

BBTS Annual  
Conference 2016

# The Generation of Red Cells from Stem Cells.

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Products  
University of Bristol

**BBTS Annual Conference 2016**

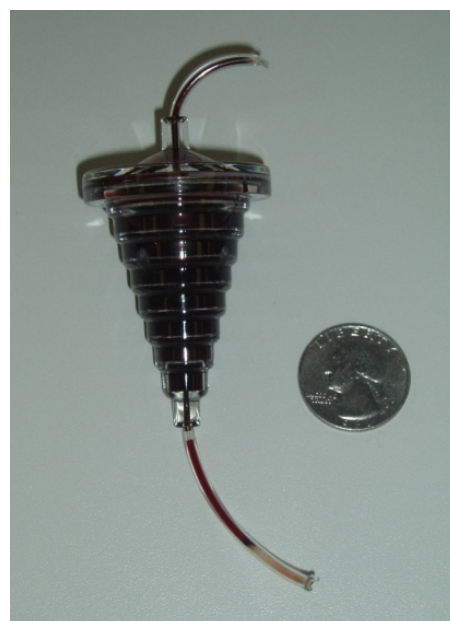
*21st - 23rd September*

# The process

$10^6$  CD34+ HPCs from a  
single apheresis cone, buffy  
coat or cord blood unit

21 days

Mature reticulocytes –  
leucocyte filtration



Multipotential  
haematopoietic stem  
cell

Proerythroblast

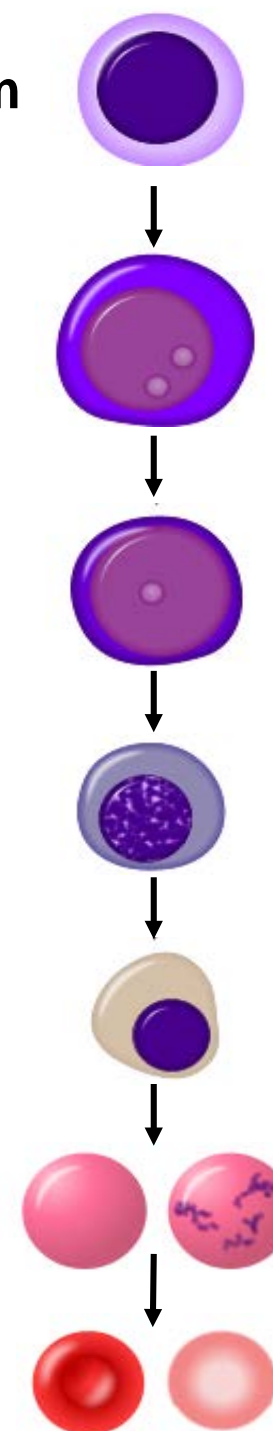
Basophilic  
erythroblast

Polychromatic  
erythroblast

Orthochromatic  
erythroblast

Reticulocyte

Erythrocyte



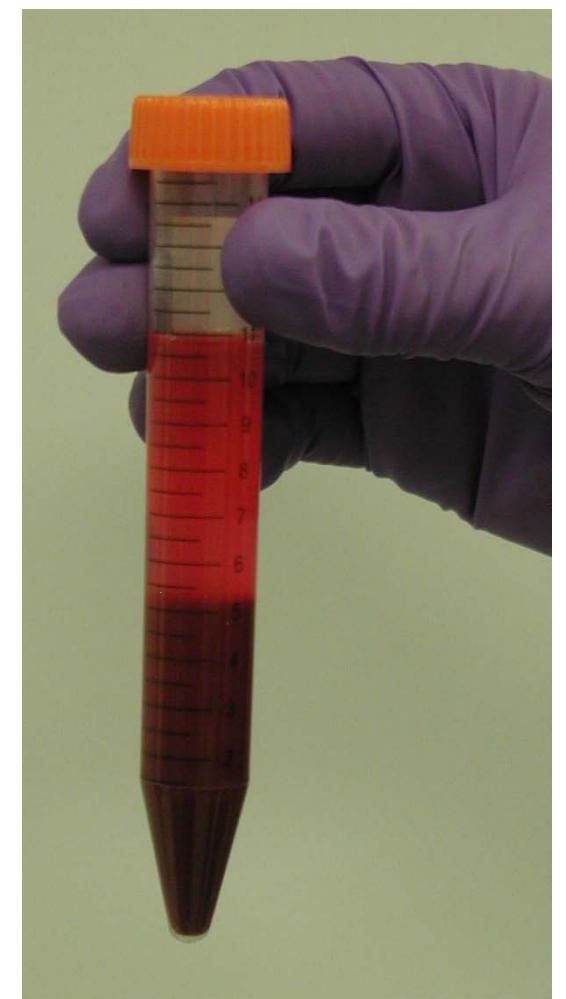


## Cultures in spinner vessels



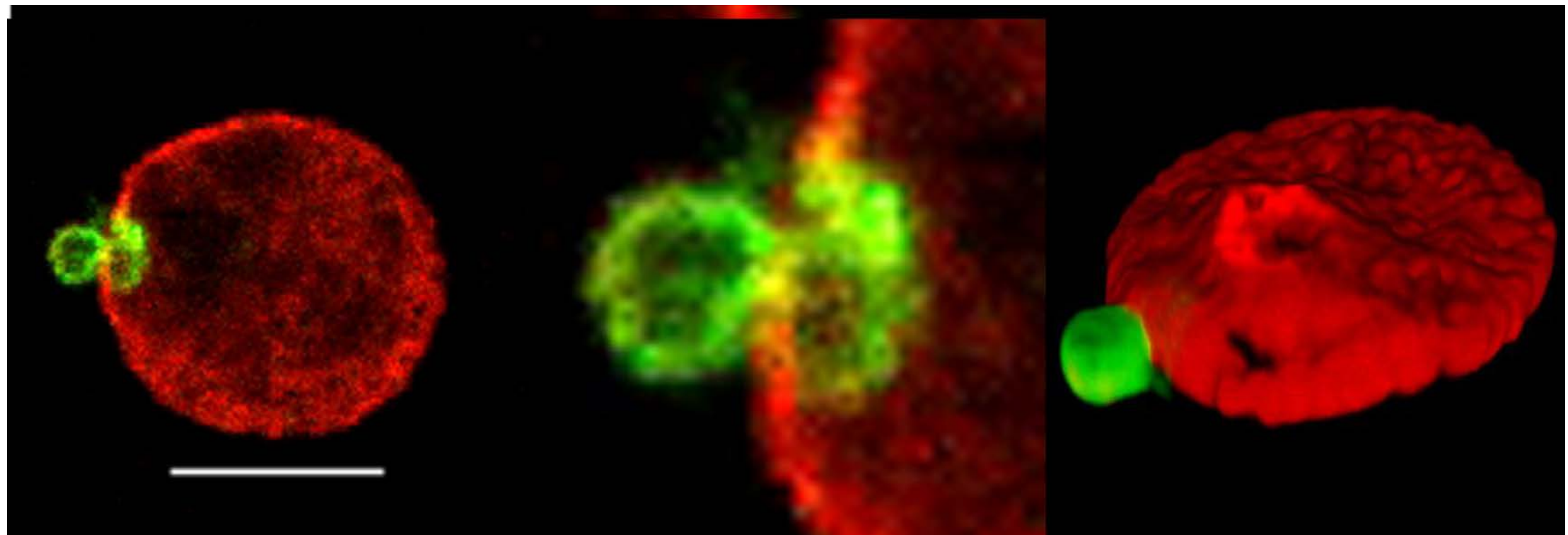
# **Mature functional human reticulocytes can be generated in a liquid culture system from peripheral blood CD34+ cells.**

- $\alpha$  and  $\beta$  Globins
- Reversible O<sub>2</sub> binding
- Normal expression of blood group active proteins and cell surface antigens
  - Absence of T and Tn antigens
  - Deformability
  - Proteome



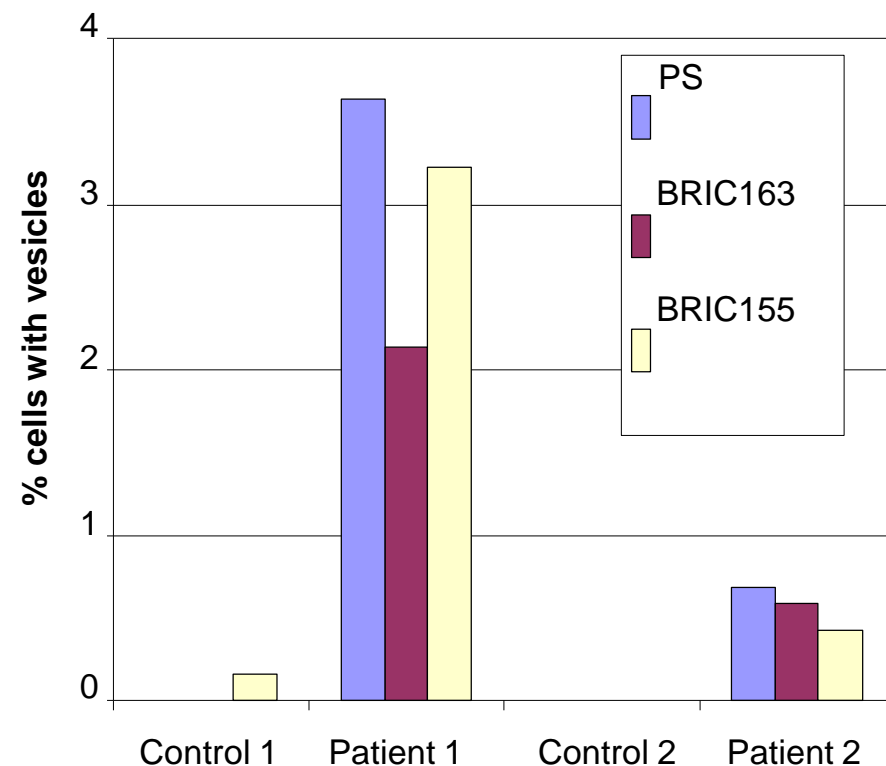


Reticulocytes reduce surface area and volume and remove residual organelles by expulsion of autophagic vesicles.



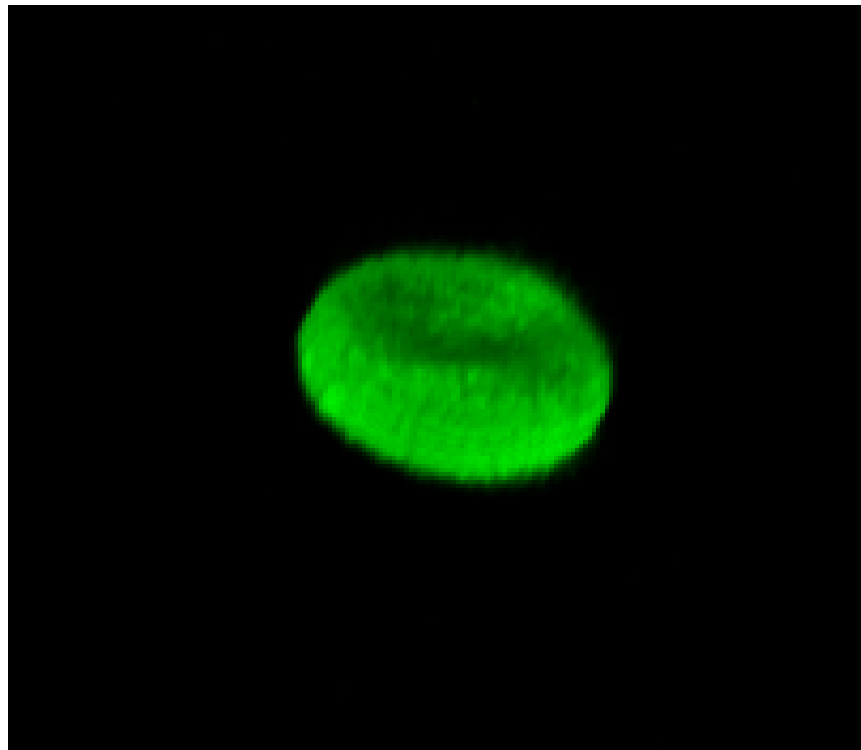
Mankelow et al. Blood 2015; 126(15): 1831

# Role of the Spleen in Vesicle Removal

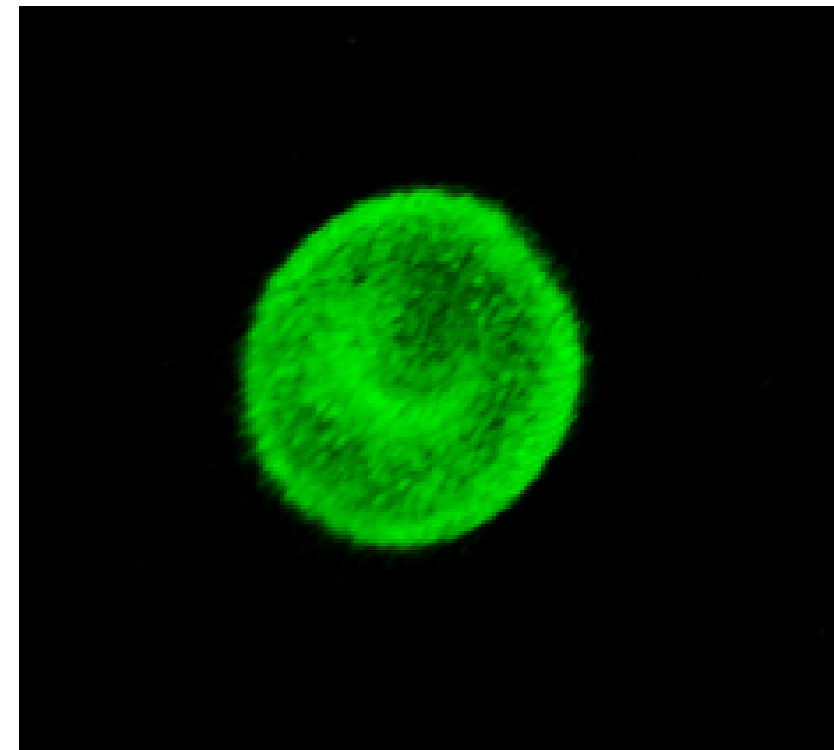


Elevated levels of vesicles on circulating red cells in splenectomised patients suggests the spleen is important for their removal.

# Adult Cultured Reticulocytes remodel *in vivo* (mouse).



10 min



24 hours

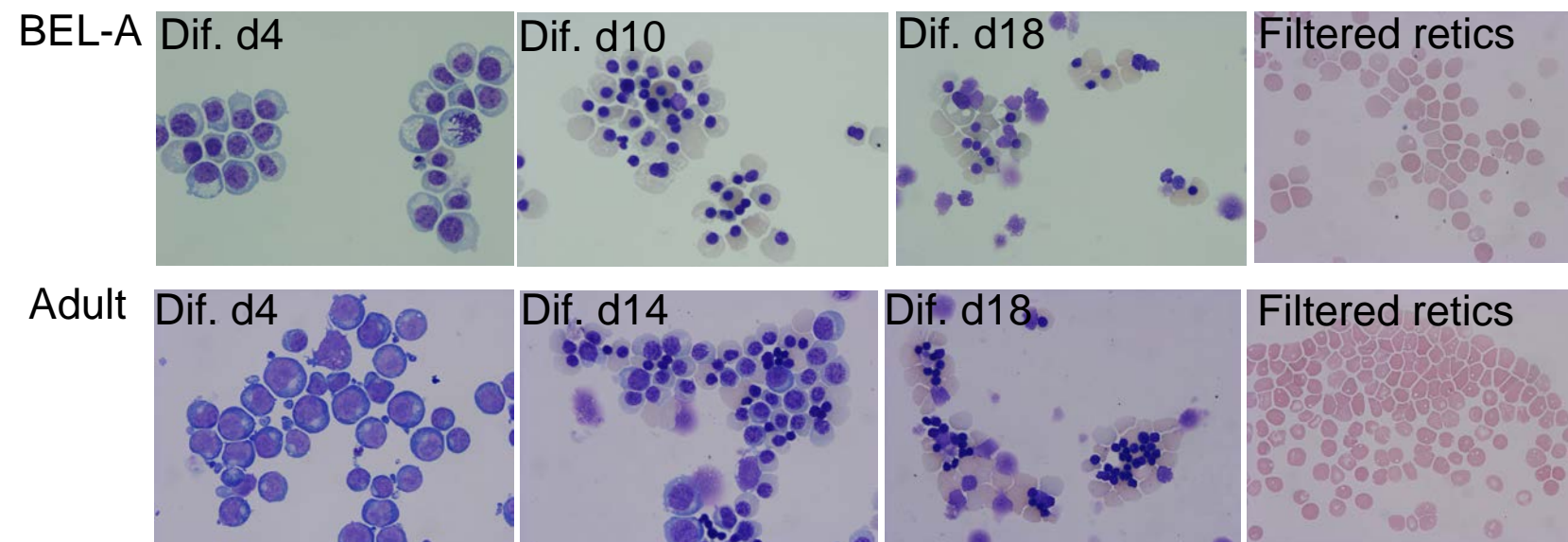
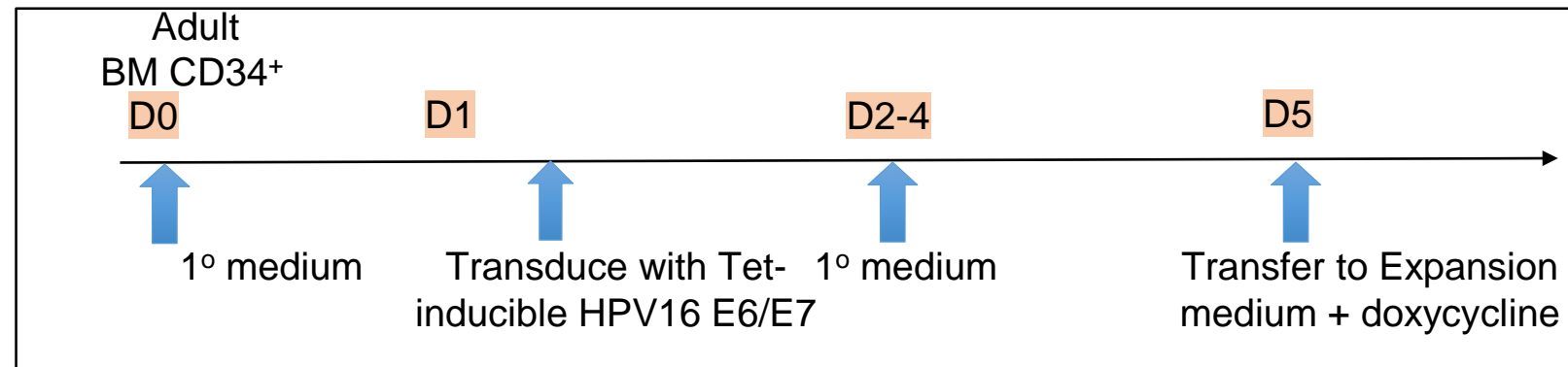
Becky Griffiths

# BUT

- Need a sustainable supply of cultured red cells of desired blood group phenotype

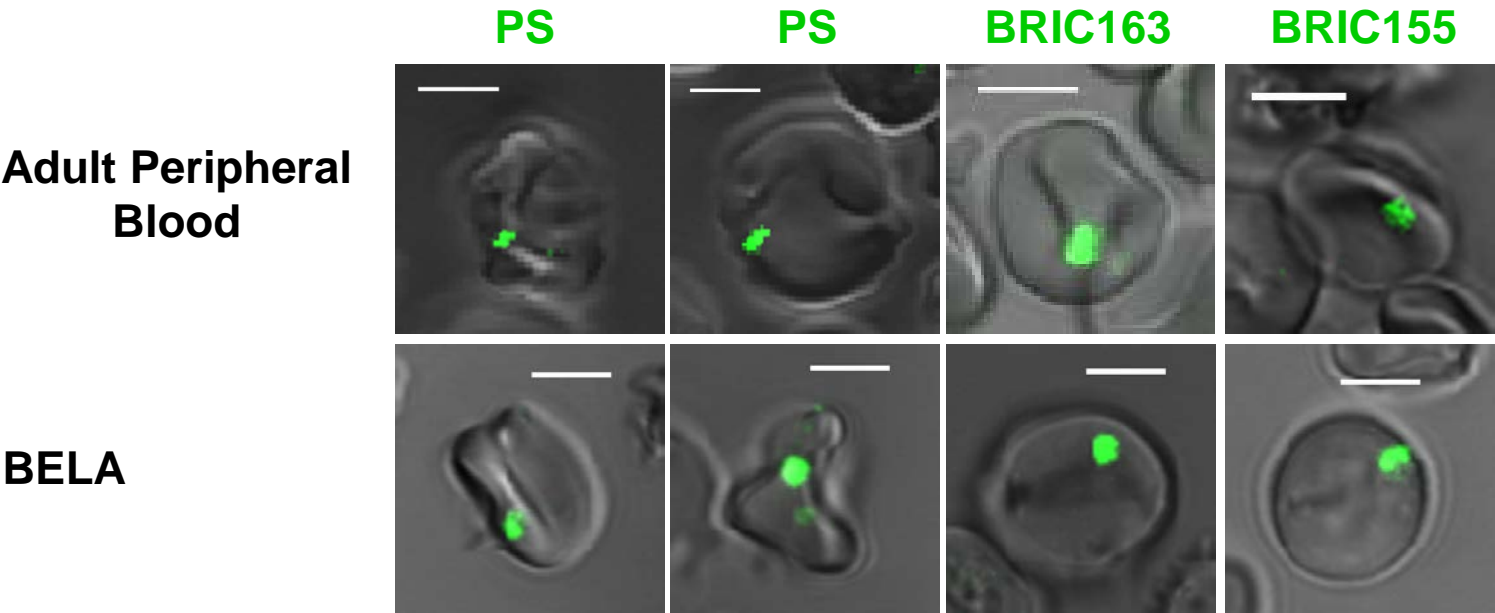


# Generation of the First Adult Erythroid Progenitor Cell Lines



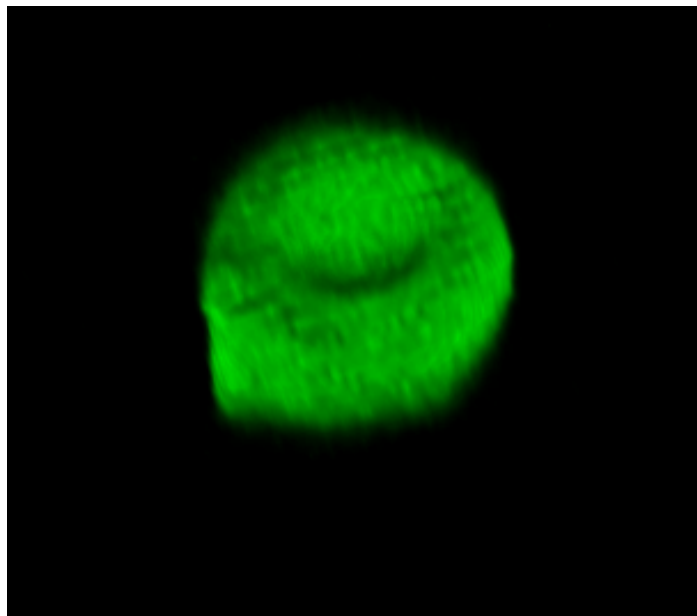
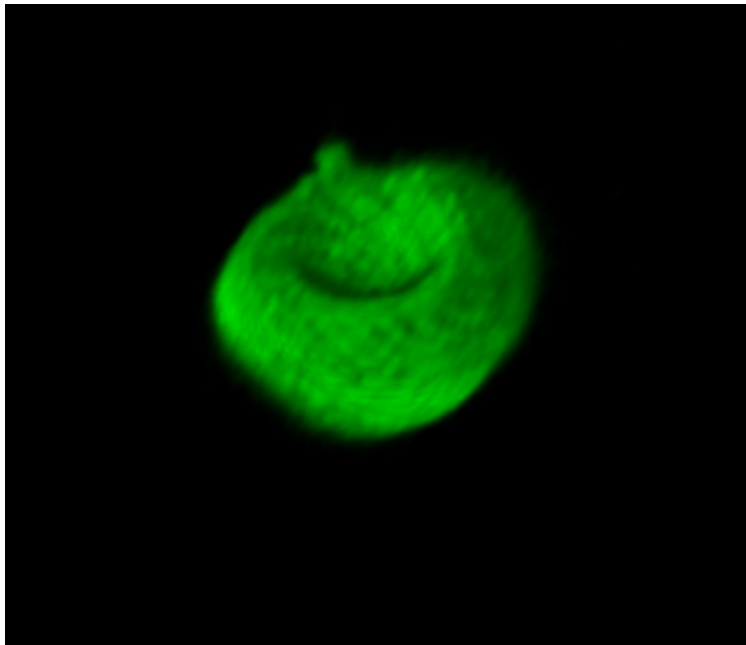
“Tom” Trakarnsanga

Live imaging of filtered reticulocytes showing presence of autophagic vesicles

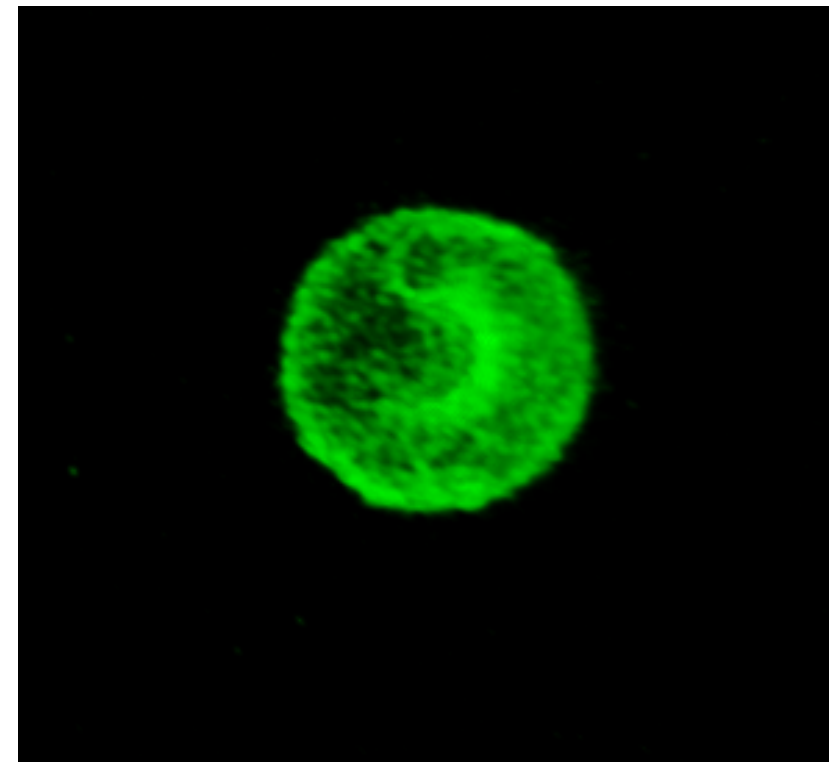
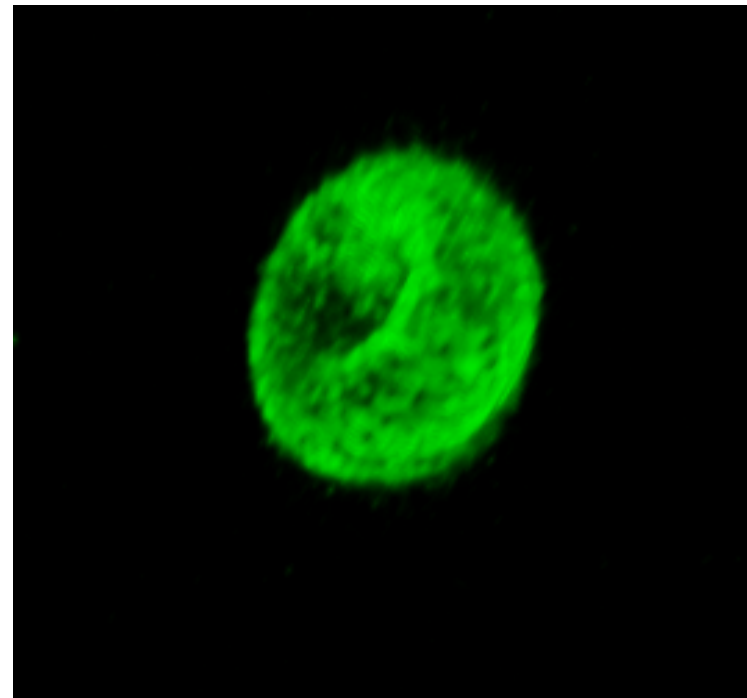


# BELA Reticulocytes in vivo (mouse)

10 min



24 hours



Becky Griffiths

# Why do we need cultured red cells?

For patients with rare blood  
groups.

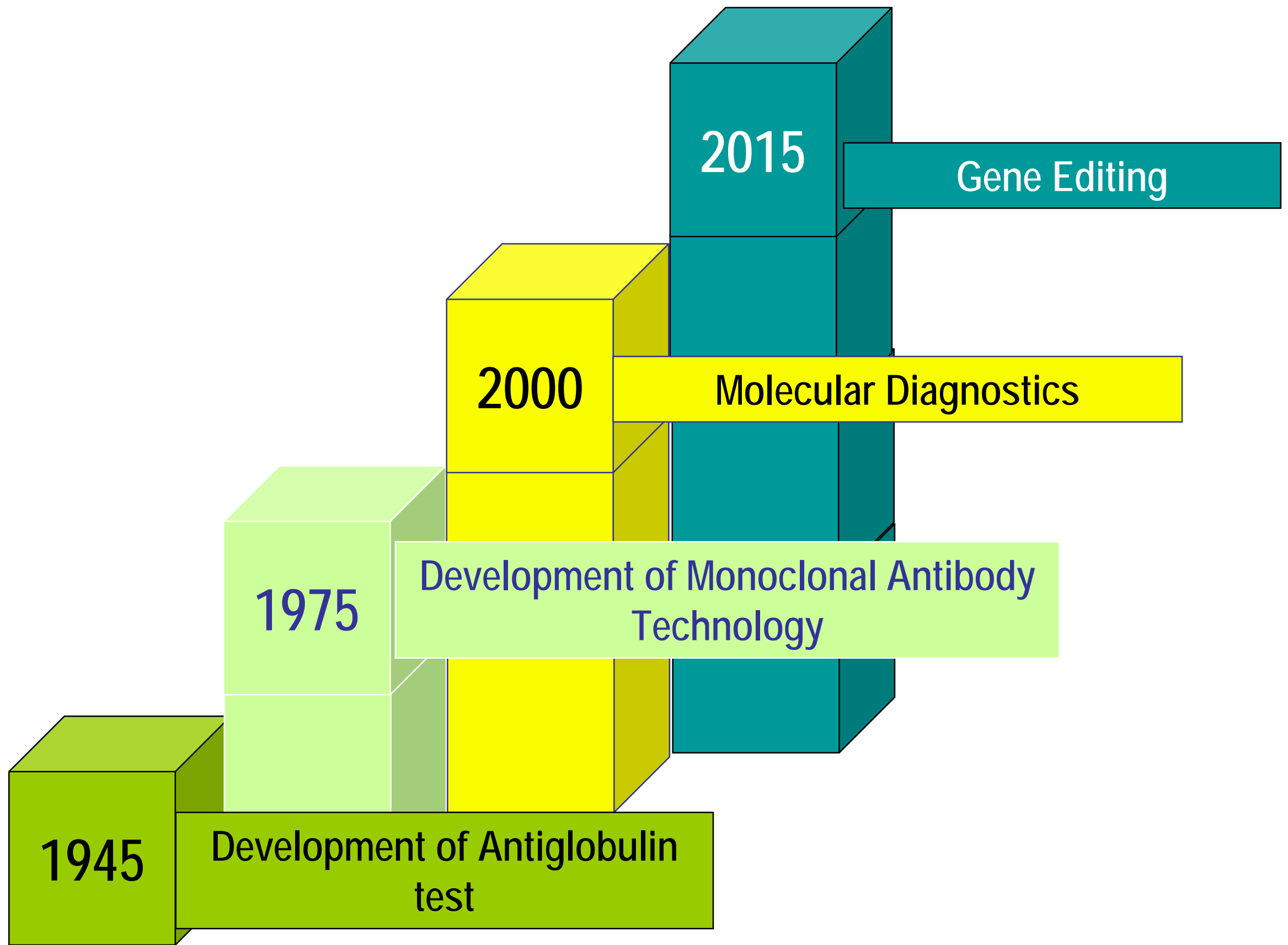
For patients with Sickle Cell  
Disease

• .

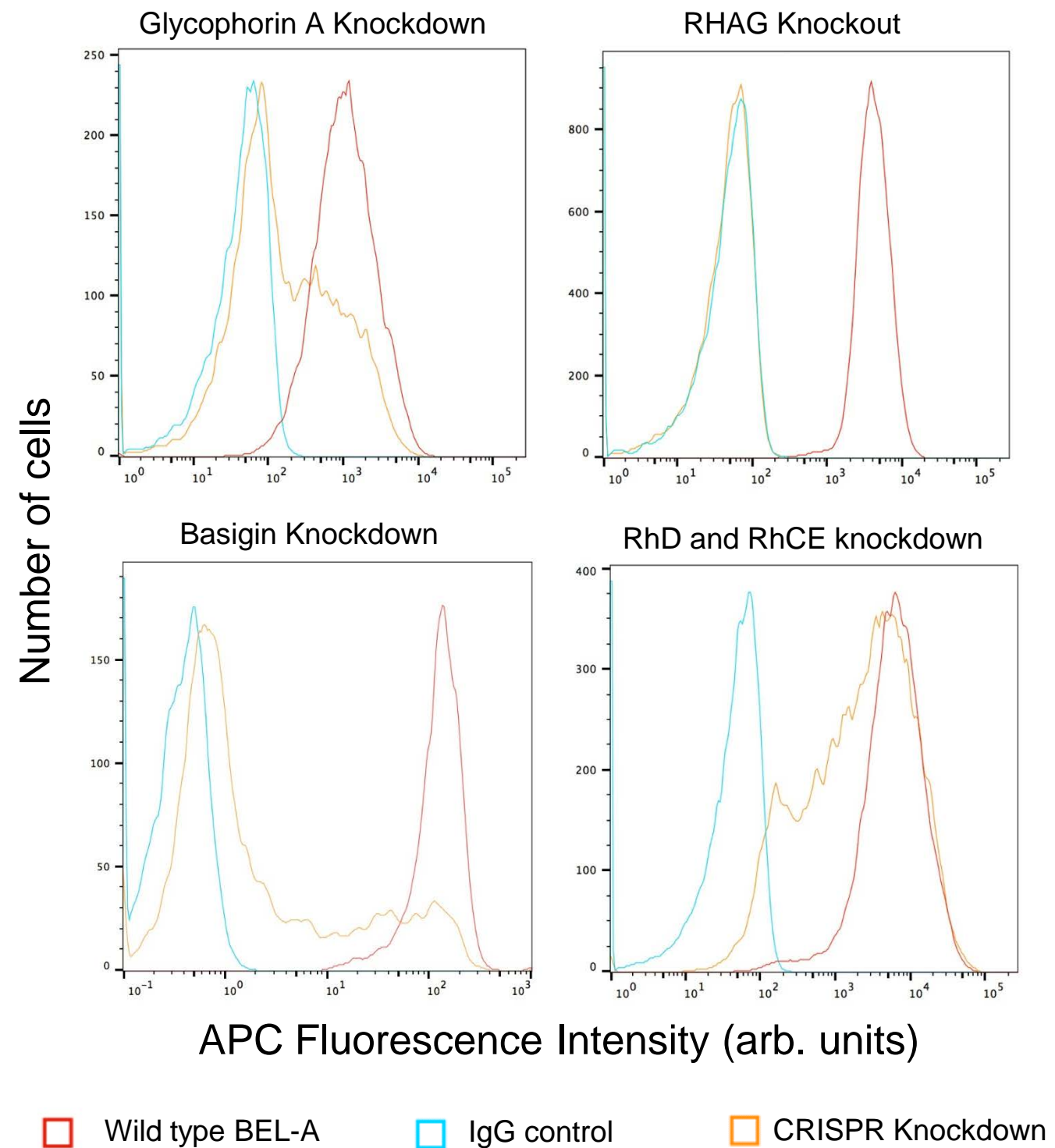
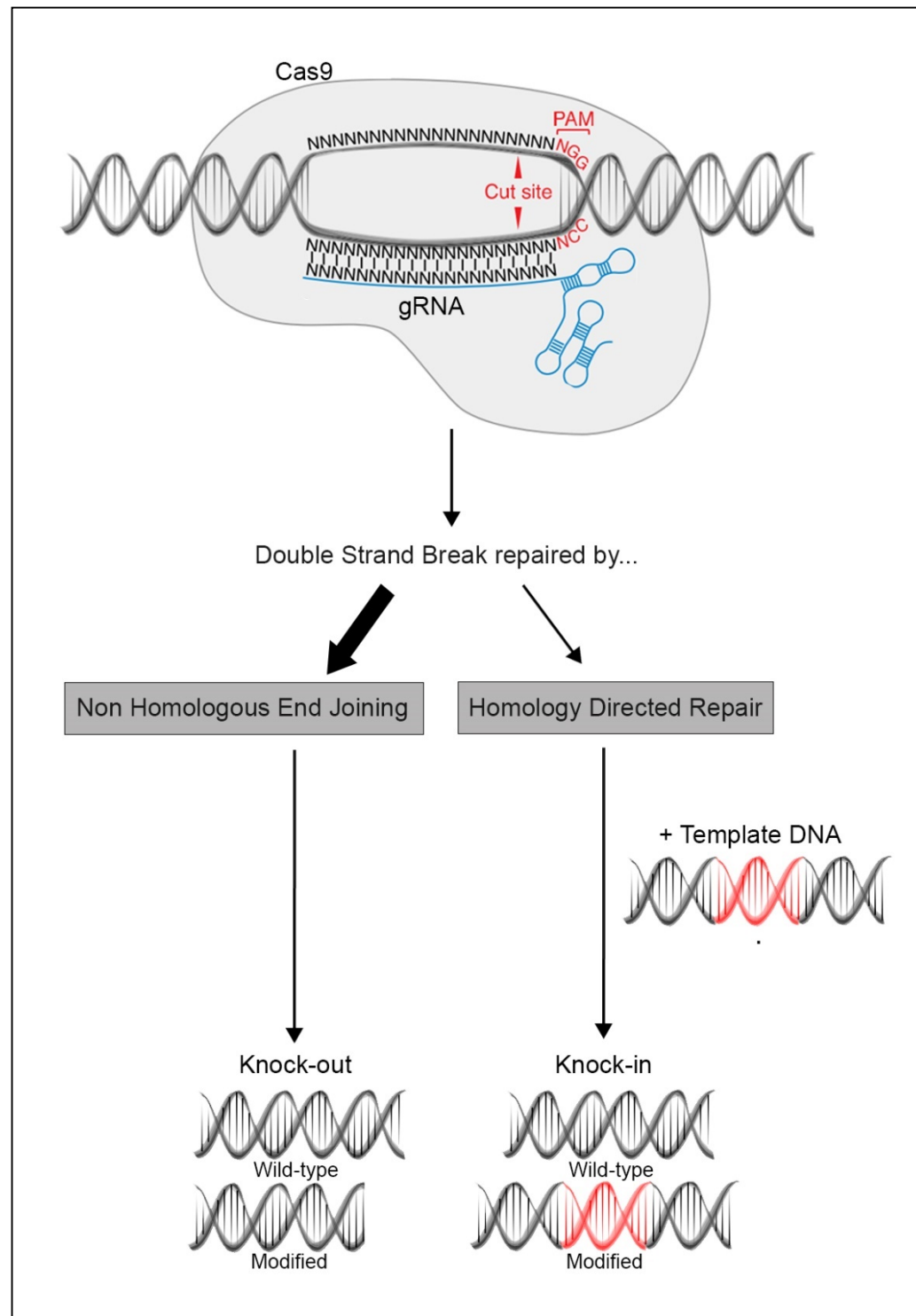
*Based on data from Dr.Fiona Regan*



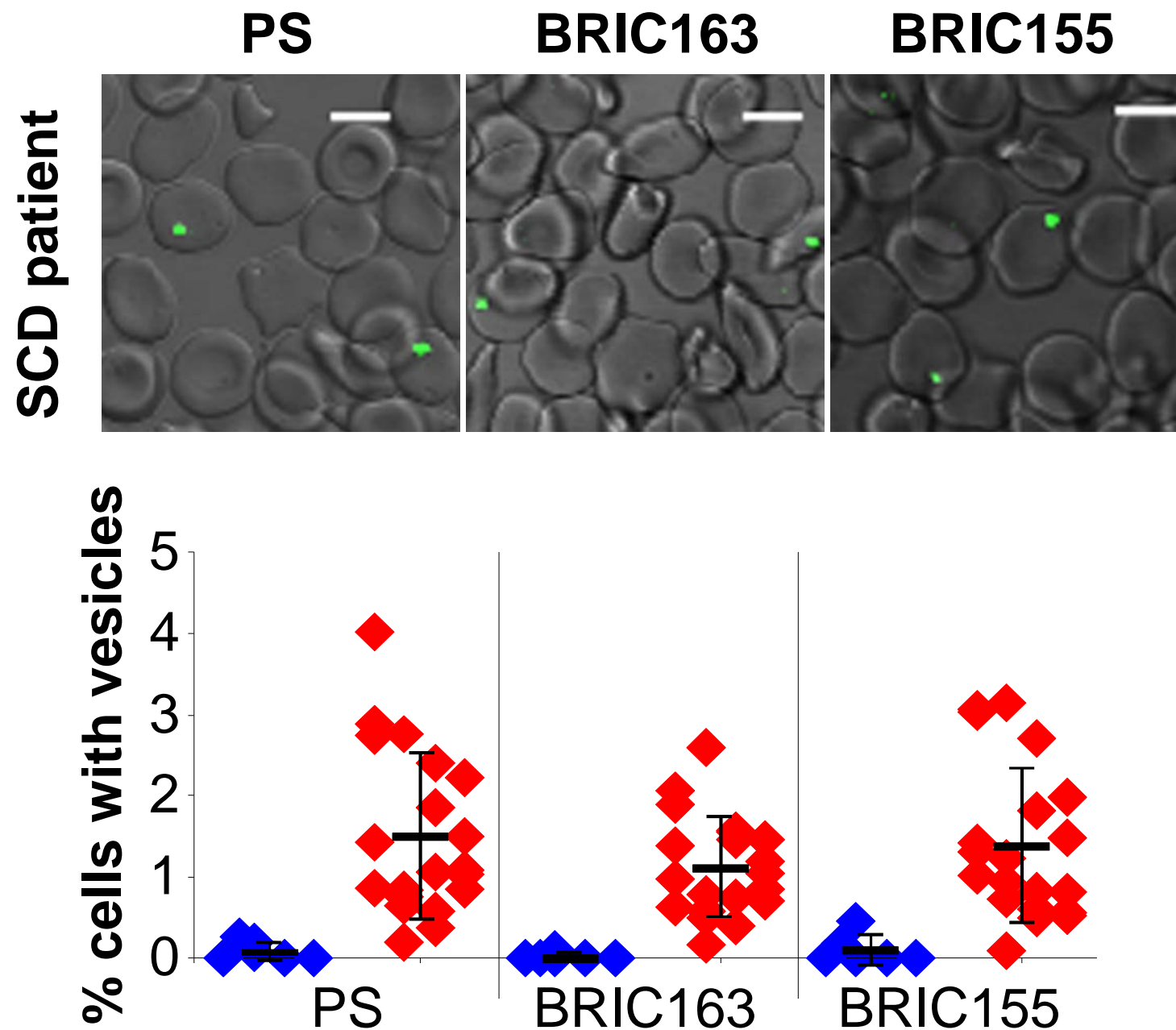
# New Technologies applied in Transfusion Medicine



# Genome editing BELA's using CRISPR



# Autophagic Vesicles from Reticulocytes remain in the Blood of Patients with Sickle Cell Disease



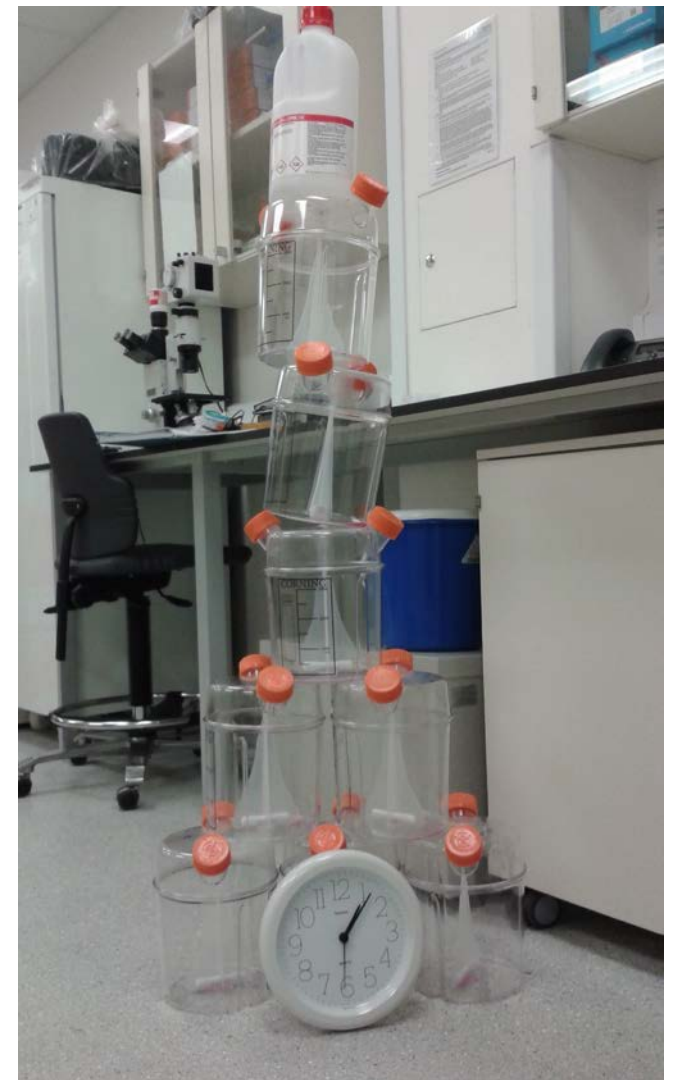
# Current Challenges

- Induce maturation of cultured reticulocytes to erythrocytes *in vitro*.
- Develop technology for scale up to therapeutic doses.





Sabine Taylor



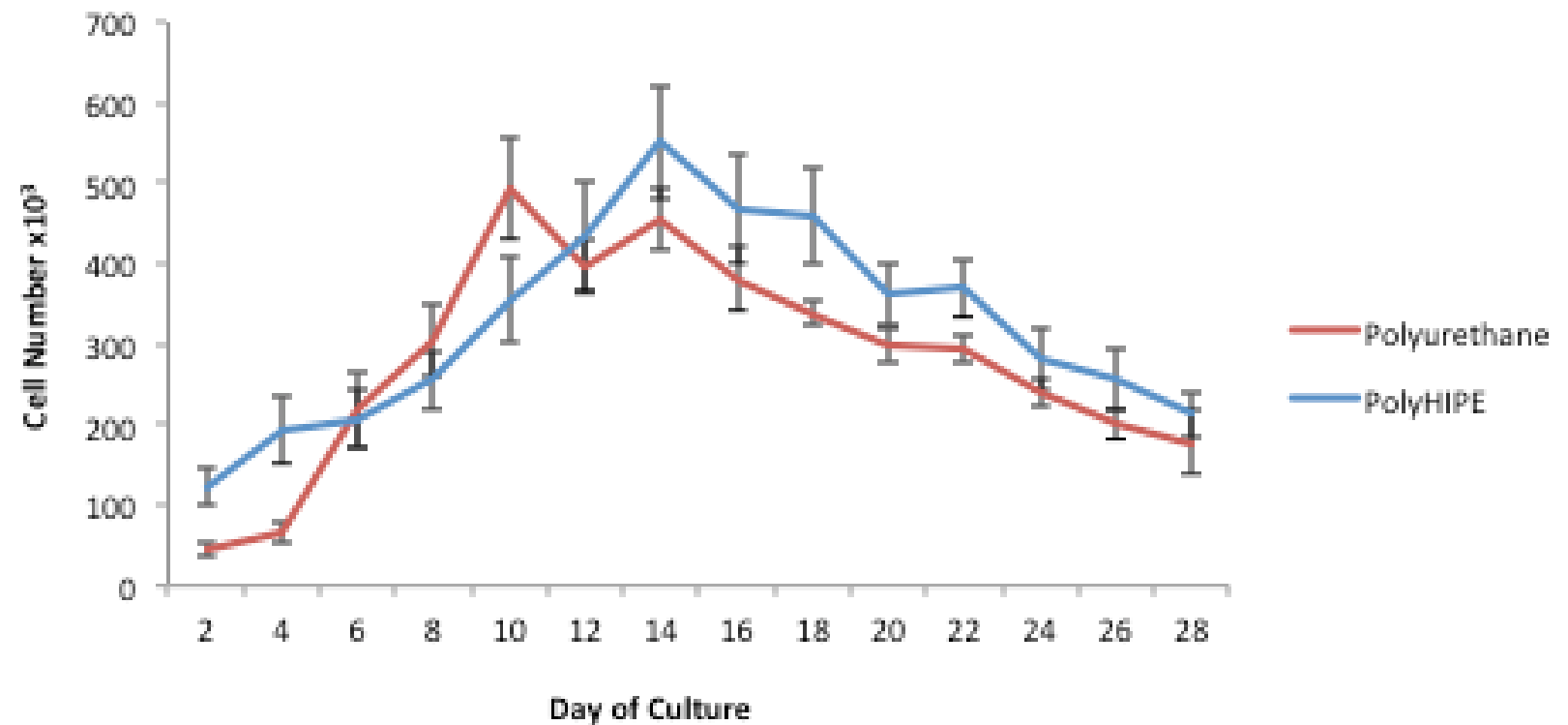
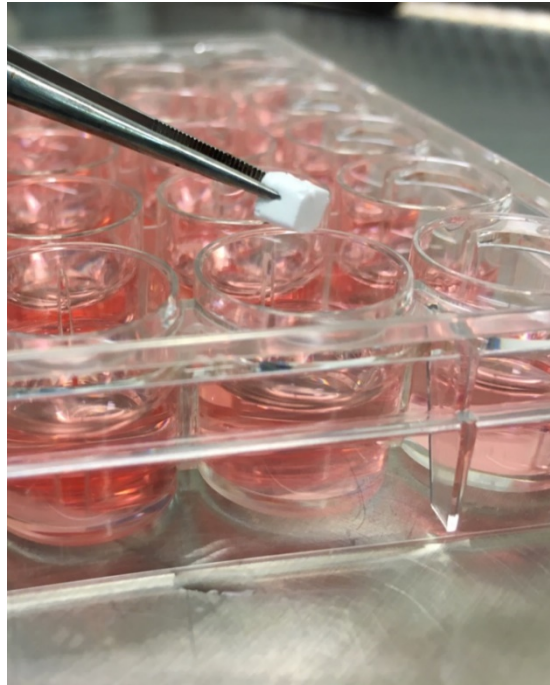
Nicky Cogan

# Develop Technology for Scale Up - two approaches

Functionalised Scaffolds

Genetic Manipulation

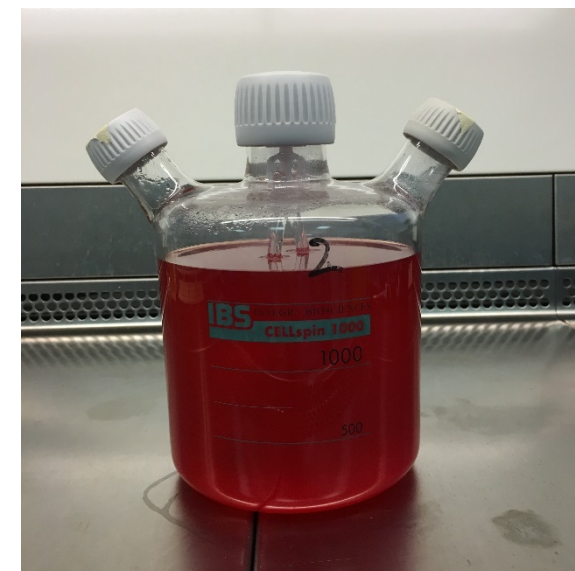
# Use of synthetic scaffolds for *ex vivo* culture



T75 with scaffold and  
40ml expansion medium



1.6 liters in  
Spinner Flask



# Summary

- Erythroid progenitor cell lines provide a sustainable supply of cultured reticulocytes with the same properties as those obtained from cultures of CD34+ cells.
- Gene Editing of Blood Group Genes will allow the manufacture of cultured reticulocytes with different rare blood group phenotypes from a single founder erythroid progenitor cell line.
- Further work is required to effect maturation of cultured reticulocytes to erythrocytes *in vitro* and to manipulate erythroid progenitor cell lines to maximise yield of reticulocytes but the technology required to do this is now available.



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NHS Blood and Transplant

Wellcome Trust

**Key Collaborators:** Yukio Nakamura, Riken Institute, Tokyo  
Sara Trompeter, UCLH, London



# NIHR Blood and Transplant Research Unit University of Bristol 2016

