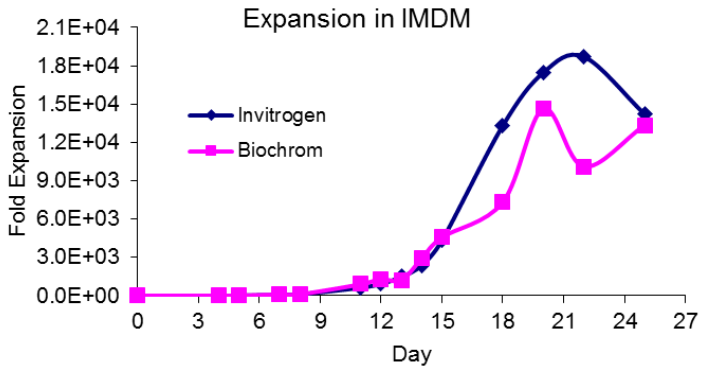
14

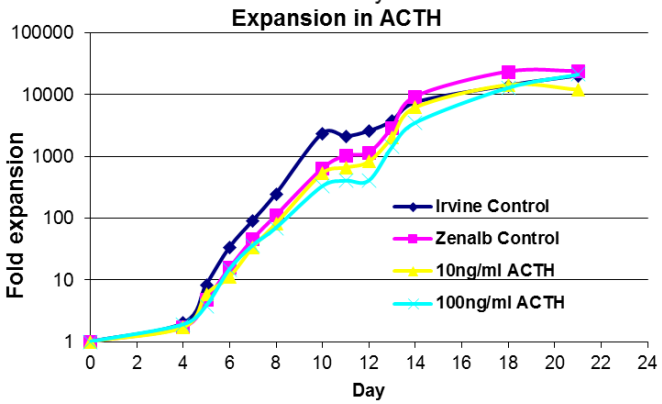
Stage 2: Serum, EPO, TfN, Insulin

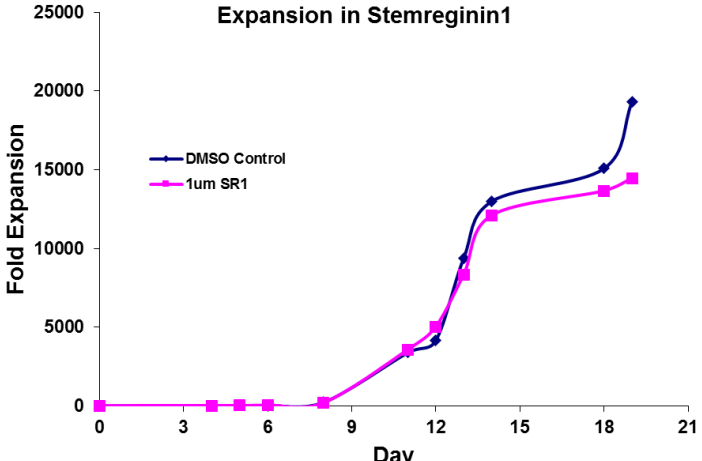
20% enucleation

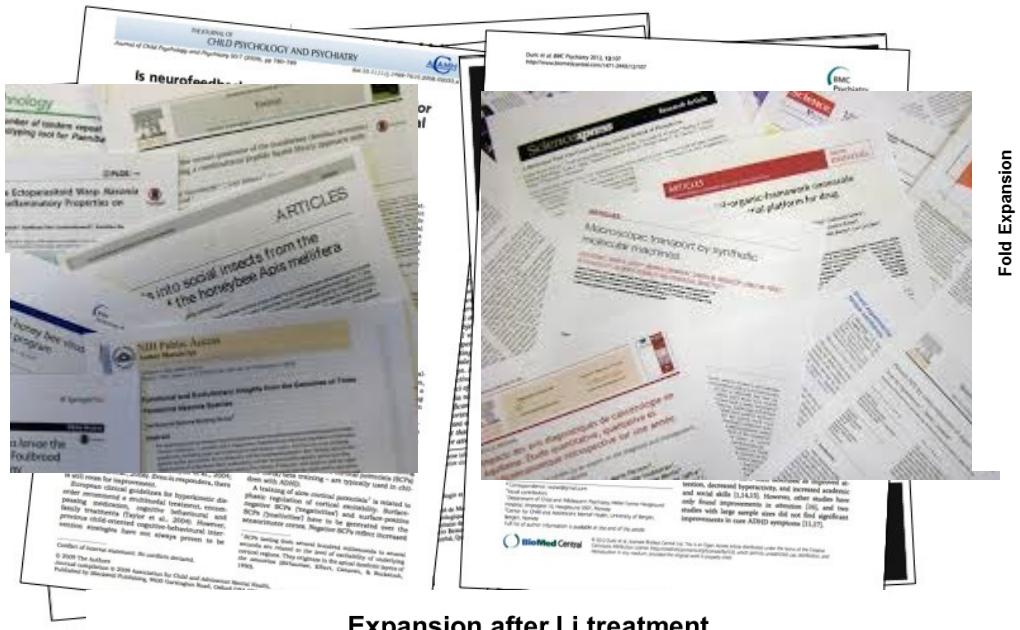


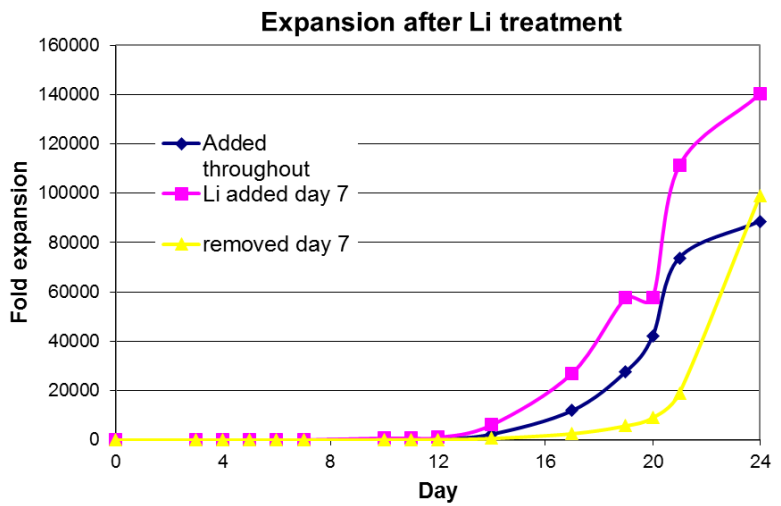
Optimizing culture conditions

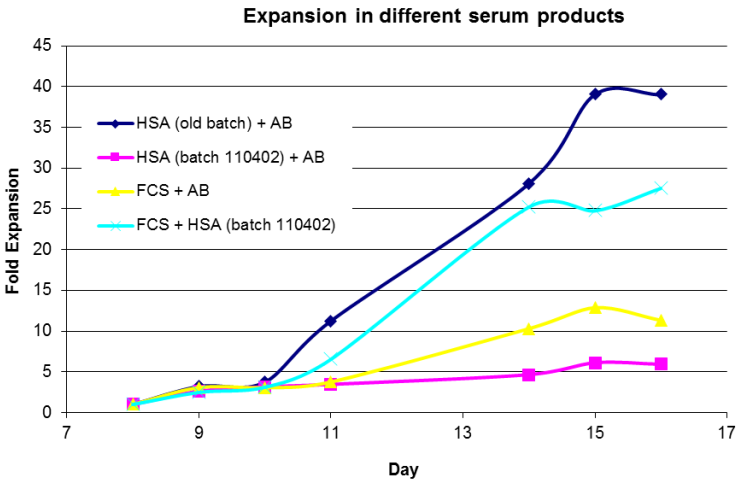


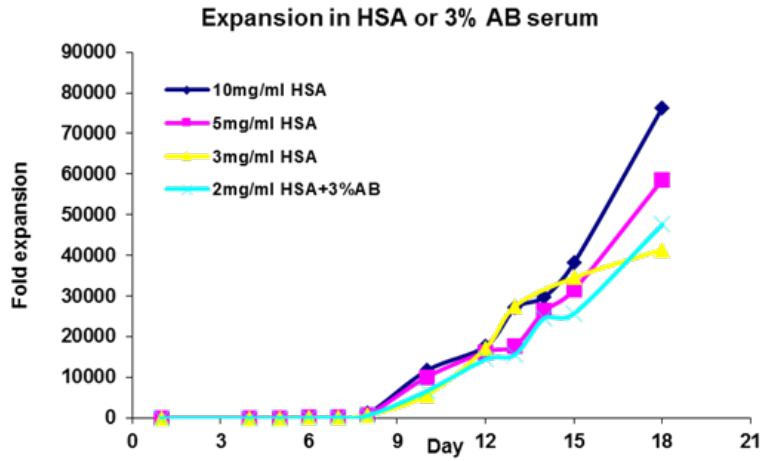




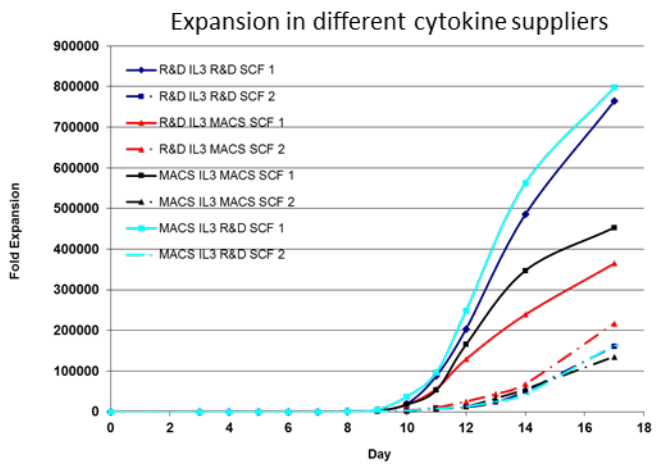


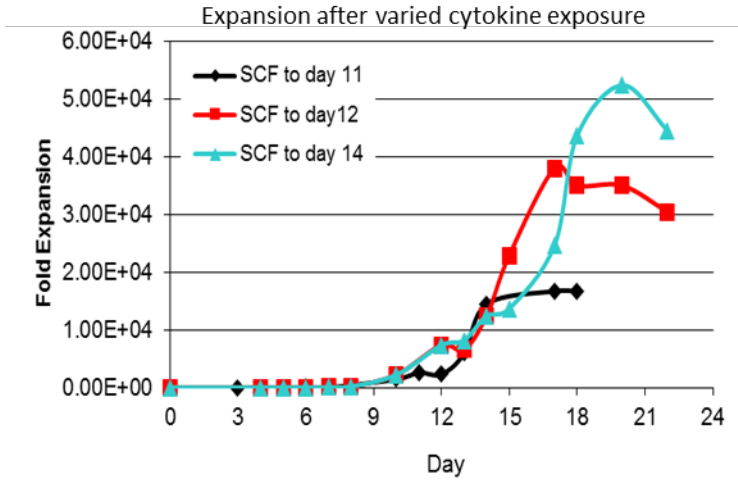


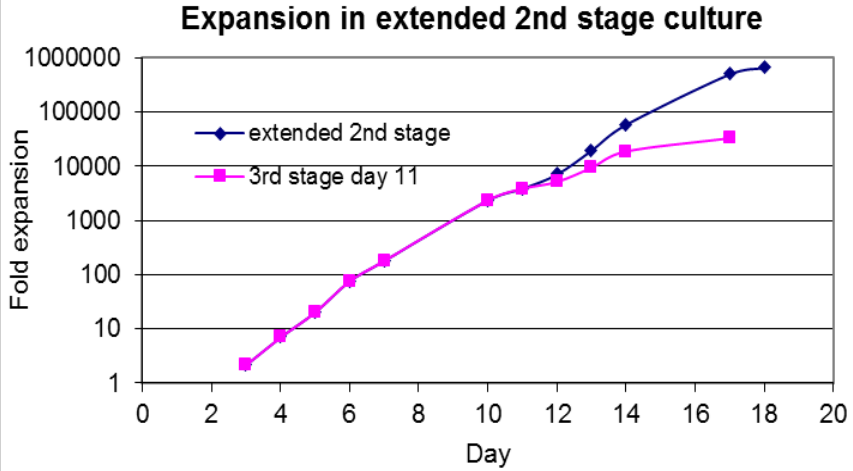


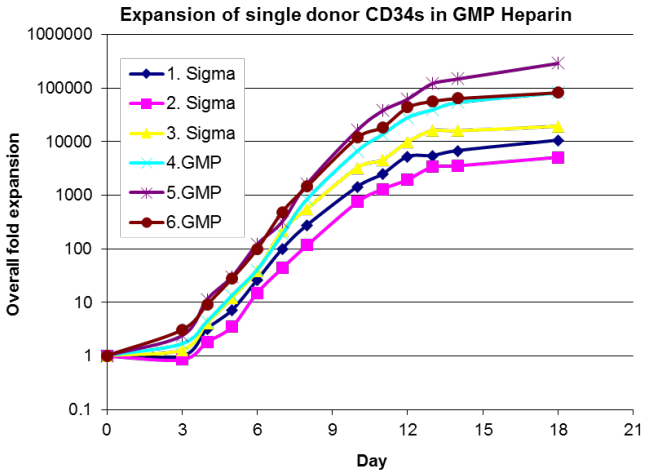


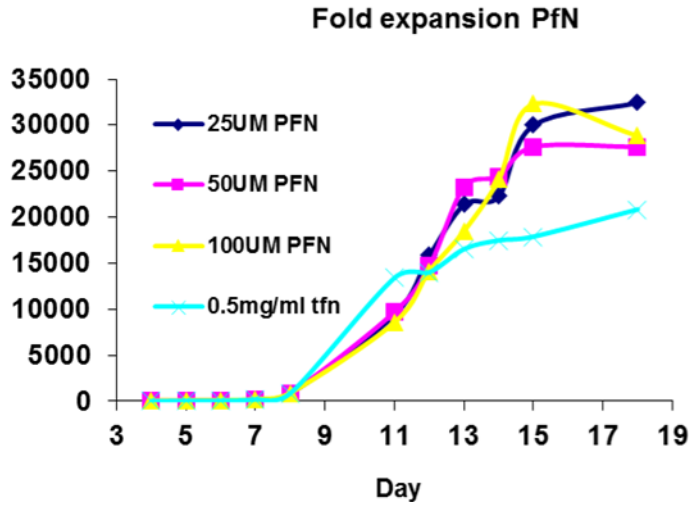
Optimizing culture conditions

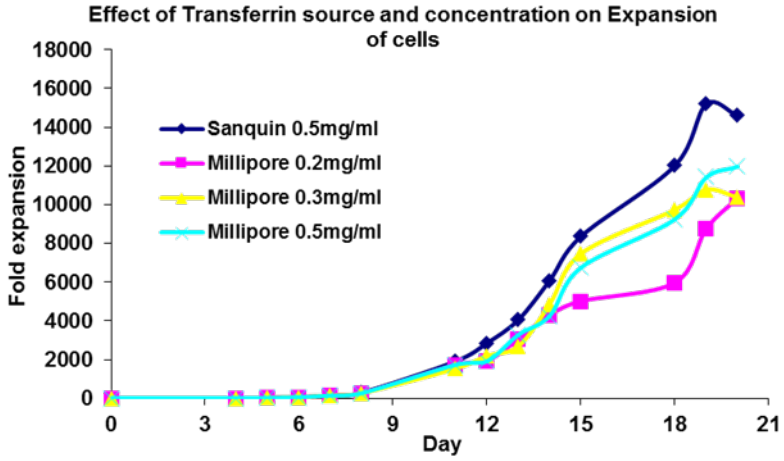




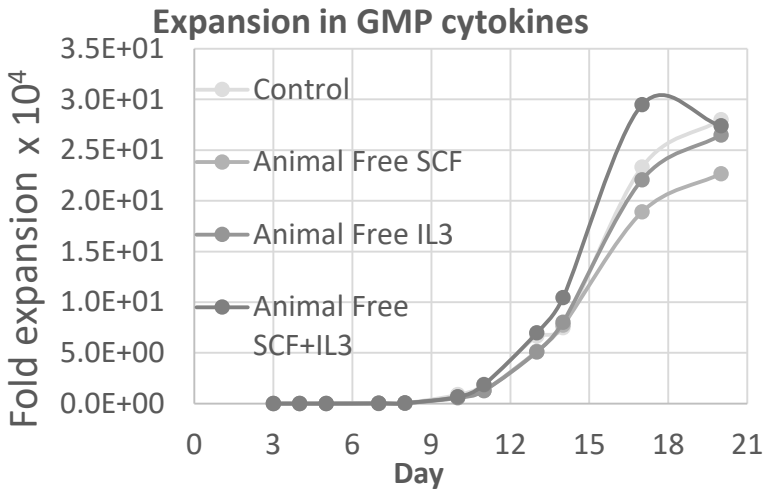


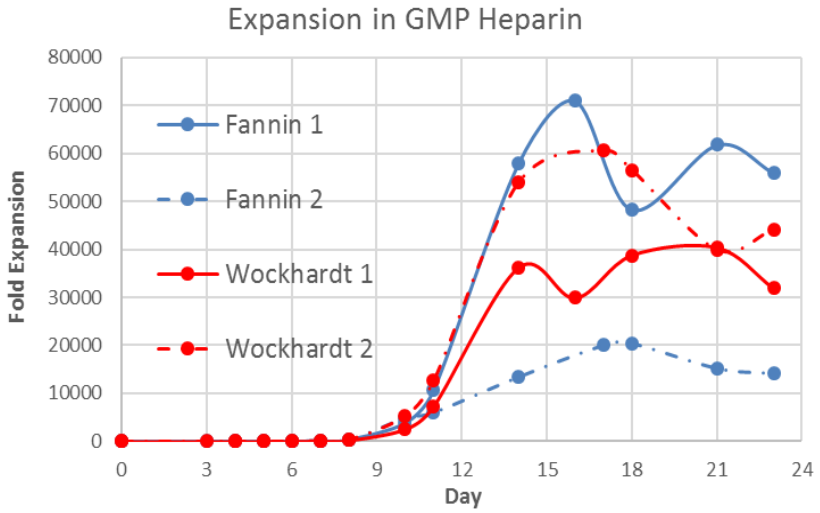


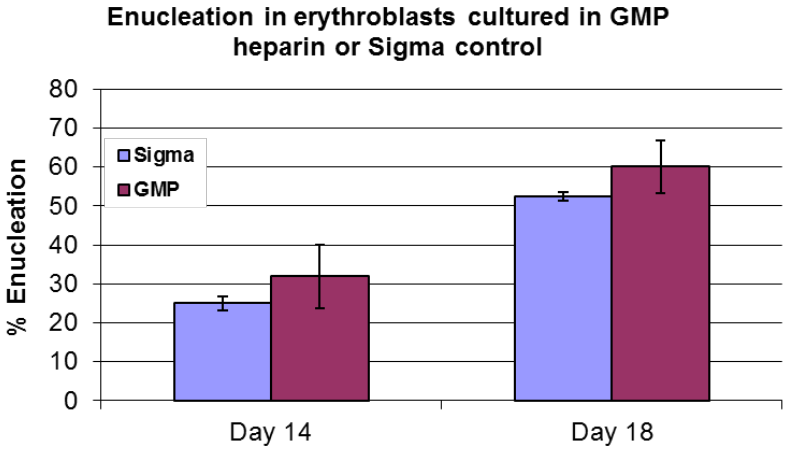


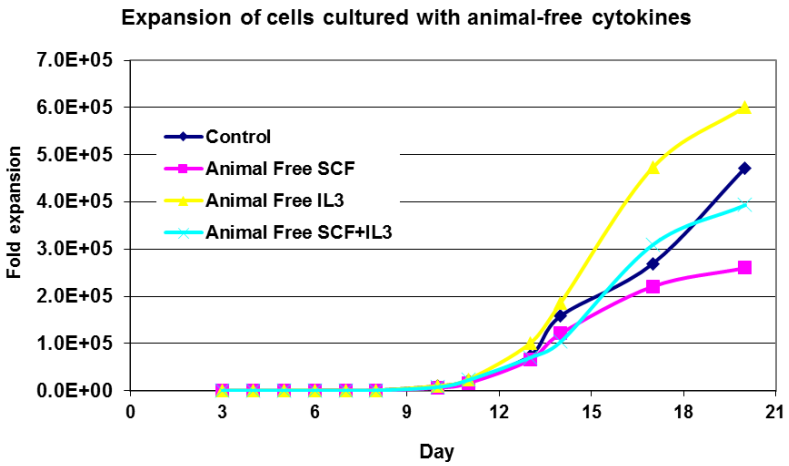


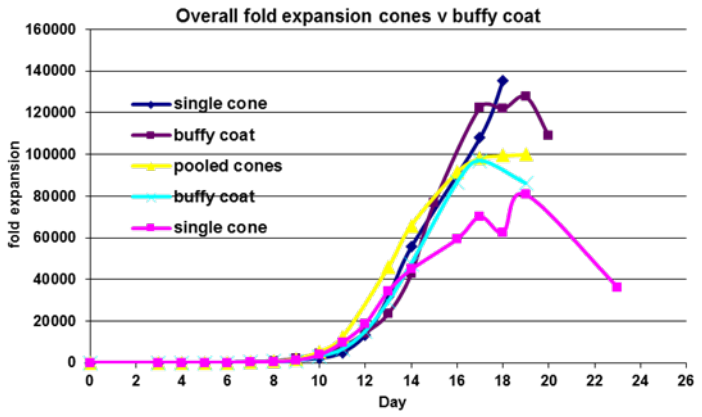
Optimizing culture conditions

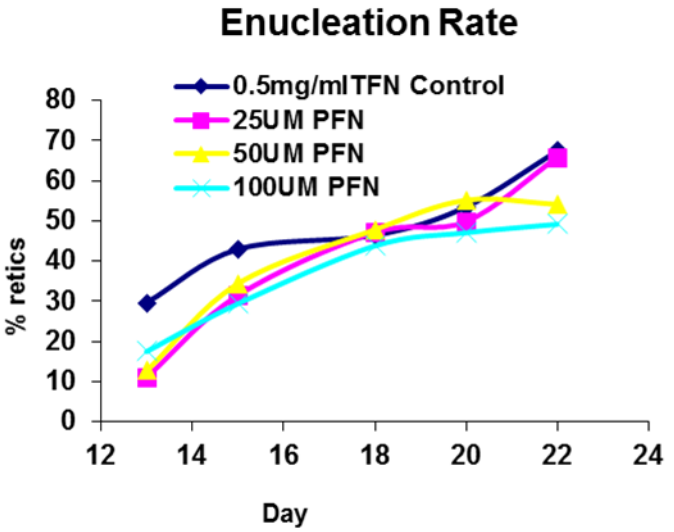












3-Stage culture process

Red Blood Cell Generation From Human Induced Pluripotent Stem Cells: Perspectives For Transfusion Medicine

Hélène Lapillonne, Ladan Kobari, Christelle Mazurier, Philippe Tropel, Marie-Catherine Giarratana, Isabelle Zanella-Cleon, Laurent Kiger, Marie Wattenhofer-Donzé, Hélène Puccio, Nicolas Hebert, Alain Francina, Georges Andreu, Stéphane Viville, Luc Douay

Haematologica October 2010 95: 1651-1659; **Doi:**10.3324/haematol.2010.023556

21 days

Stage 1
SCF
IL3
EPO
Transferrin

IMDM+glutamax, AB serum, HSA, insulin & heparin

Stage 2
SCF
EPO
Transferrin

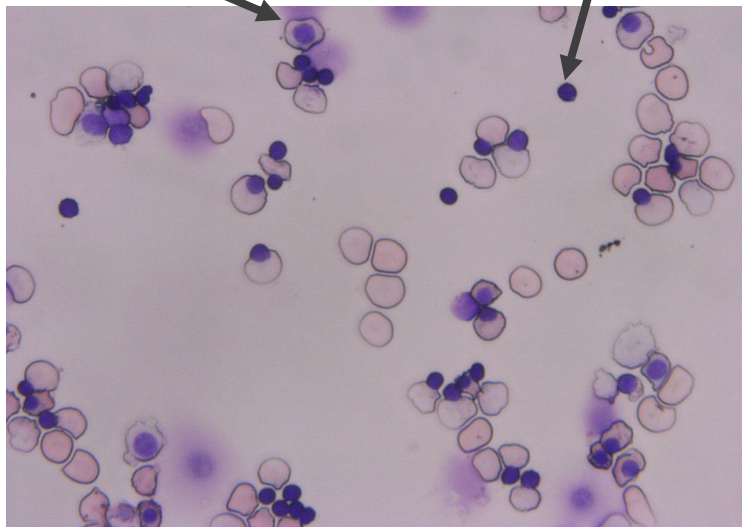
Stage 3
EPO
Transferrin

$X > 10^4$

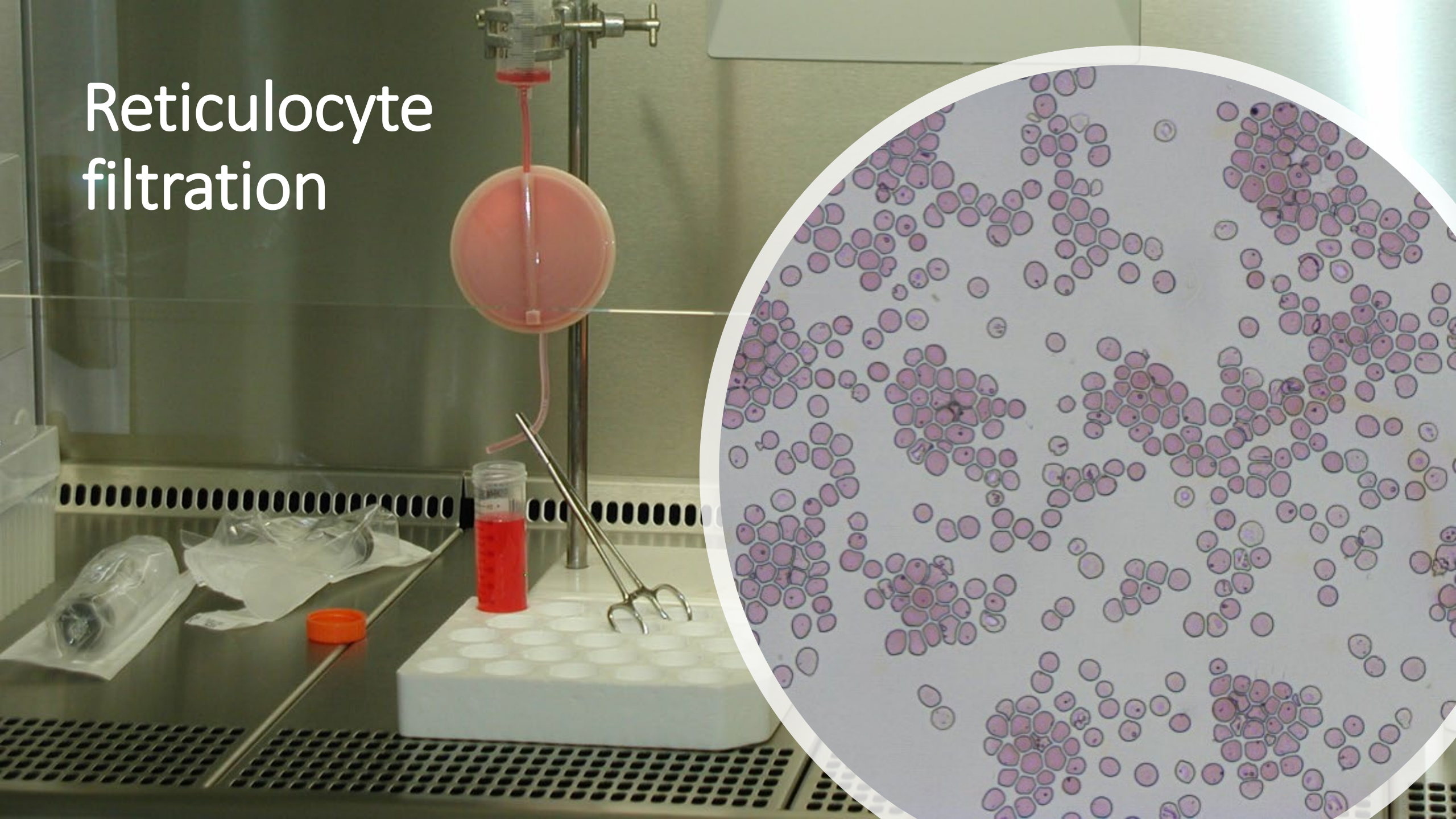
Residual nucleated cells

Up to 90% enucleation

Free nuclei

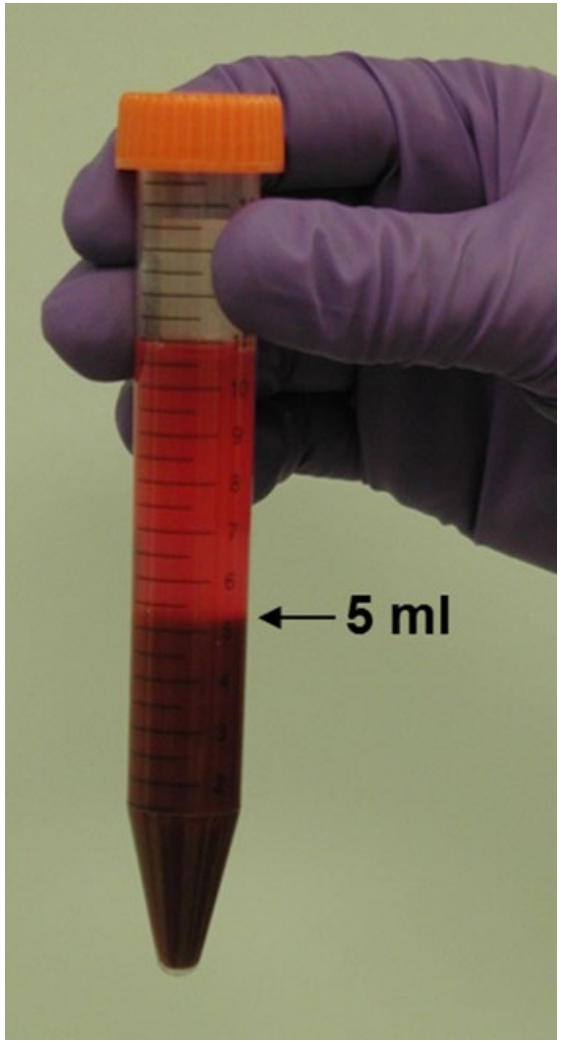


Reticulocyte filtration

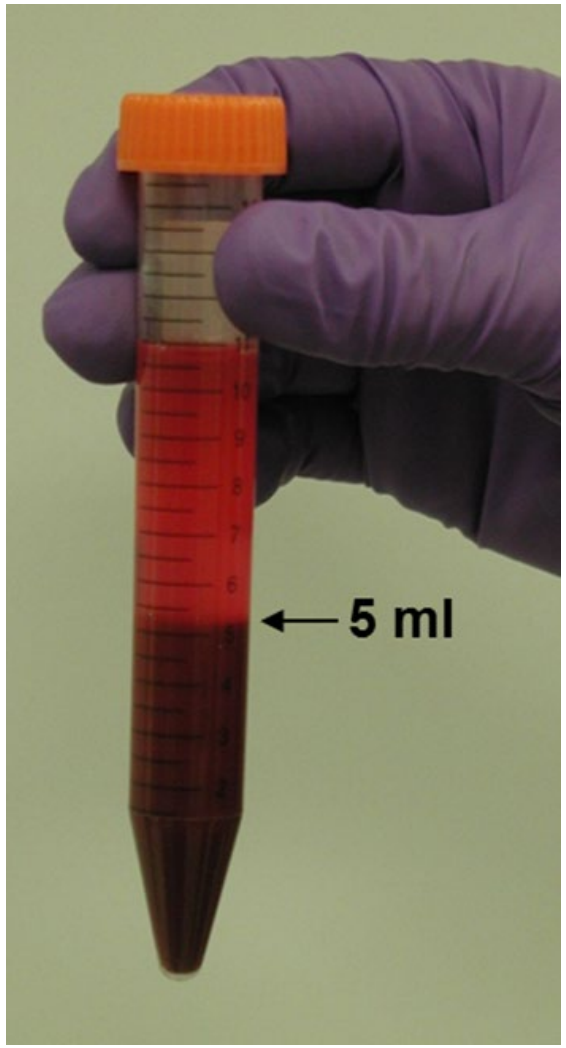


Volume reduction

- 5ml packed pellet pure reticulocytes
- In-man trial feasible
- <u>GMP</u>



Good manufacturing practice (GMP)



Medicines & Healthcare products Regulatory Agency

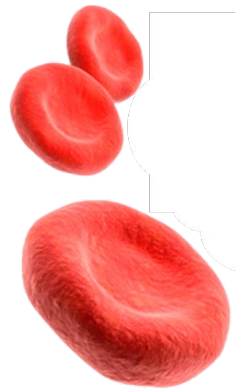
- Guidelines, standards and regulations
- Ensure quality and safety
- Covers every step of the process

- Raw materials
- Manufacturing
- Testing
- Shipping
- Storage

Modifications required for GMP compliance

Issues with current process:

- Open CD34+ isolation
- Untraceable starting material
- Reusable glass culture vessels
- Open filtration process
- Open volume reduction
- 18 hour harvest step

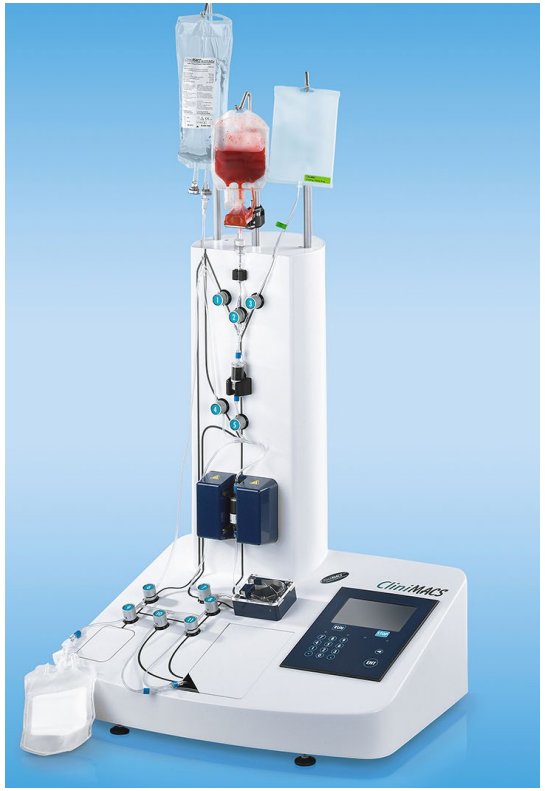


CD34^{+} isolation method

Apheresis cone

Open magnetic bead separation

Whole blood

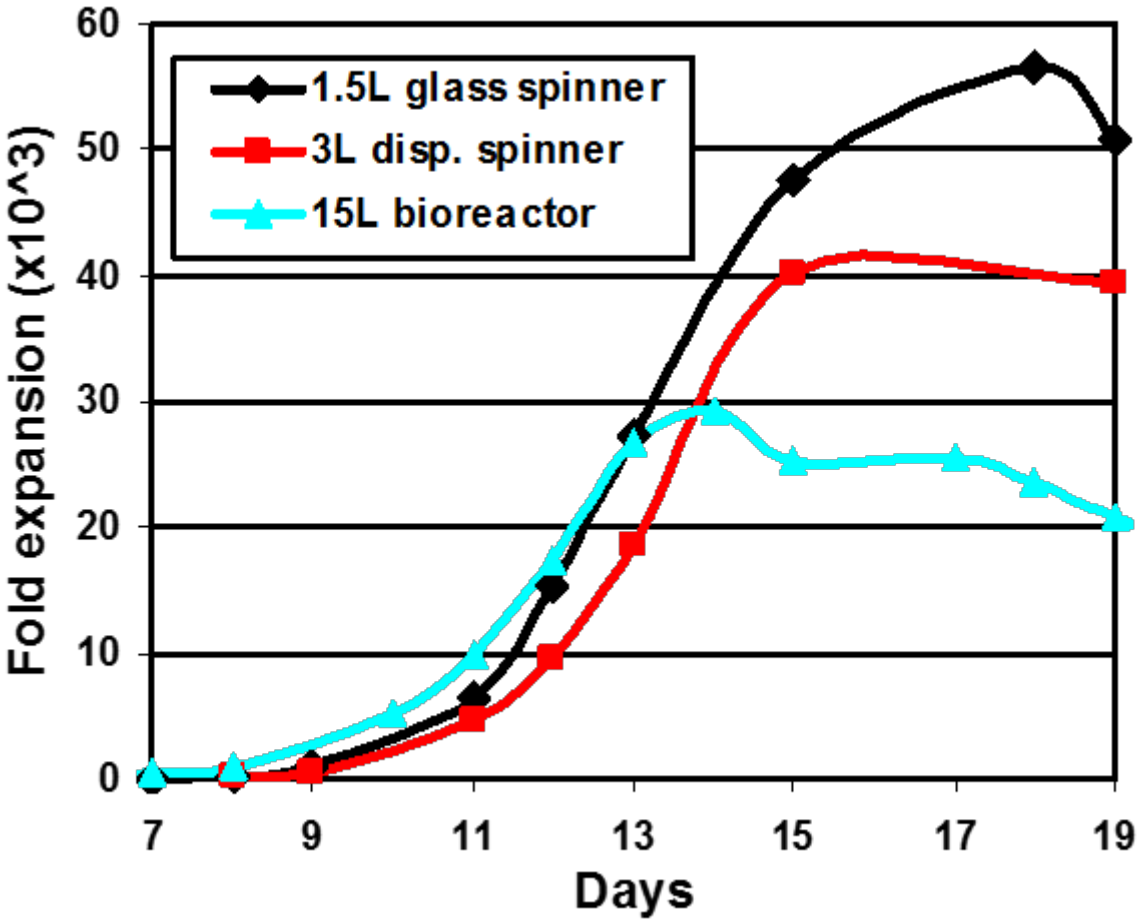


CliniMACS

Identification of culture vessel

1.5L reusable glass spinner

2x 15L bioreactors

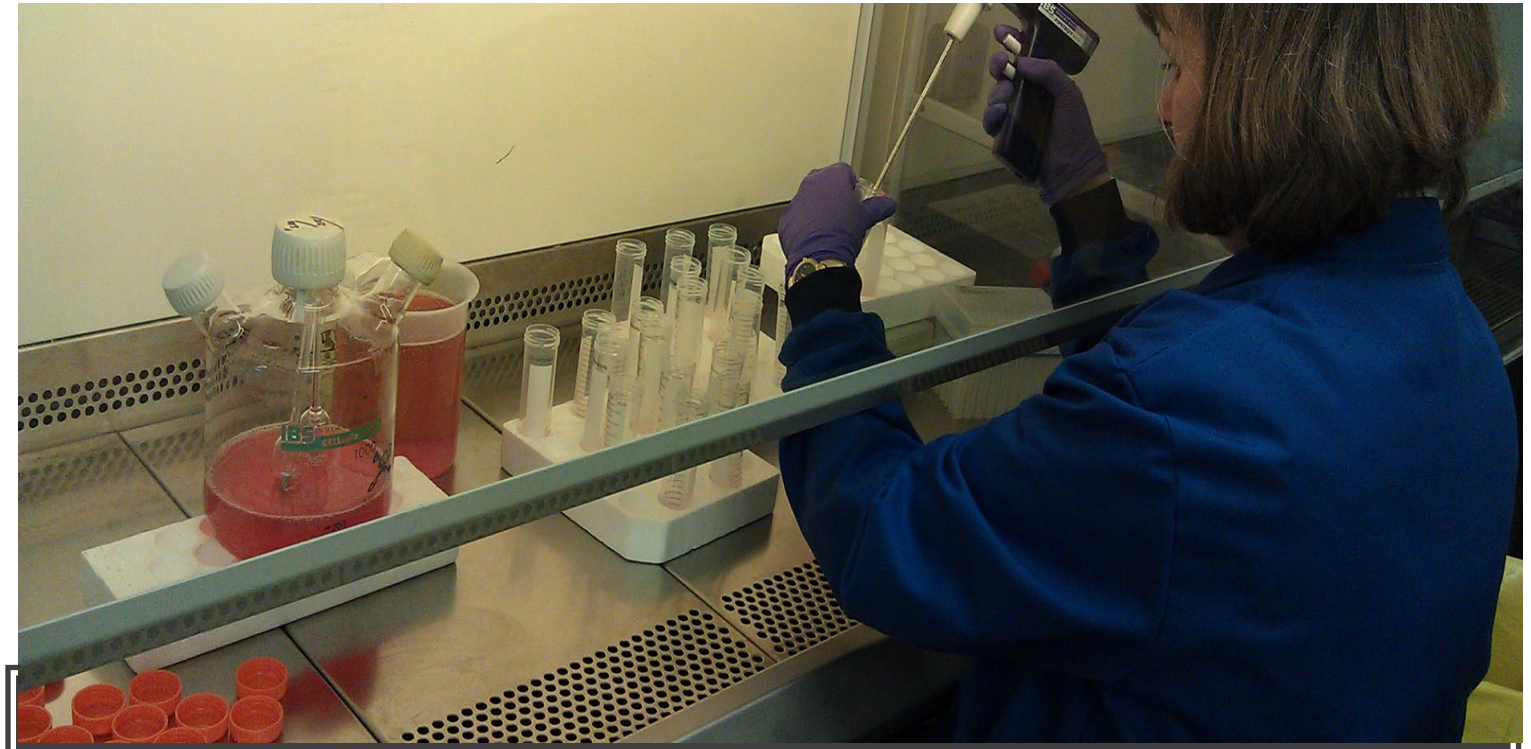


3L disposable spinner

Reticulocyte filtration

Time: 91 Minutes
Recovery: 66%

Time: 49 Minutes
Recovery: 60%



Volume reduction

Cell Saver Elite operation

Waste bag for culture supernatant

Concentrated in centrifuge bowl

Filtered reticulocytes

Optimizing the Cell Saver Elite

Fill speed ml/min	100	200	250	300	500
% Cells lost to waste fraction	0.3	1.7	5.2	6.4	47

150ml/min

Default setting

- GMP process trialled in the R&D lab
- Significant improvements in yield and harvest duration
- ATU: Successful engineering and validation runs
- Regulatory approvals in place

RESTORE Clinical trial

Recovery and Survival of Stem Cell Originated Red Cells

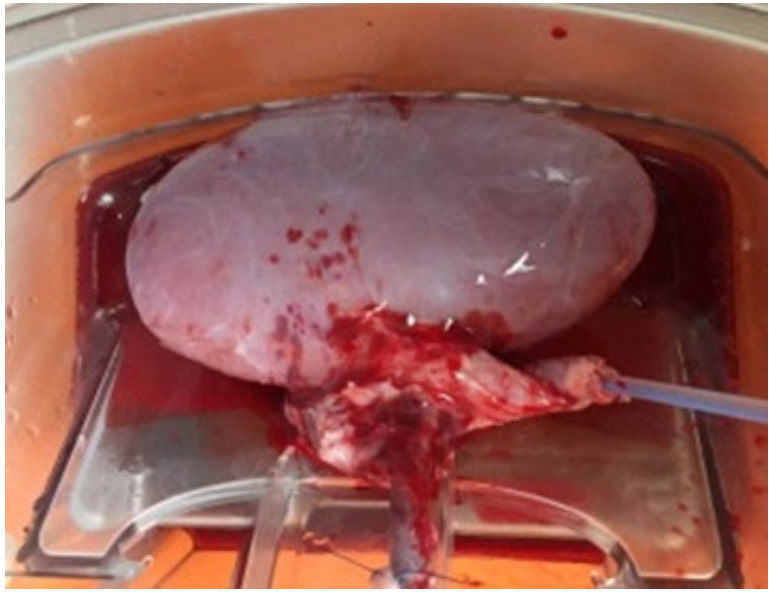
Aim: Assess the recovery and survival of a mini-dose of red blood cells derived from $CD34^{+}$cells isolated from adult blood vs standard donated red blood cells

In a standard blood transfusion, 20-25% of the cells are cleared from circulation within the first 24 hours

Cultured RBCs may have longer survival in the circulation of the volunteers than the standard donated RBCs because they are all new nascent cells with a 120 day lifespan

Increasing the yield

- Further changes to culture process, cytokines and growth factors – improved progenitor cell expansion
- Improved reticulocyte maturation and purification
- Identifying donor factors
- Immortalized erythroid cell lines: BEL-A



Future perspectives

- Universal modifications
- Cytokine-independent expansion
- Enhanced RBCs for new therapeutics
- Organ reconditioning

Conclusions

- Largest quantities of cultured red cells reported
- Clinical trial
- Feasible transfusion therapy
- Other applications

Thank you

Partners

NIHR | Blood and Transplant Research Unit in Red Blood Cell Products at University of Bristol

NHS Blood and Transplant

The BTRU in Red Blood Cell Products is part of and funded by the NIHR and is a partnership between University of Bristol and NHSBT in collaboration with the University of Warwick, the University of Bath and the University of the West of England

University of BRISTOL

UNIVERSITY OF BATH

WARWICK
THE UNIVERSITY OF WARWICK

UWE BRISTOL
University of the West of England

The proposed Clinical Trial additionally will involve:

UNIVERSITY OF CAMBRIDGE

NIHR/Wellcome Trust
Cambridge Clinical Research Facility

Department of Nuclear Medicine
Addenbrooke's Hospital

This research is co-funded by NIHR and NHSBT

Funded by NIHR