Genetic Engineering of Blood Products (RBCs)

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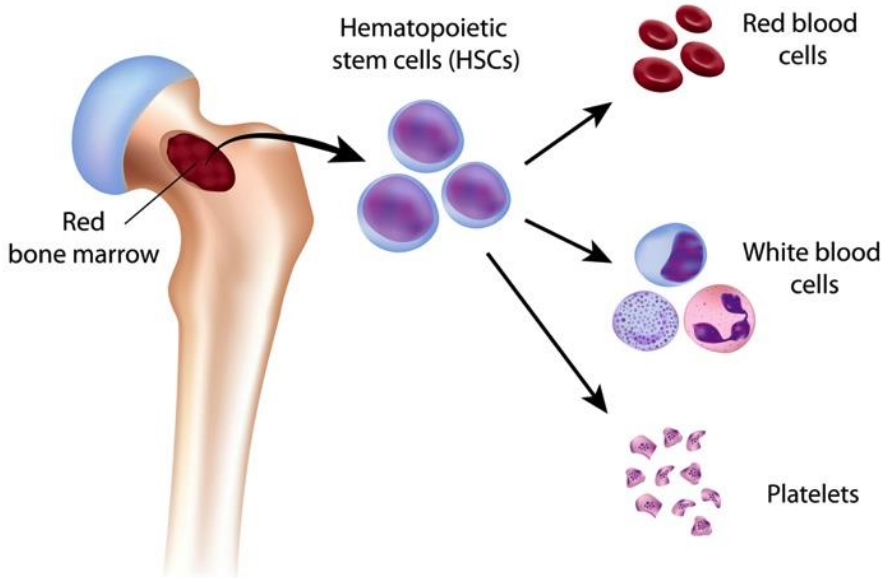
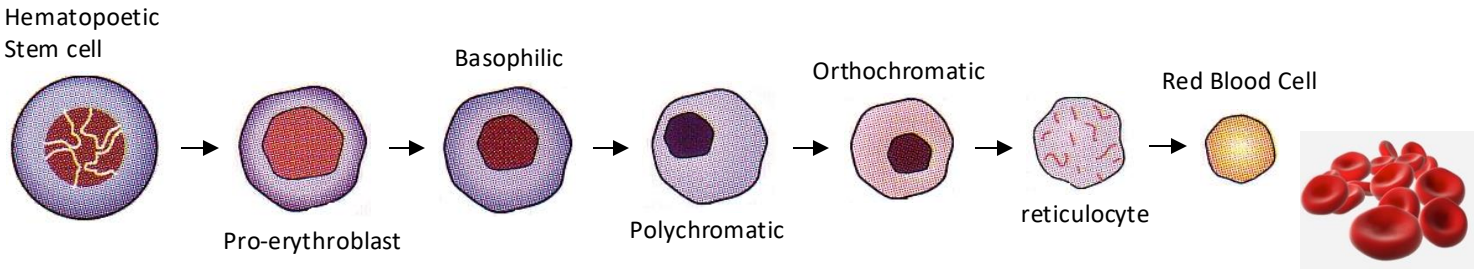
University of Bristol, UK

- Growing RBCs in lab
- Sustainable erythroid lines as source of RBCs
- Genetic engineering of RBCs
- RBCs as drug delivery and targeting agents

Erythropoiesis in vivo





Primary aim of in vitro grown RBCs for unmet clinical need

MDS
Transfusion-dependent patients } erythrocyte alloimmunization, iron overload

Rare blood types

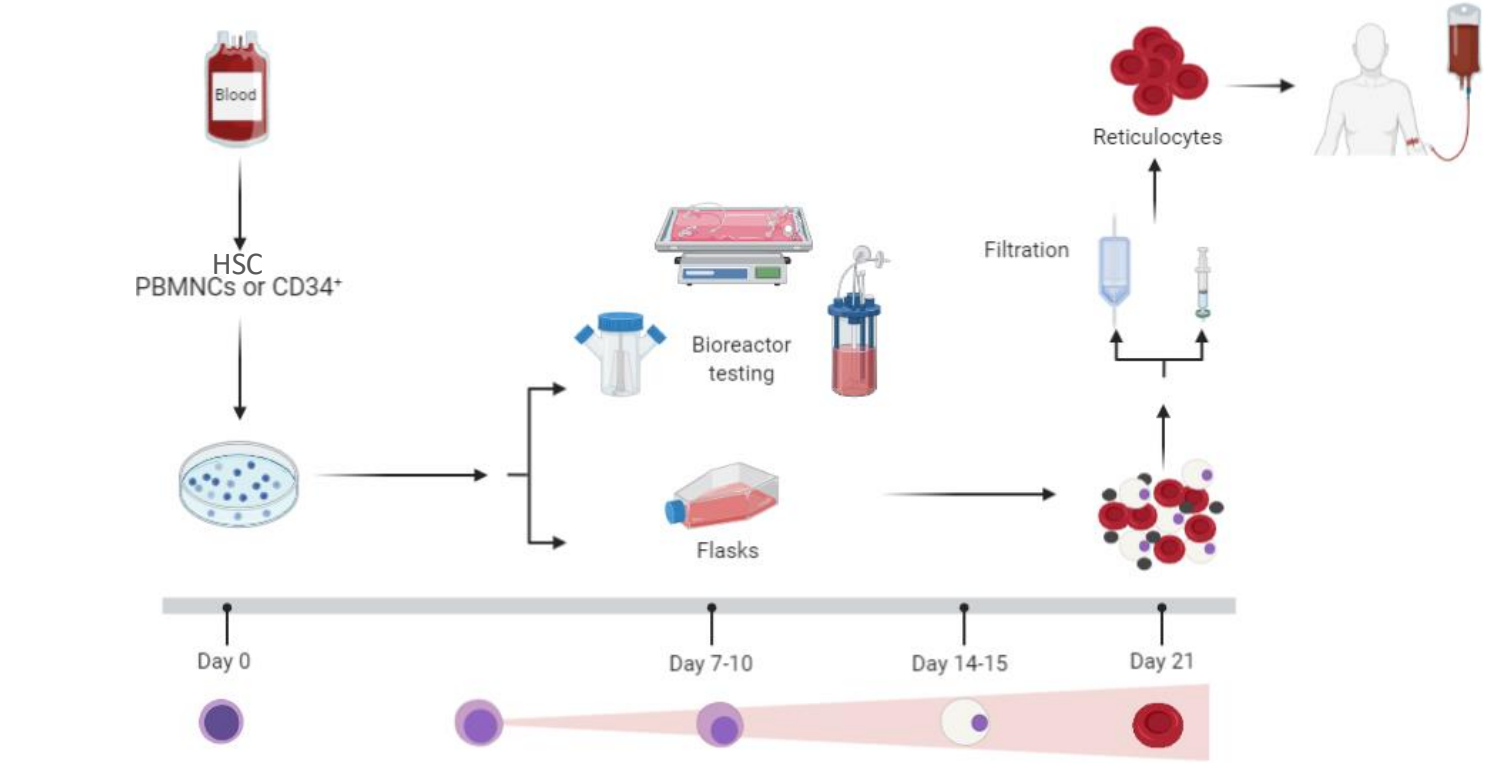
Younger cells – reduce transfusion frequency and dose, reduced infectious risks

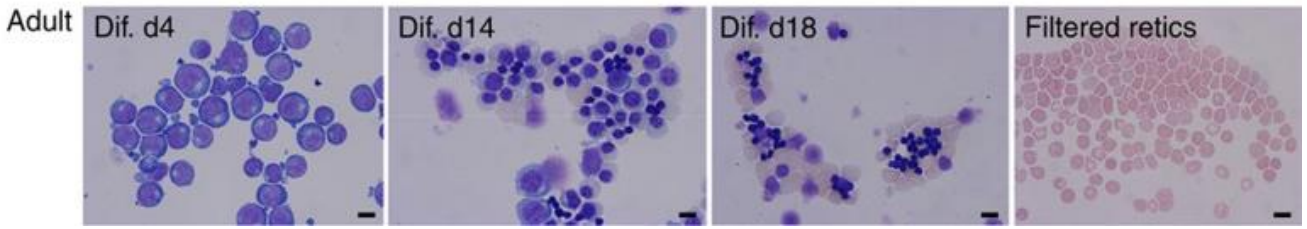
Research tool to study erythropoiesis in health and disease

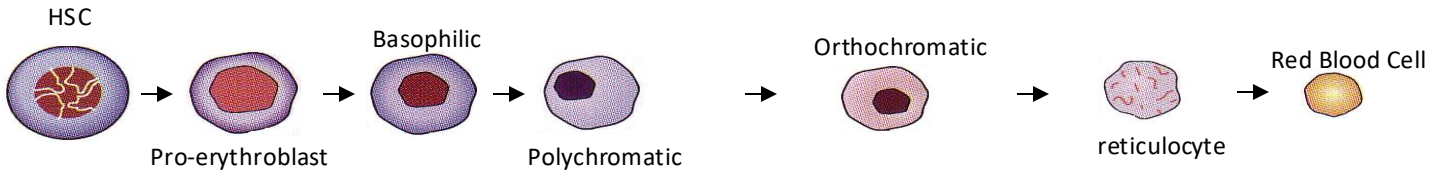
Progenitors for *in vitro* generation of Red Blood Cells

- Bone marrow HSC
- Peripheral blood HSC
- Cord blood HSC
- iPSCs
- ESCs

In vitro culture of red blood cells

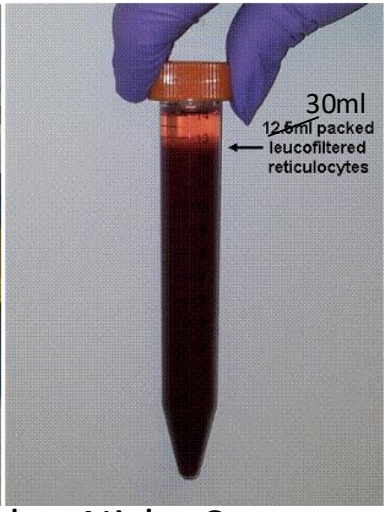






Growing cells at scale under GMP

70L



Good Manufacturing Practice (GMP) Compliant

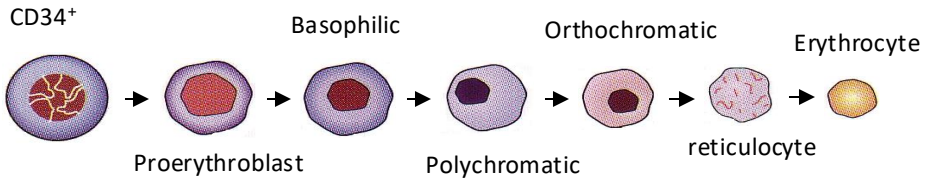
Images courtesy of Sabine Taylor, Nicky Cogan and Prof. David Anstee (NHSBT)

RESTORE: Recovery and Survival of Stem Cell Originated Red Cells

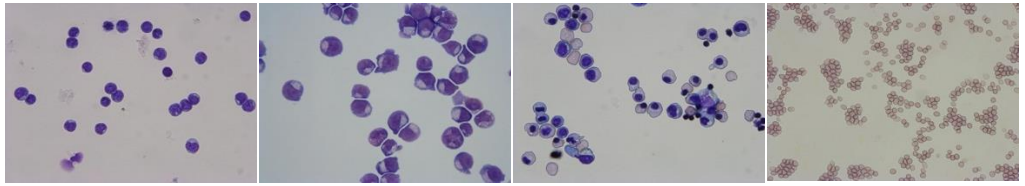
- Phase 1
- Allogeneic

Led by Prof Cedric Ghevaert and Dr Rebecca Cardigan

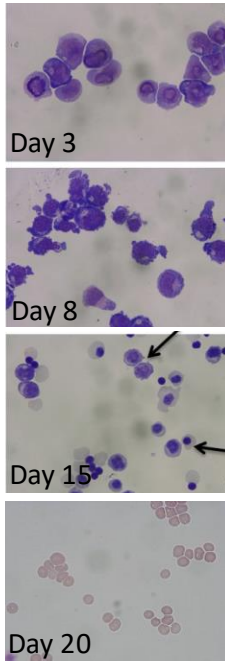
Hurdles with cultures



Adult PB CD34+ cells

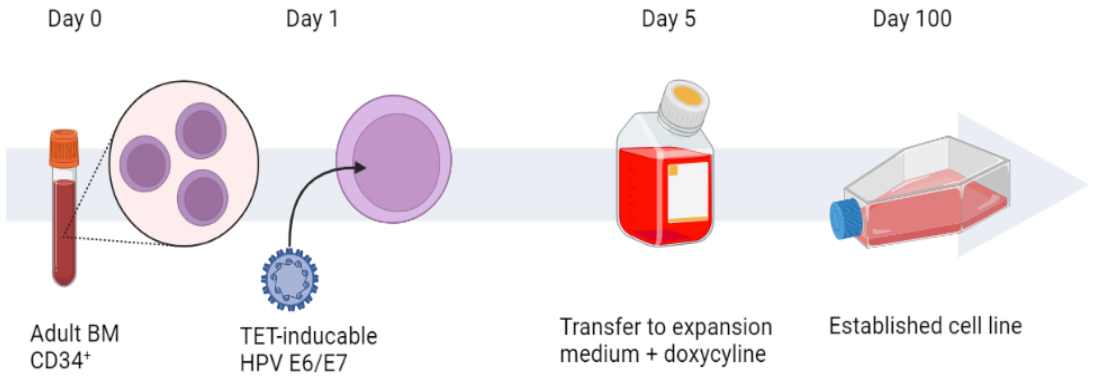


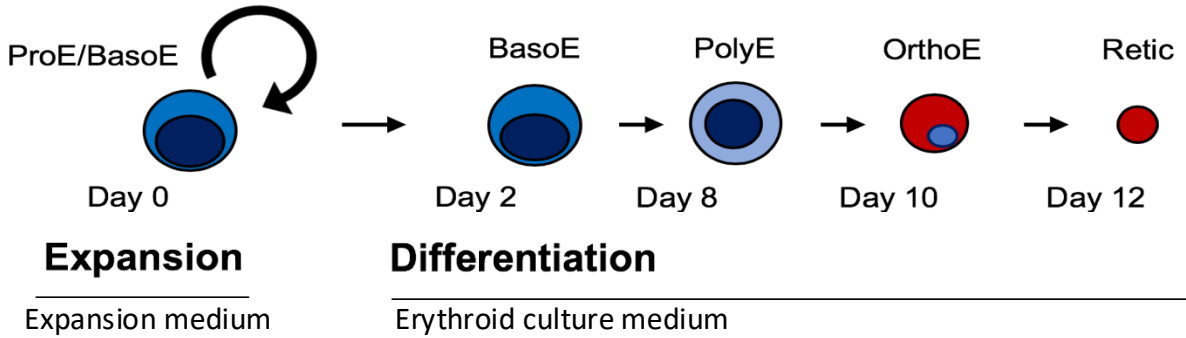
Cord Blood CD34+

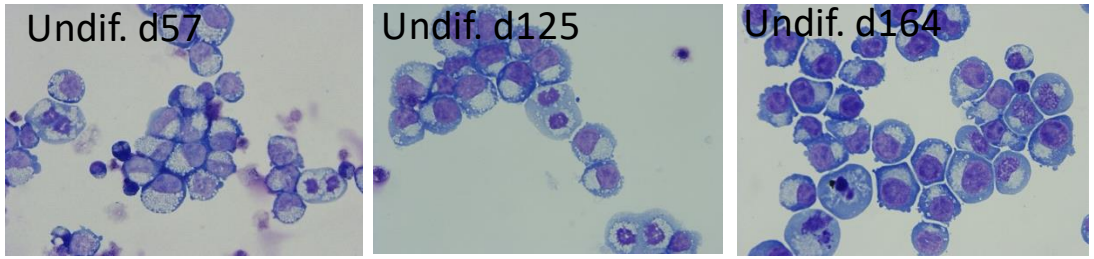


Scalability
Repeat HSC donations
Available blood groups

Creating immortalised adult erythroid line (BEL-A)

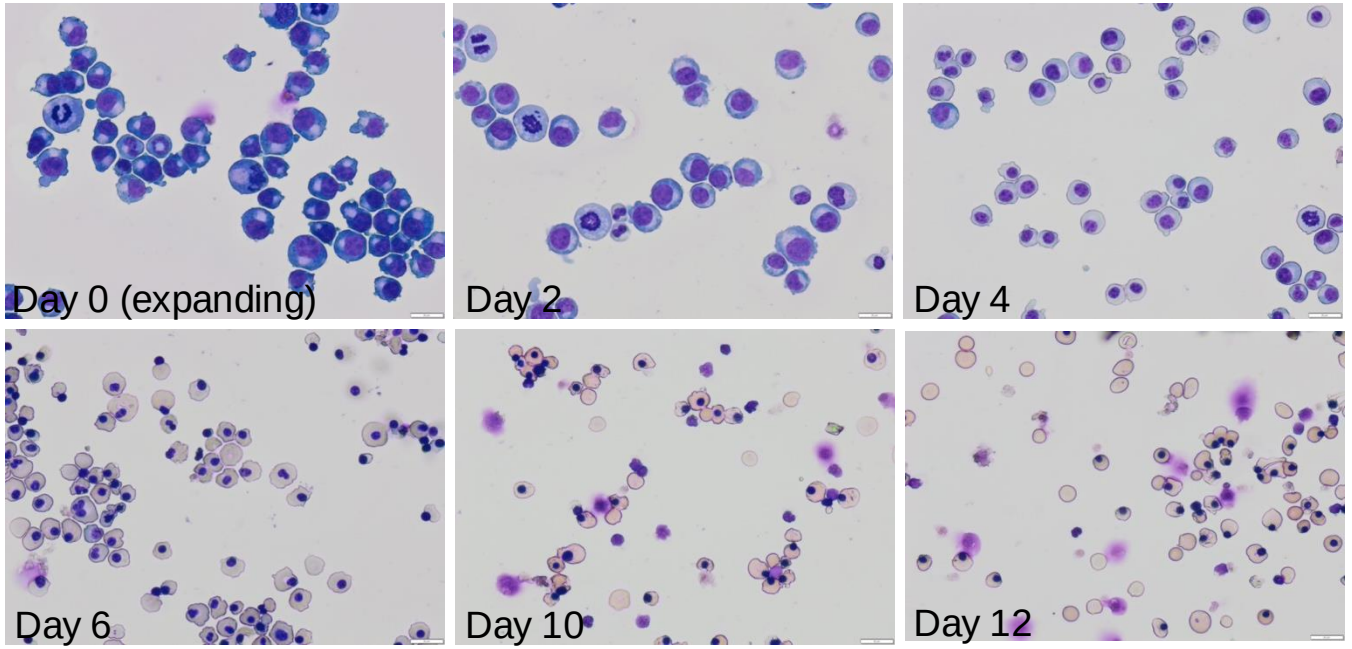






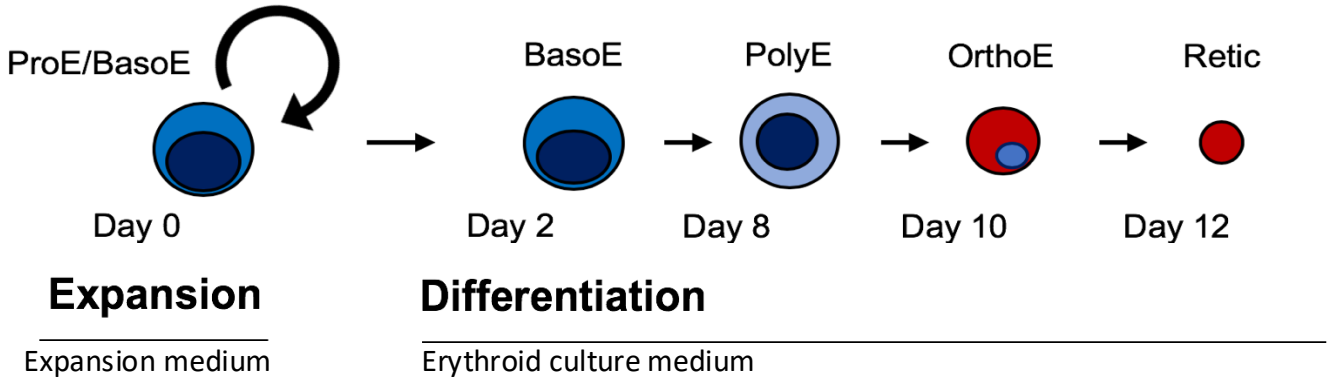
Line unchanged over time

>d450



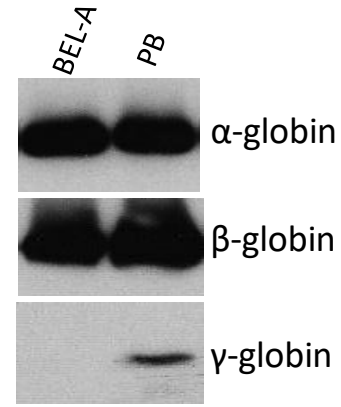
>40% enucleation

Differentiated BEL-A cells have adult phenotype



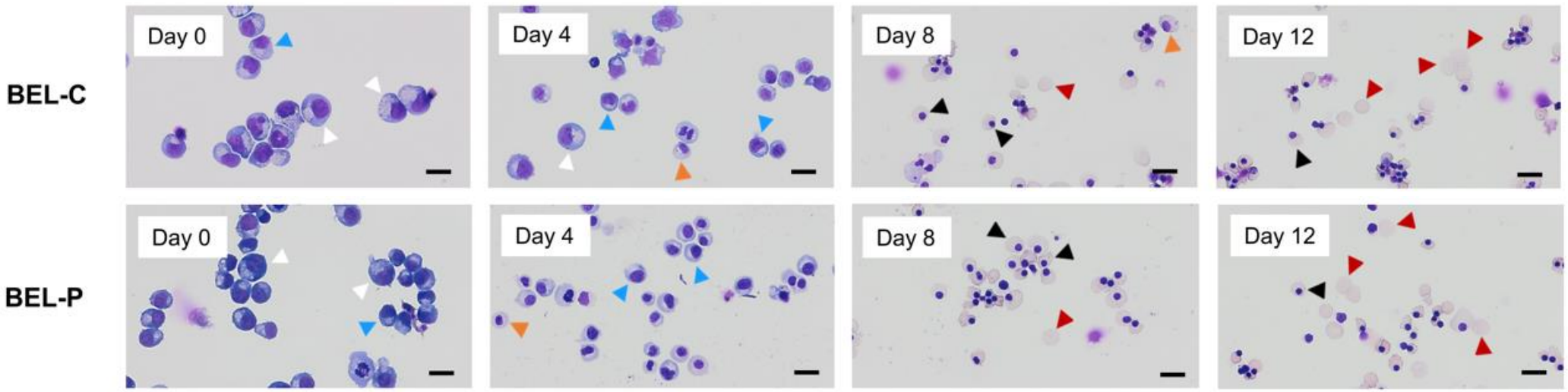
Extensively Characterised

- **Trakarnsanga *et al* Nat Comm 2017**
- **Daniels *et al* Haematologica 2019**

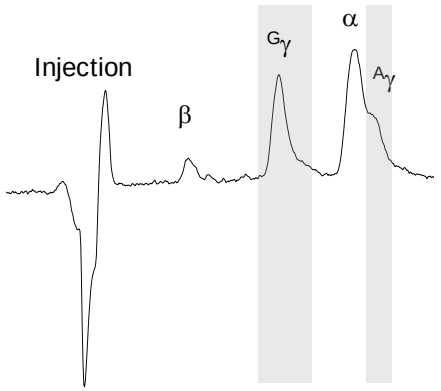


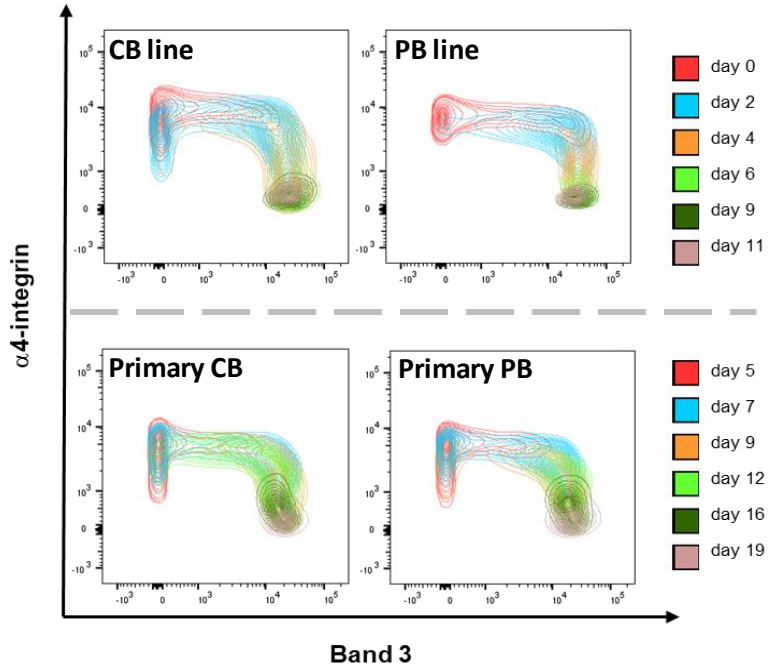
- First immortalised adult human erythroid line, recapitulates adult erythropoiesis, produces reticulocytes
- Do not require repeat collection of donor stem cells
- 12 different immortalised erythroid cell lines using same methodology – from BM, PB, CB $CD34^+$ cells
- Create with chosen blood group phenotype – line has phenotype of the original donor
- **Provides a scalable, sustainable source of red blood cells**

Lines from more accessible Peripheral & Cord Blood CD34+ recapitulate phenotype of donor erythroid cells

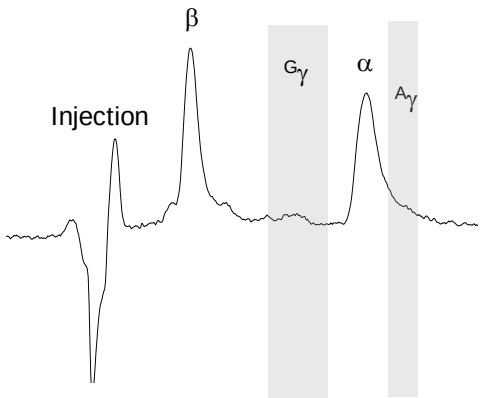


Cord blood line



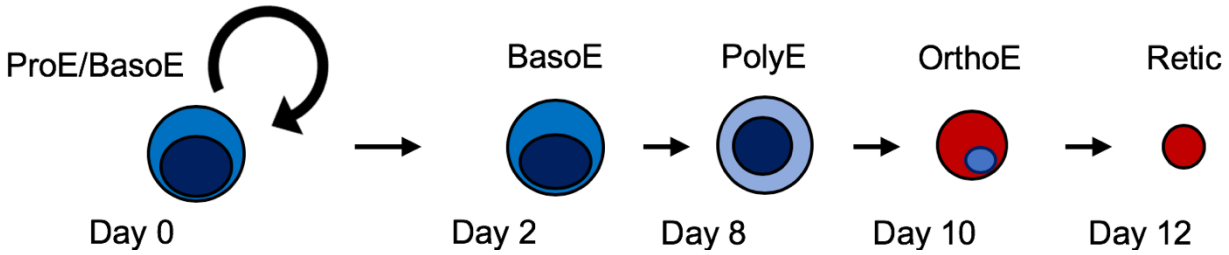


Adult PB blood line



Daniels et al 2021 Mol Ther Methods Clin Dev 12;22:26-39

Differentiated BEL-A cells have adult phenotype



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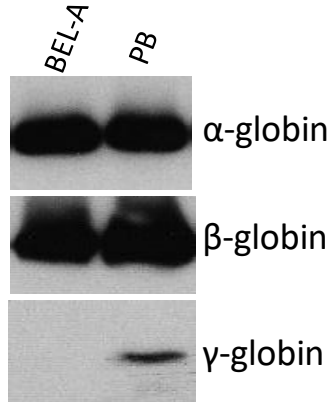

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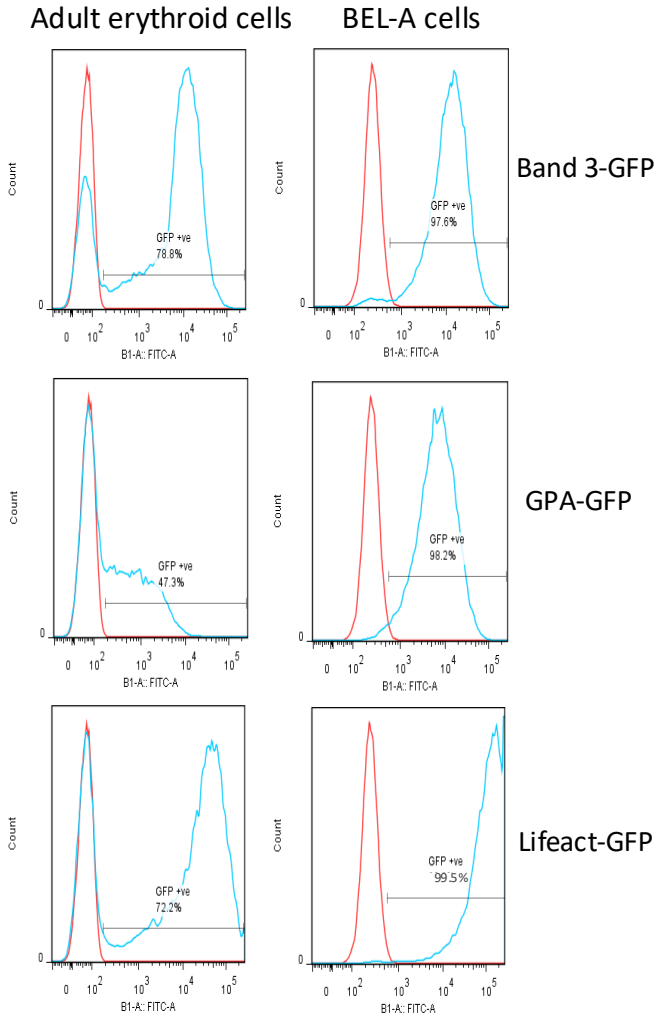
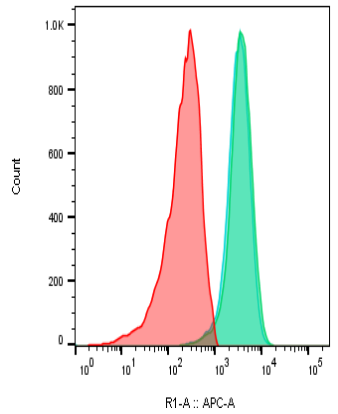
Overexpression

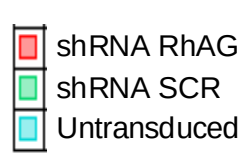
Transduction efficiency



shRNA knock down

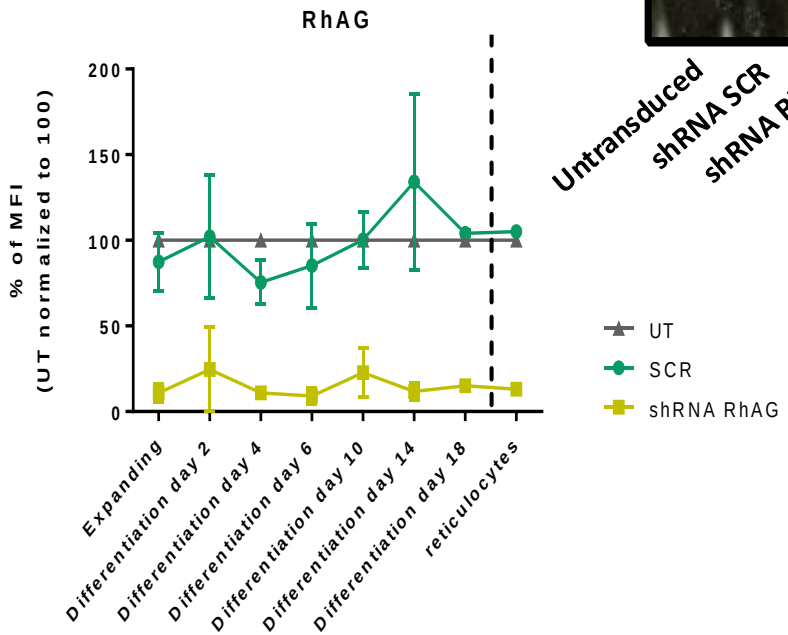
Knock down of RhAG



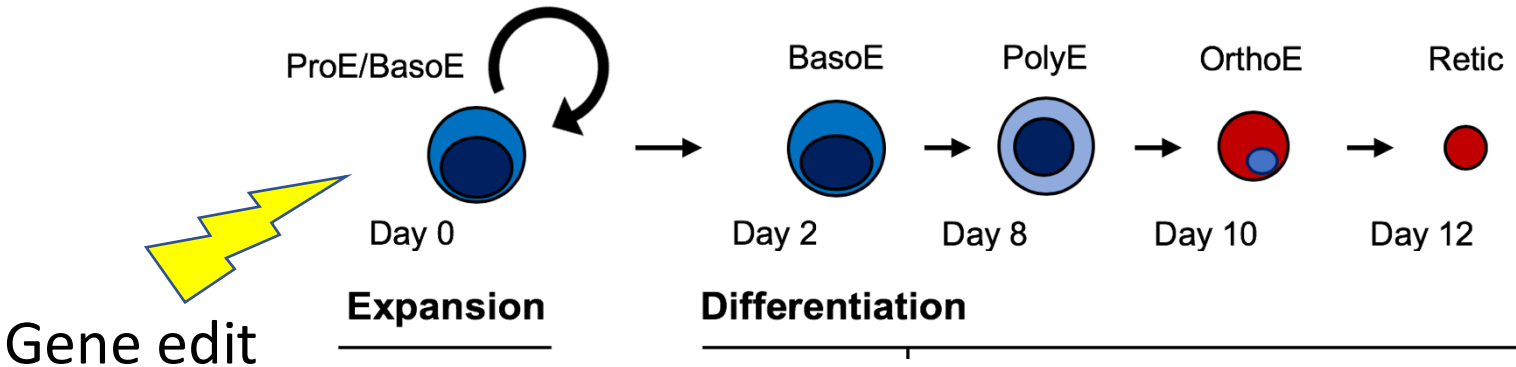


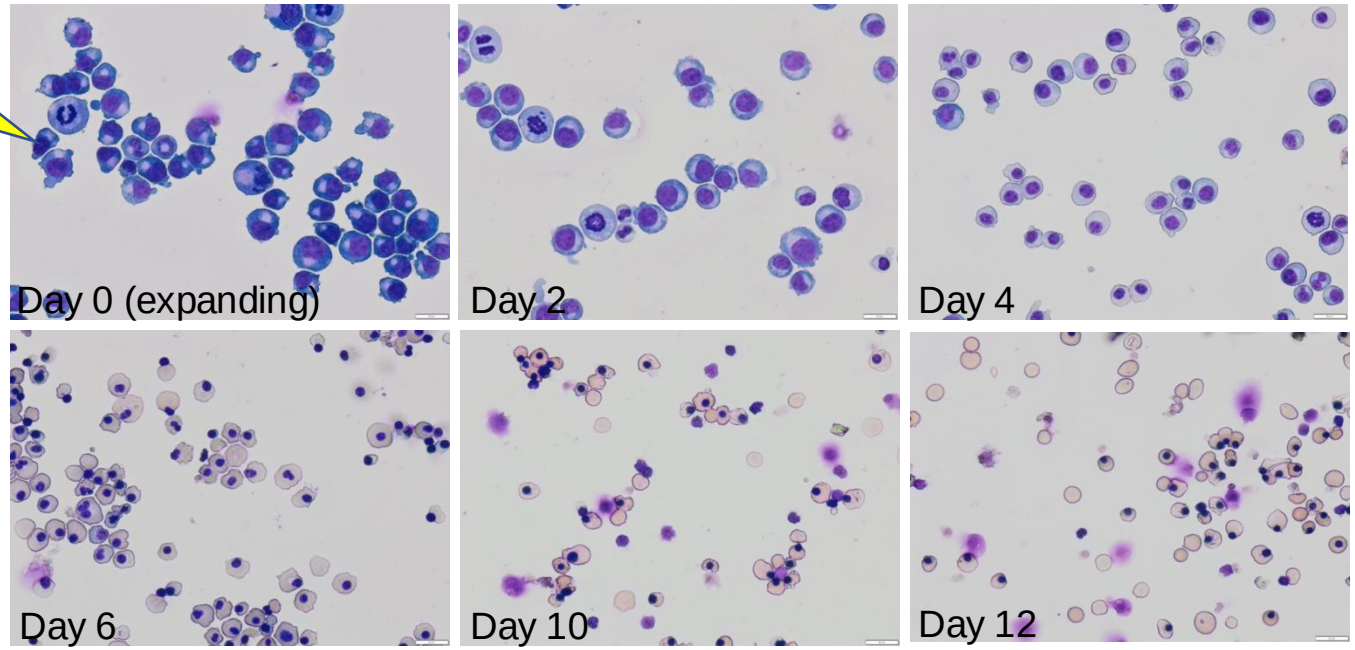
Western blot

Flow Cytometry



Platform for CRISPR gene editing of BEL-A cells



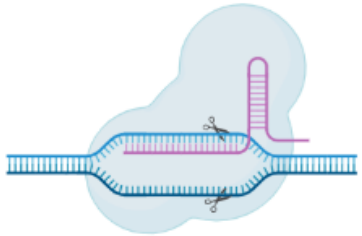


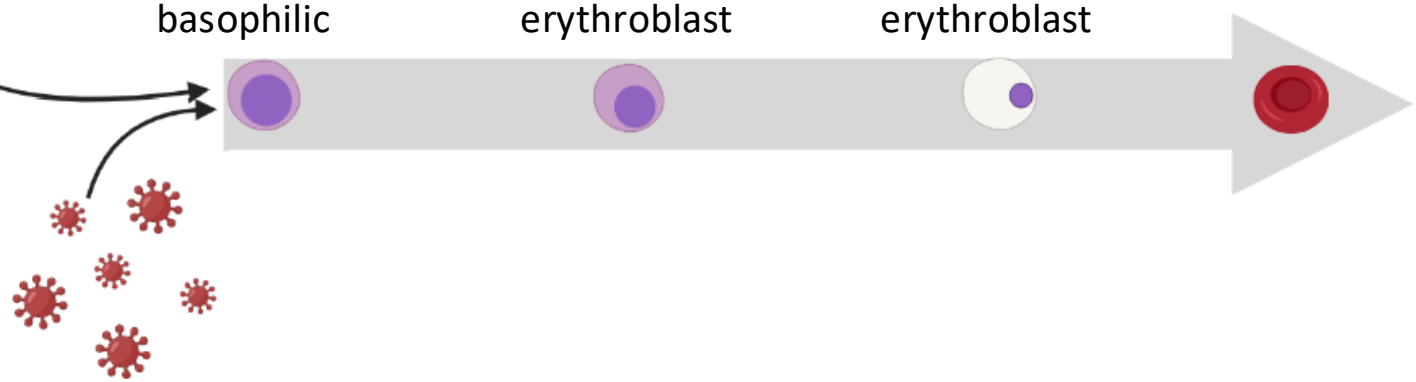
>40% enucleation

Platform for CRISPR gene editing of BEL-A cells

CRISPR gene KO and introduction of specific mutations and tags (and CRISPRa)

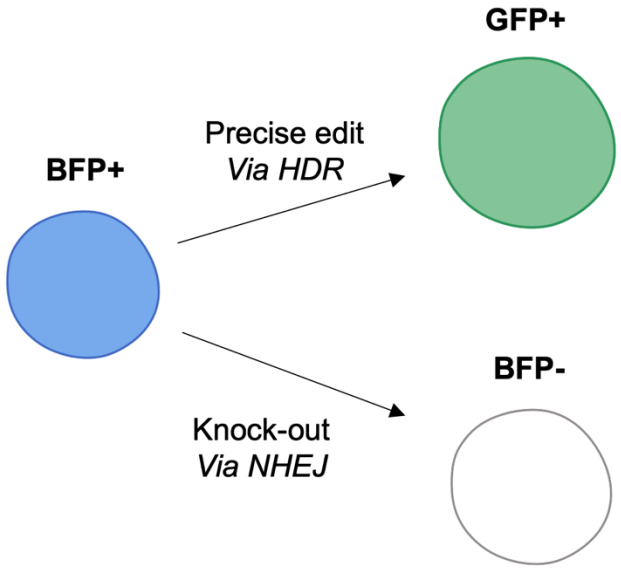
Lenti ➡ nucleofection of plasmids ➡ nucleofection of RNPs

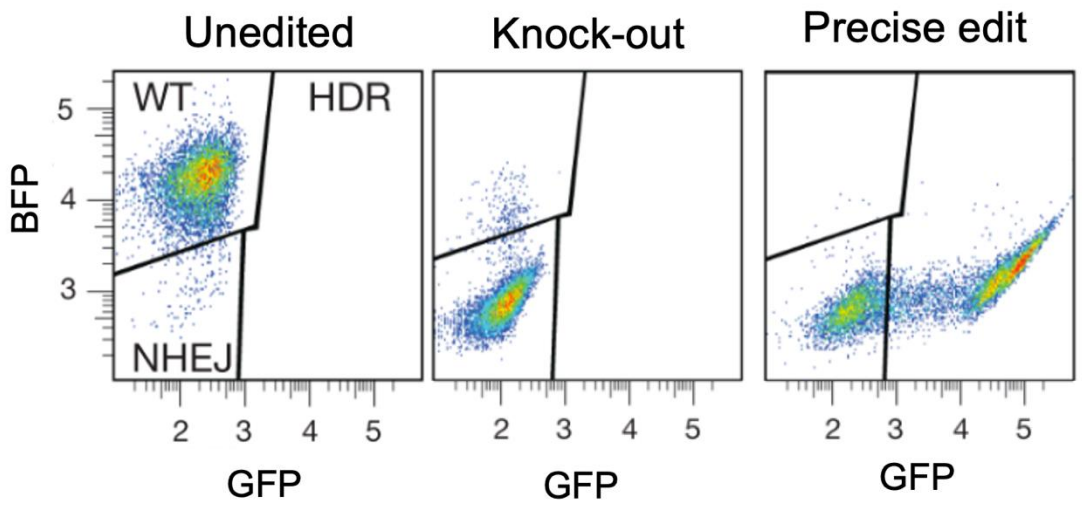




Lentiviral over expression and shRNA knock down

CRISPR Fluorescent Conversion Assay to validate reagents to improve HDR efficiency





Up to 70%

Up to 50% endogenous genes

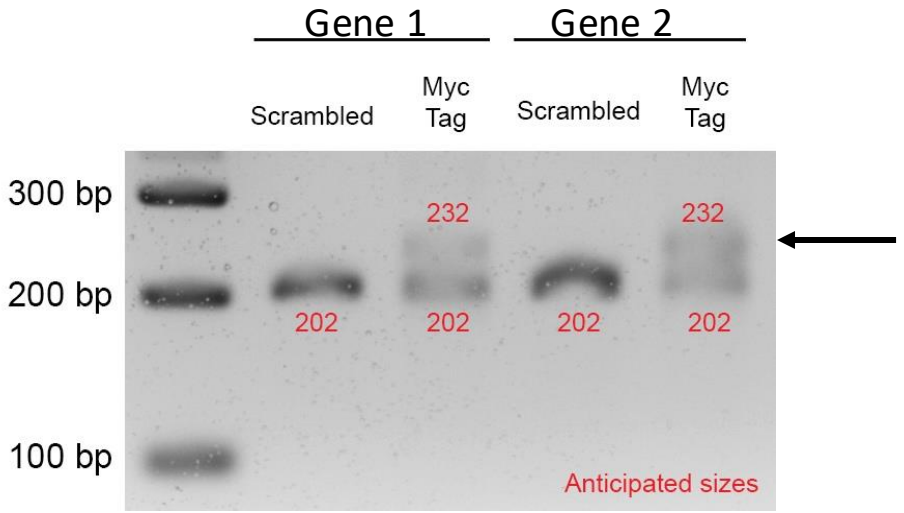
BFP: …CCACCCTGACCCATGGCGTGCAGTGCTTCAGCCGCTA…
GFP: …CCACCCTGACGTACGGCGTGCAGTGCTTCAGCCGCTA…

3 bp edit from BFP to GFP

Applications of BEL-A gene editing

RBC disease lines from BEL-A as research tools and drug screening platforms

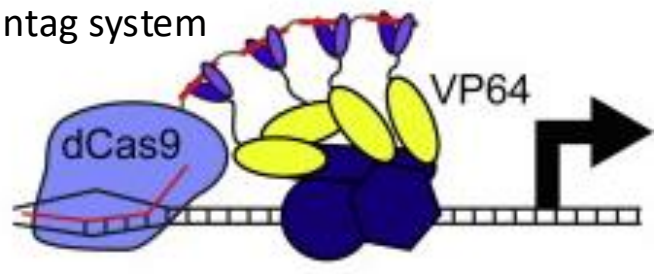
- β-thalassemia lines CD41/42 –TTCT, IVS1-1, IVS1-100
- α-thalassemia major lines
- Sickle cell disease lines β6Glu->Val
- CDA IV lines E325K KLF1



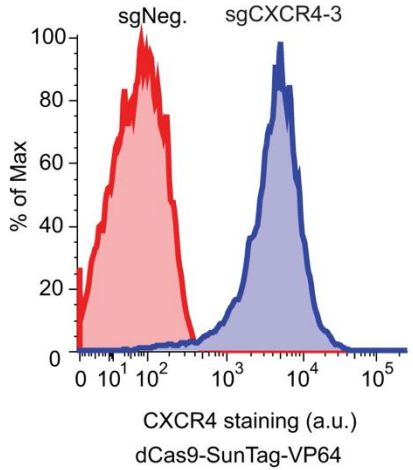
myc tag introduced at 5’ of genes

CRISPRa to induce/increase expression of endogenous genes in BEL-A

Suntag system



Tanenbaum et al Cell 159:635-646



Improving compatibility and properties of BEL-A erythroid cells

Improved compatibility or
make more “Universal blood”

Improved efficiency

Improved survival/storage characteristics

Improved oxygen carrying capacity and delivery

Defining the clinical requirement

Survey for problematic transfusion requirements where matched blood could not be fulfilled or supplies scarce

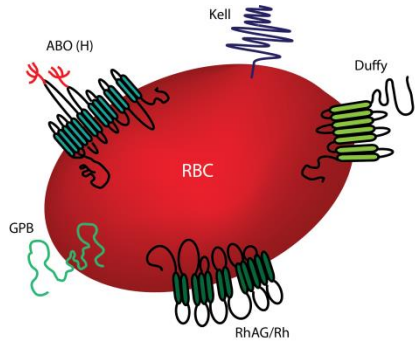
Blood Group System	Patients with Alloantibodies	Alloantibodies identified
MNS (GPB)	22	U, S, s
Rh	19	D, C, c, E, e, Hr^B, hr^B, Hr_0, MAR, C^w
Duffy	10 (+2)	Fy^a, Fy^b, Fy3
Kell	10	K, k, Kp^a
ABO (H)	8	H (Bombay Phenotype)
Lutheran	3	Lu^a, Lu^b
Kidd	3	Jk^b

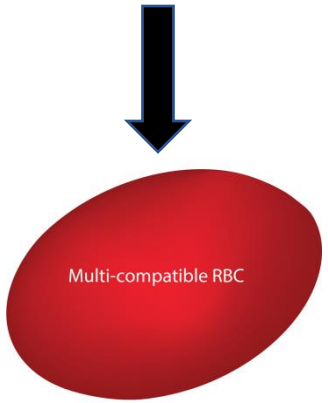
18 patients (mainly sickle cell) have alloantibodies to more than one blood group
48 of 56 patients could be serviced by the removal of just 5 blood group proteins

Individual null phenotypes occur naturally without clinical phenotype (mild for Rh null)

(11/2014-01/2015) + (05/2015-05/2016)

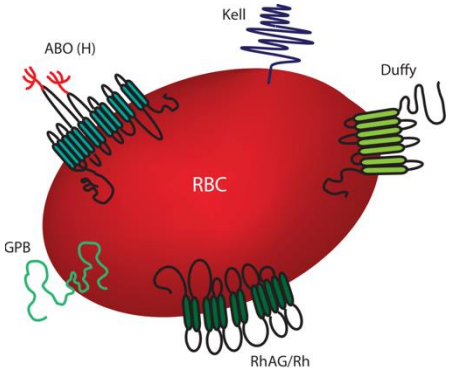
Raw data courtesy of Dr. Fiona Regan NHSBT, London.

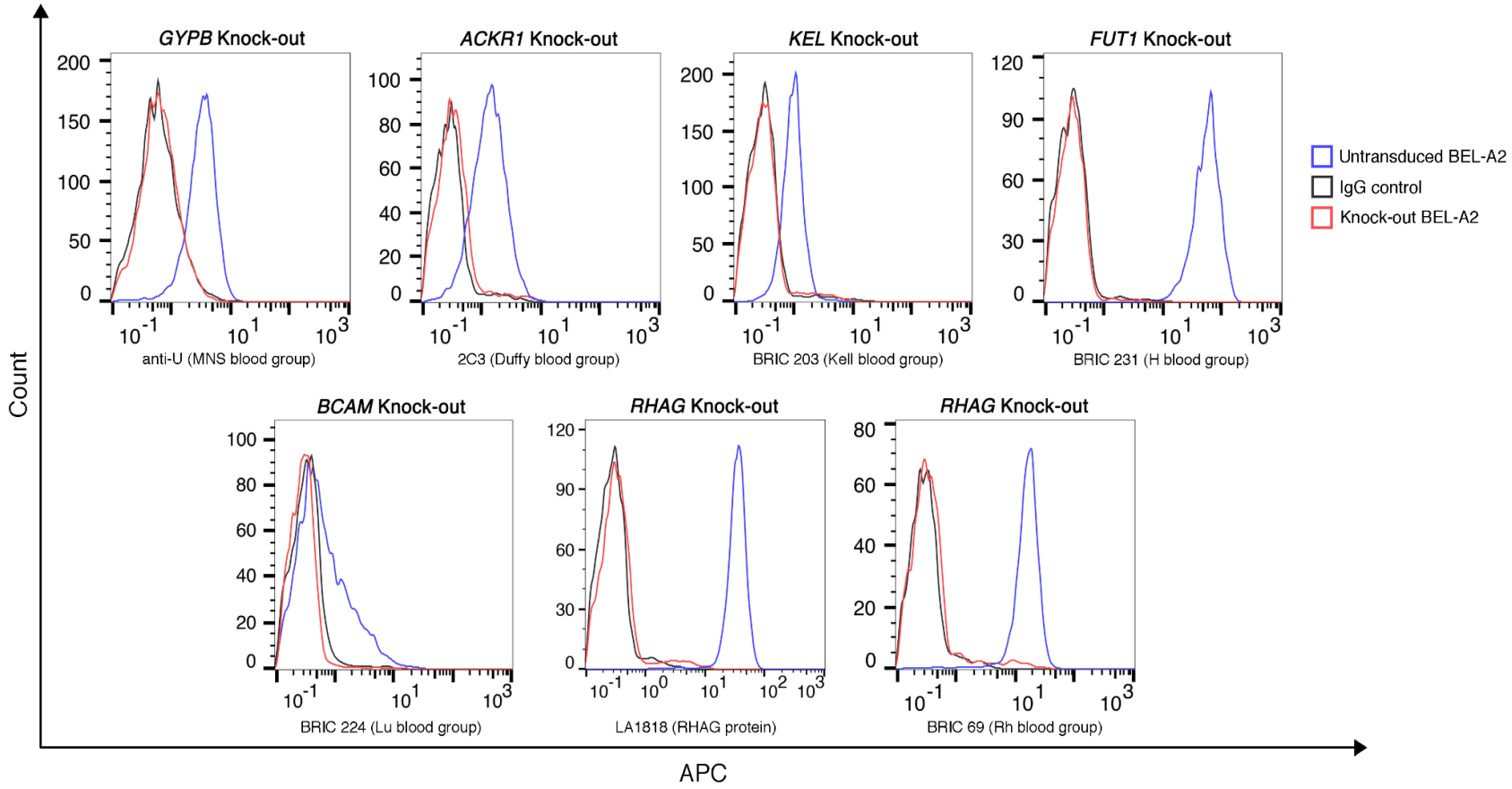




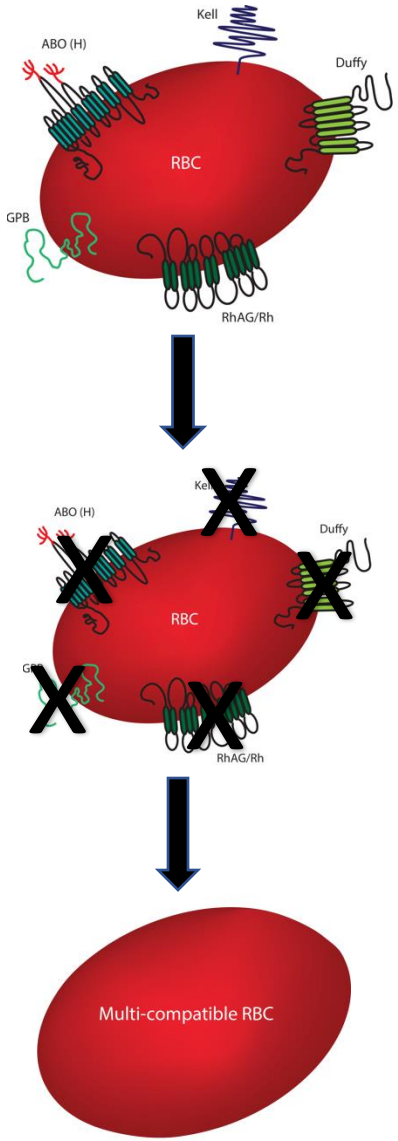
- Generate lines from individuals with relevant rare blood types
- Gene editing to customise cells with individual or combined multiple null phenotypes to broaden transfusion compatibility

Production of a bank of BEL-A sublines with single blood group knockouts

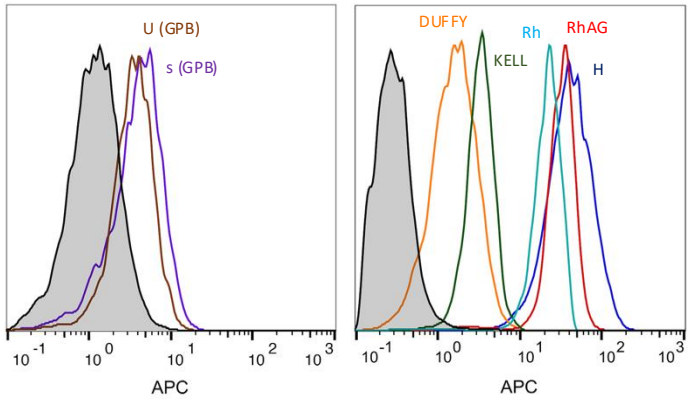


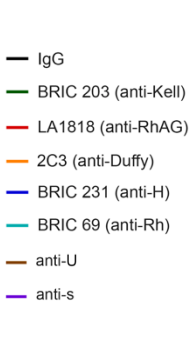


Engineering a more compatible red blood cell

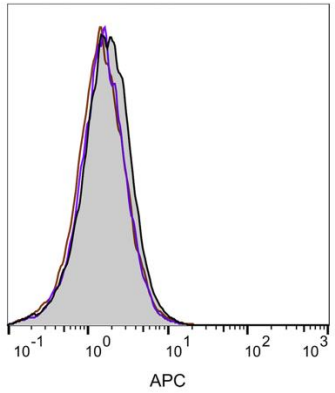


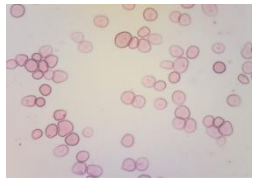
Control BEL-A Derived Reticulocytes

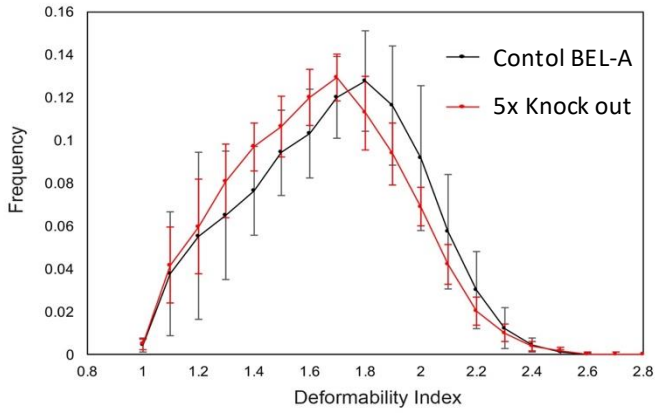


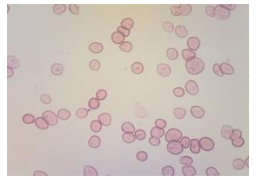


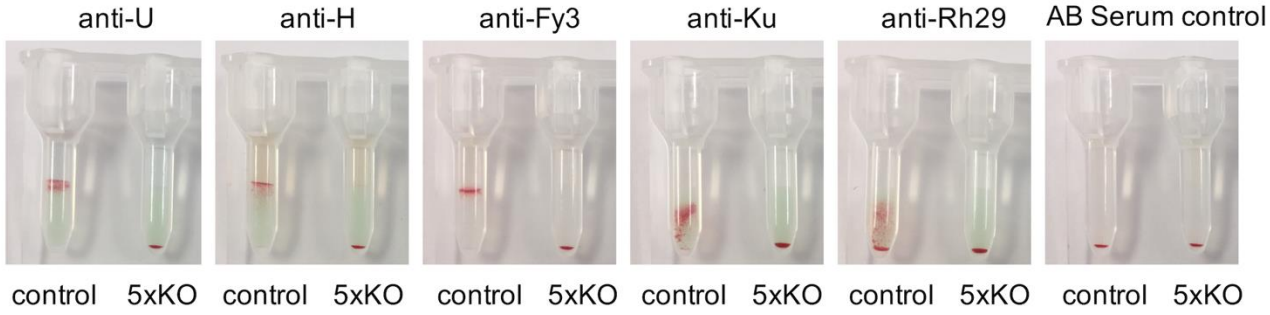
5x Knockout (GPB, Kell, Duffy, Rh_{null}, Bombay) Reticulocytes





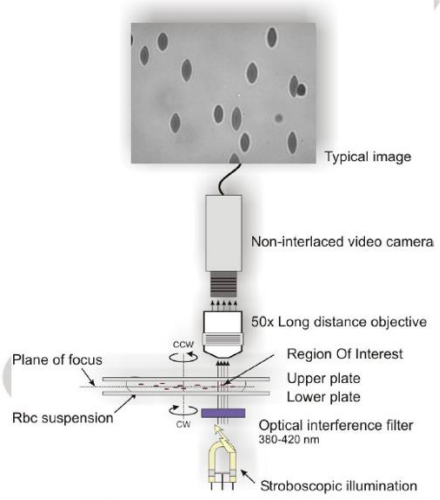






FLOW cytometry
Comparative proteomics

Automated Rheoscope Cell Analyser (ARCA)



Hawksworth *et al* EMBO Mol Med 2018

RBCs as drug delivery and targeting agents

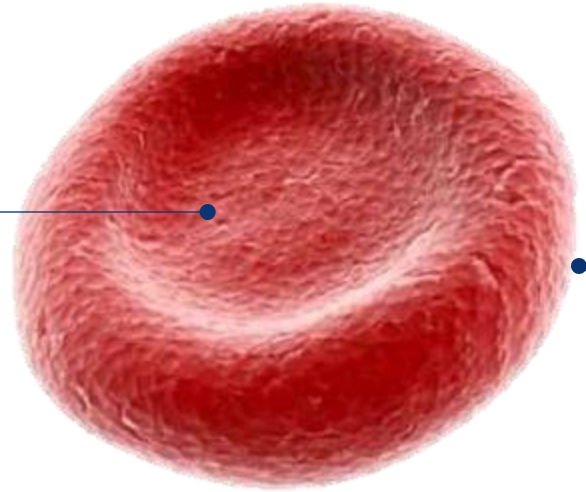
Engineering RBCs to add new functionality to solve clinical problems

Requires fewer cells than transfusion
Survival time
Biocompatibility
Immune system evasion
Biodegradability

Express enzymes & transporter systems

Functionalise cells to:

- Breakdown toxic metabolites in blood
- Replenish enzymes
- Deliver therapeutic proteins
- Reduce drug toxicity



Remove, replace or modify surface proteins

- Attachment to factors that remove compounds in circulation
- Recognition of pathogens
- Improved targeting to sites e.g. wounds/clots/cancer
- Enhanced delivery of drugs

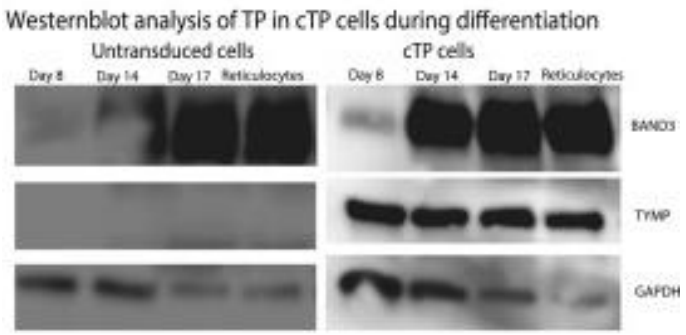
Engineer enzymes to improve kinetic parameters

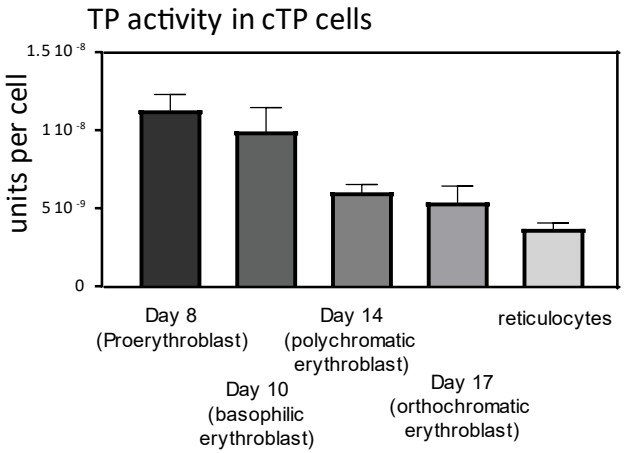
Engineering erythroid cells to express & retain functional thymidine phosphorylase

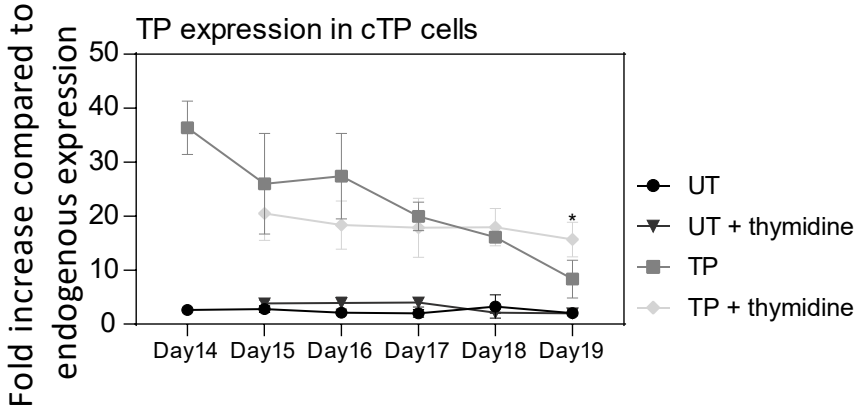
Deficiency of thymidine phosphorylase causes Mitochondrial Gastrointestinal Encephalopathy (MNGIE)

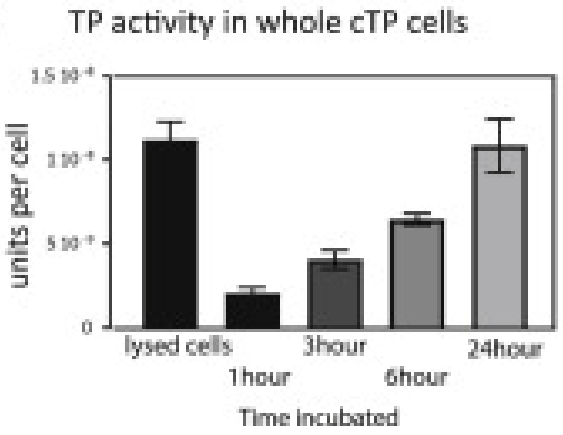
Patients have very high levels of thymidine in the body, which damages mitochondrial DNA and affects the gastrointestinal and nervous systems

Hypothesis: expression of thymidine phosphorylase in red blood cells could break down excess thymidine









Meinders et al Mol Ther Methods & Clinical Development 2020

RBCs as drug delivery and targeting agents

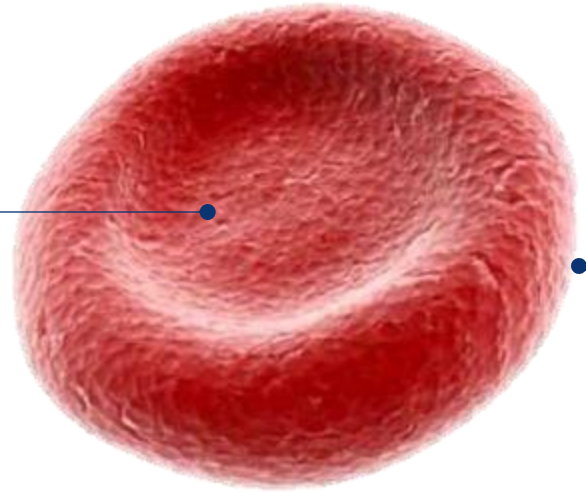
Engineering RBCs to add new functionality to solve clinical problems

Express enzymes & transporter systems

Functionalise cells to:

- Breakdown toxins in blood
- Replenish enzymes
- Deliver therapeutic proteins
- Reduce drug toxicity

Engineer enzymes to improve kinetic parameters

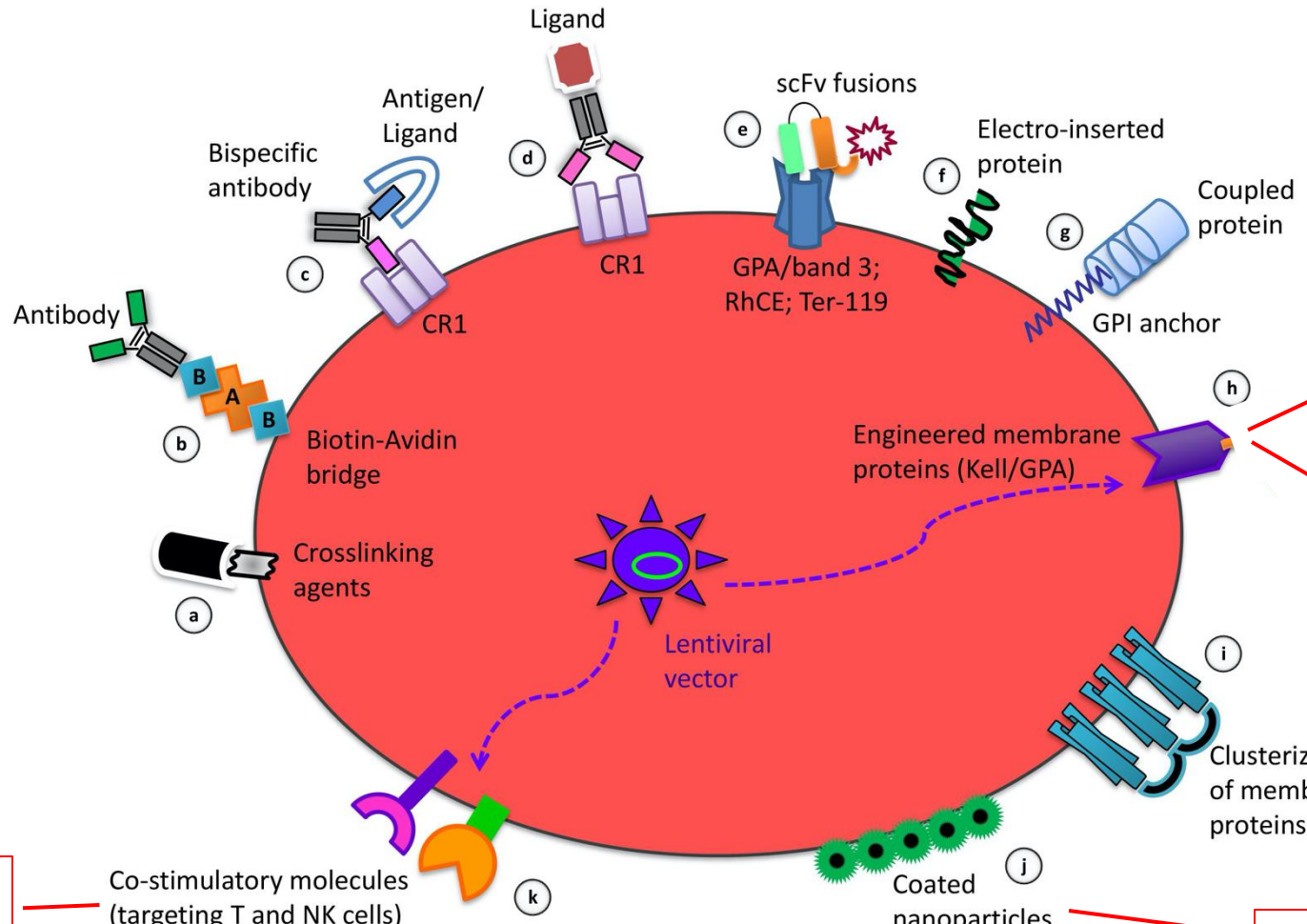


Remove, replace or modify surface proteins

- Attachment to factors that remove compounds in circulation
- Recognition of pathogens
- Improved targeting to sites e.g. wounds/clots/cancer
- Enhanced delivery of drugs

Tagging surface of RBCs

Ectopic expression of tagged membrane protein/hybrid protein
Tag endogenous gene using CRISPR
Or tagging surface mature RBC



Viral traps

prolonged protection against bacterial toxins

targeting to liver and spleen

Cancer treatment –solid tumors

Deliver increased levels of NPs.

Modified from Rossi et al 2019 Front. Physiol. 10:1070

RBCs as drug delivery and targeting agents

Expression of enzymes & transporter systems

Functionalise cells to:

- Breakdown toxins in blood
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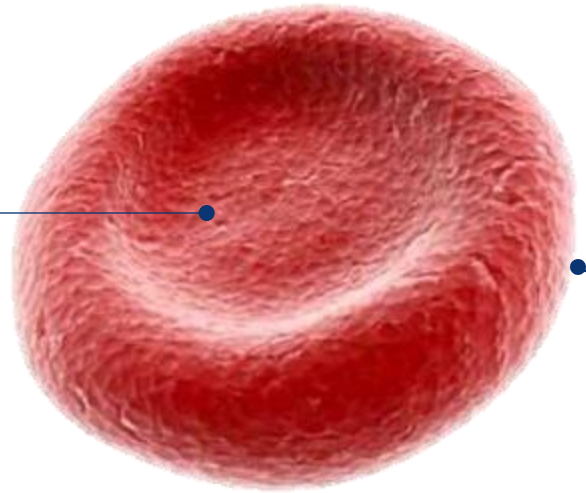
Engineer enzymes to improve kinetic parameters



Modification, replacement or addition of surface proteins

- Attachment to factors that remove compounds in circulation
- Recognition of pathogens
- Improved targeting to sites e.g. wounds/clots/cancer
- Enhanced delivery of drugs

Summary

- We can grow red blood cells in the laboratory, with identical features to endogenous cells
- Developed immortalised erythroid cell lines with phenotype of donor, providing a sustainable supply of banked red cells
- Genetic engineering of BEL-A to create desirable or multi-compatible red cells by gene knockout - with knock ins now possible meaning selected blood group polymorphisms can be introduced. Engineered cells can be banked
- Genetic engineering for enhanced red cell properties
- Genetic engineering to utilize red cells as drug delivery and targeting agents

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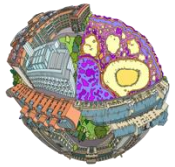
Sabine Kupzig
Nicola Cogan

RESTORE Team

NHS Blood and Transplant


BrisSynBio
biomolecules to biosystems
from understanding to design

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

[dstl]

MRC Medical Research Council

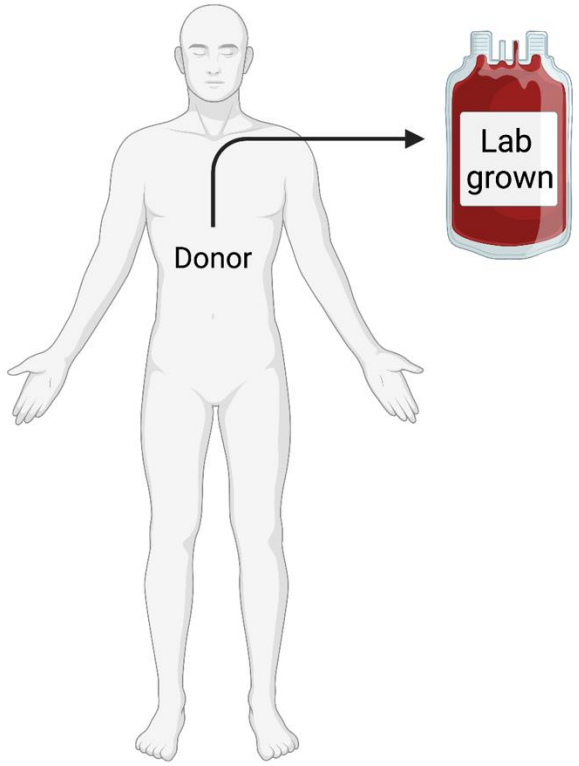
wellcome trust

Nanocarriers

Natural Polymers			
• Chitosan • Pullulan • Dextran • Hyaluronic acid	• Cycloamylose • Glucan • Curdlan	• Dextrin • Gelatin • Hydroxypropyl cellulose	• Alginate • Heparin • Chondroitin sulfate

Synthetic Polymers
• Polyethylene glycol • Polyacrylamide • Polyglycerol • Various polypeptides

Donor selection for lab grown blood



Considerations:

Currently ISBT recognises ~378 blood group antigens of which 345 fall into one of ~43 Blood group systems

Type O blood is accepted as the most ”Universal”

After ABO antigen the RhD antigen is most immunogenic followed by

K, E, c, $Fy^{a,}$ Jk^{a} and S antigens

It has been calculated using previous blood requirements for France that 2 donor derived iPSC cell lines could theoretically cover 98.6% of patient blood needs in France*

*Peyrard et al Transfus Med Rev 2011