



Gekweekte rode bloed cellen, de toekomst?

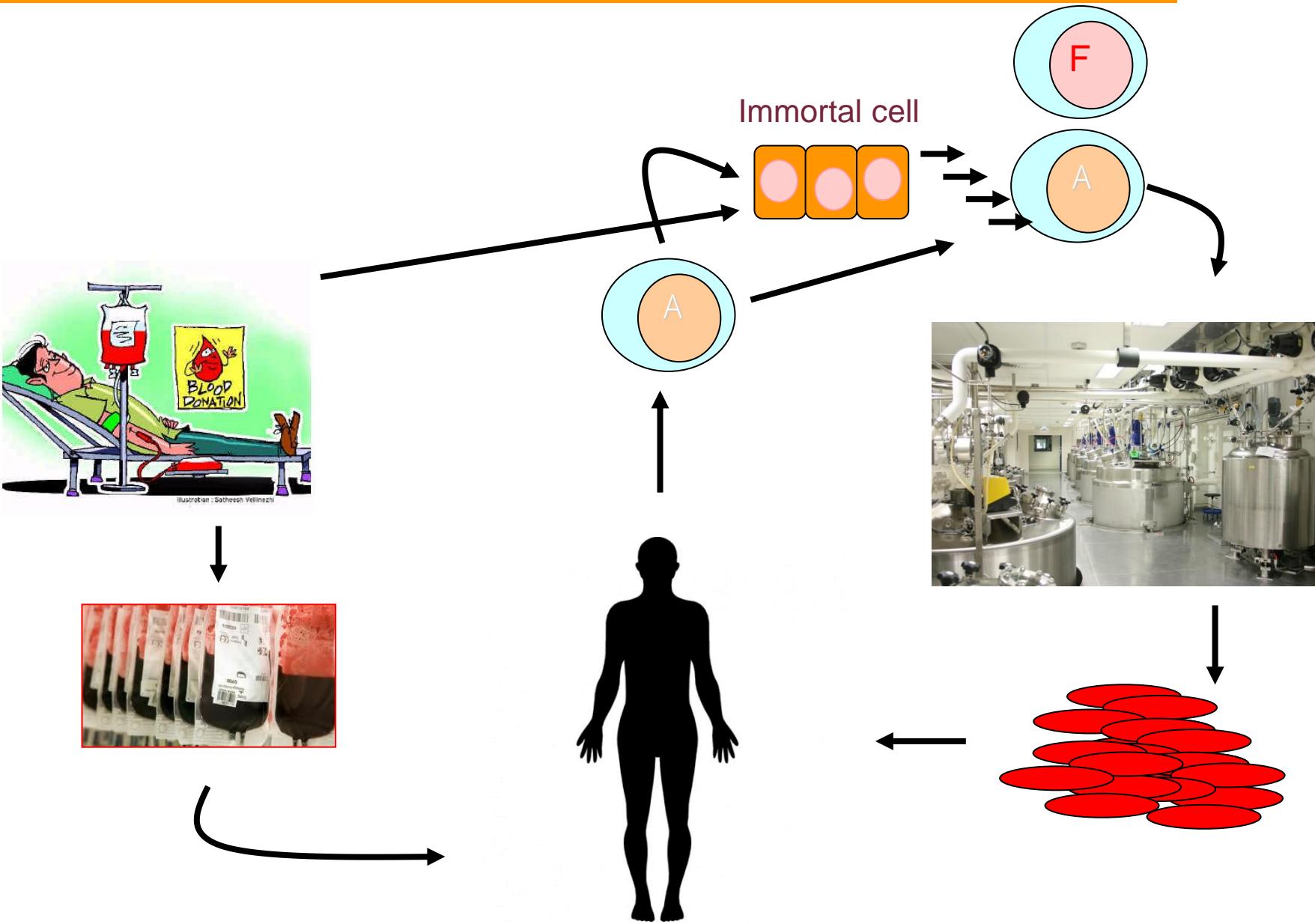


RESEARCH | DIAGNOSTICS | PHARMACEUTICALS

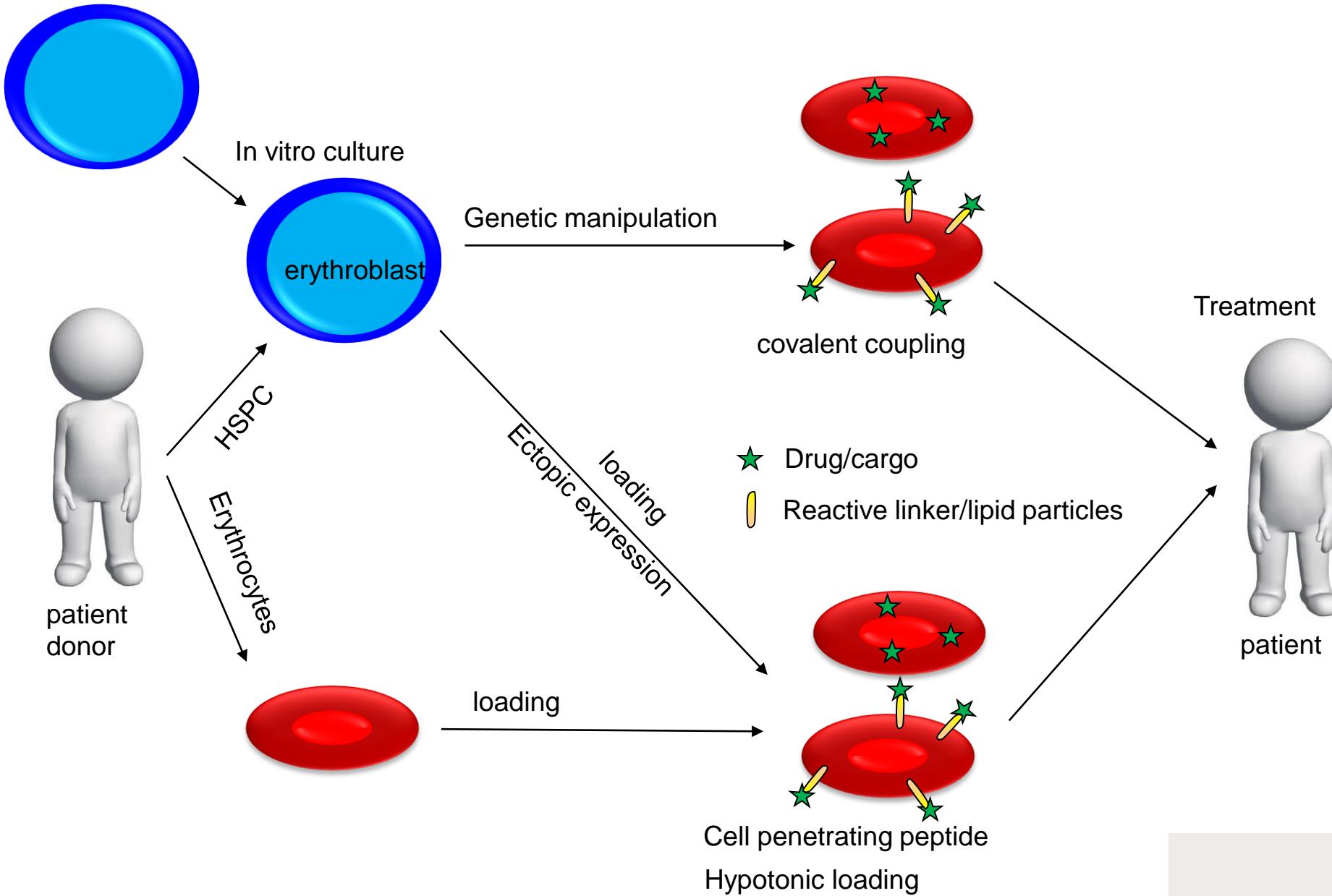
Why?**To avoid****allo-immunisation
blood borne disease****Novel therapeutic applications****To be less donor dependent****For whom?****Patients****with (very) rare bloodgroup antigens
requiring recurrent transfusions
(Thalassemia, Sickle disease)****Functionally enhanced, therapeutic RBC****Basic research on erythropoiesis**

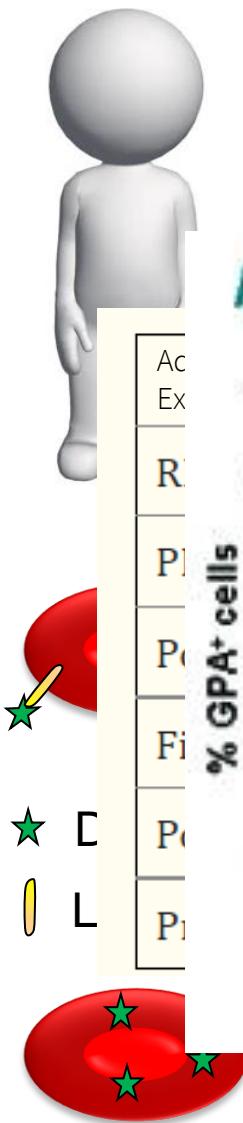
70-75% of all body cells
production: 2 500 000 /sec
 2×10^{12} erythrocytes / transfusion unit

In vitro produced erythrocytes: the future!?



Immortalized cells (iPSC, erythroblasts)

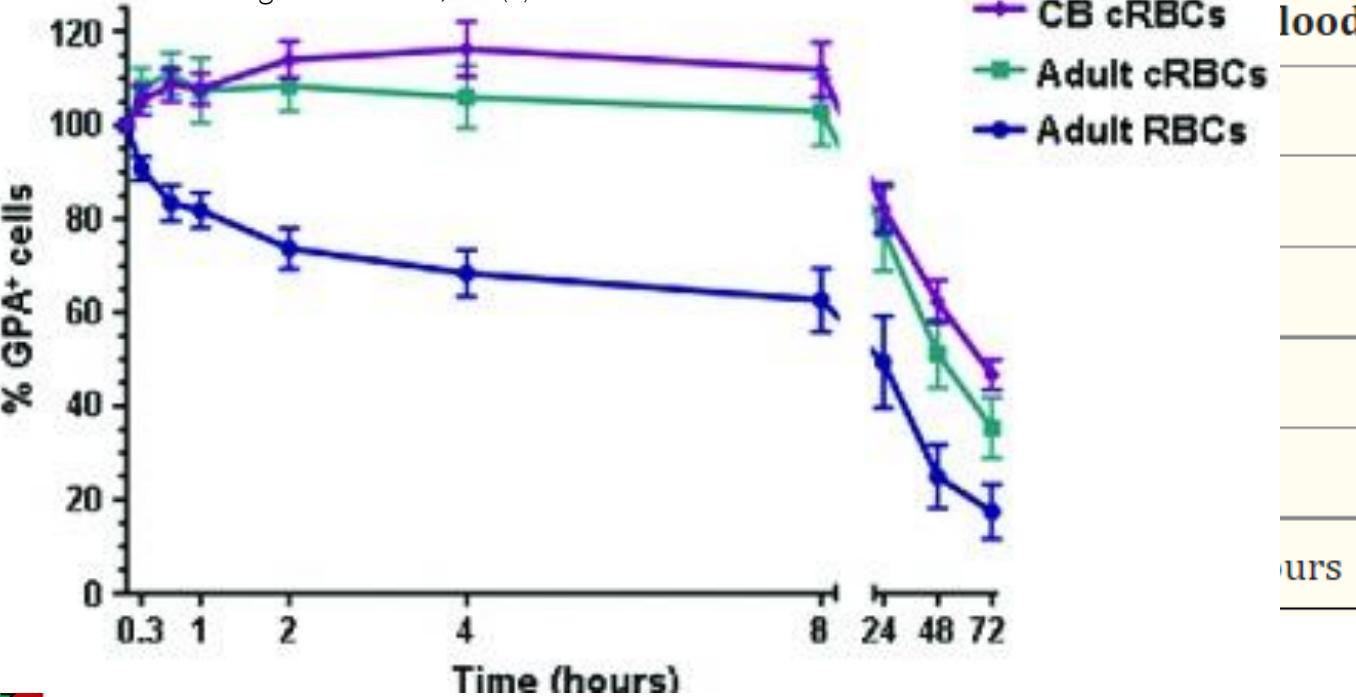




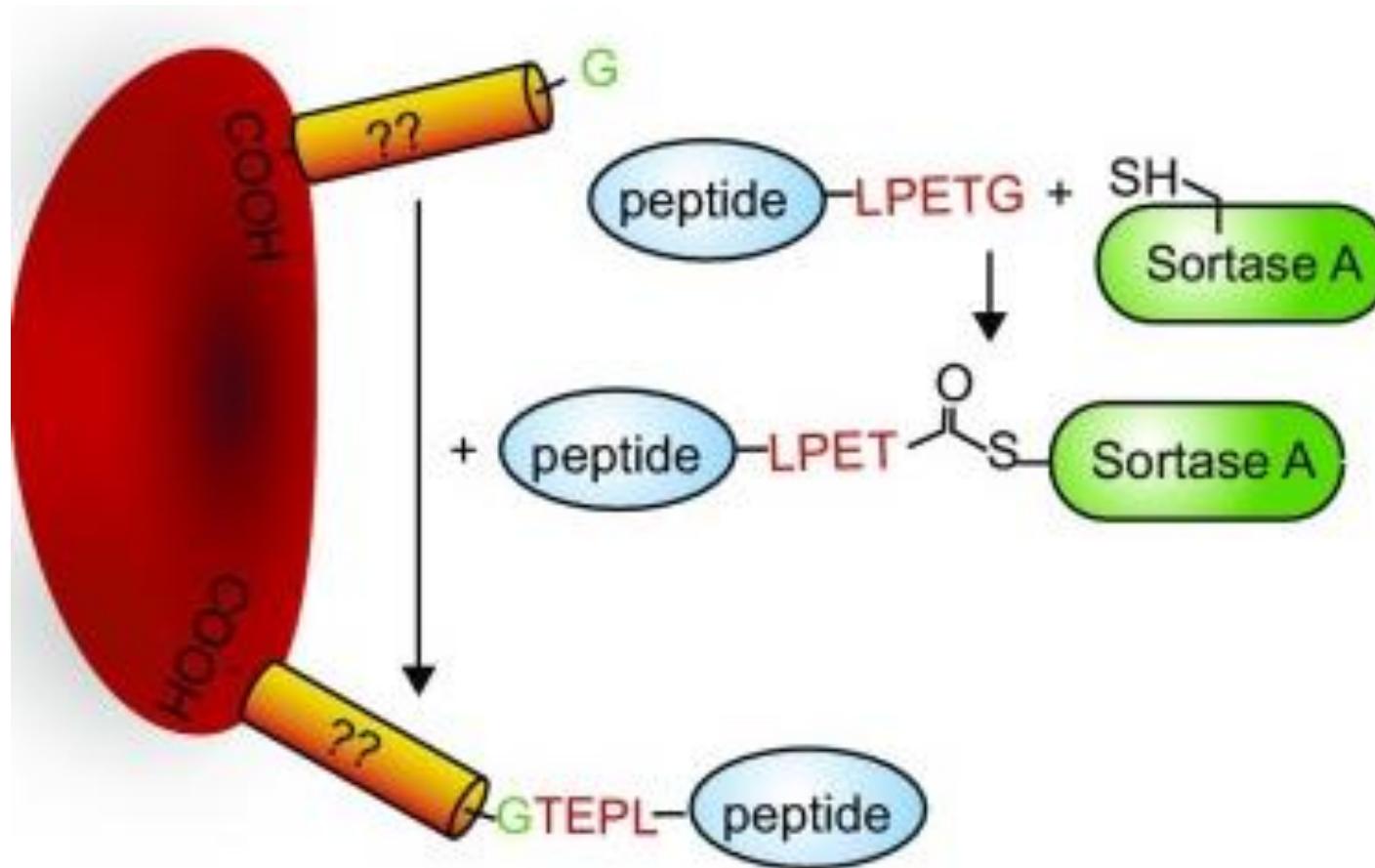
Why: Half life of chemotherapy

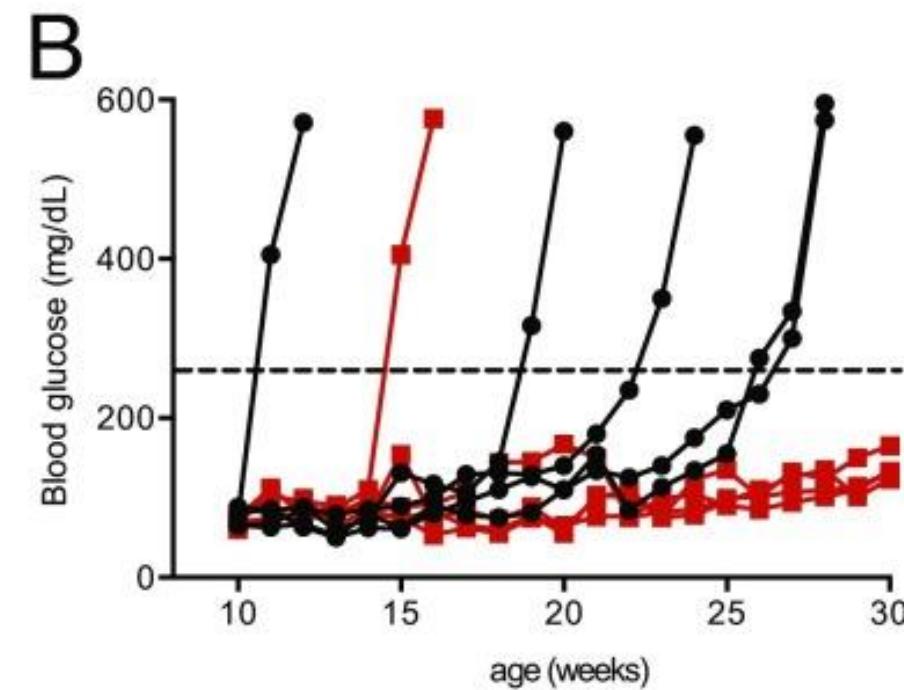
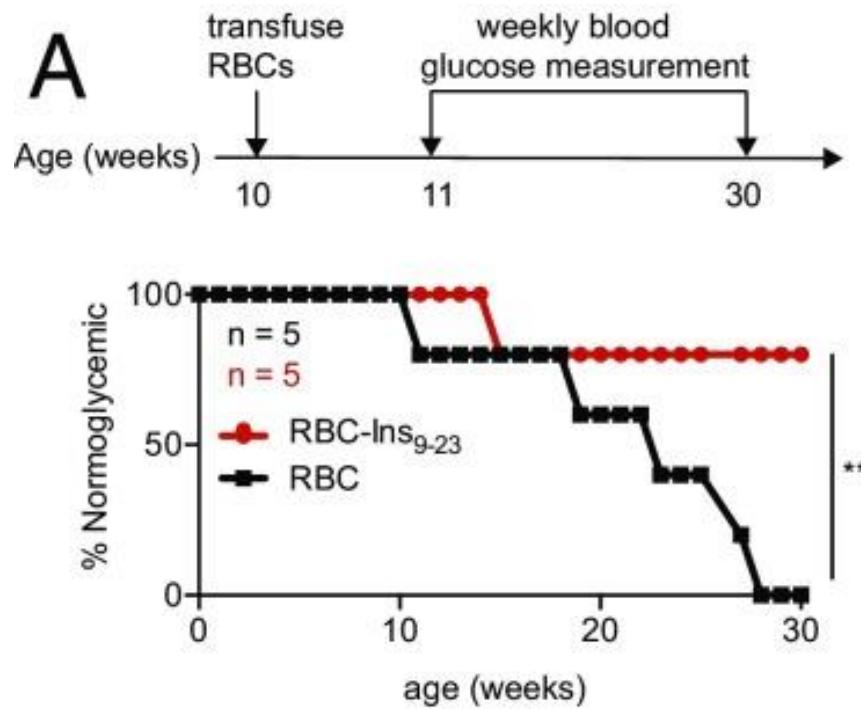
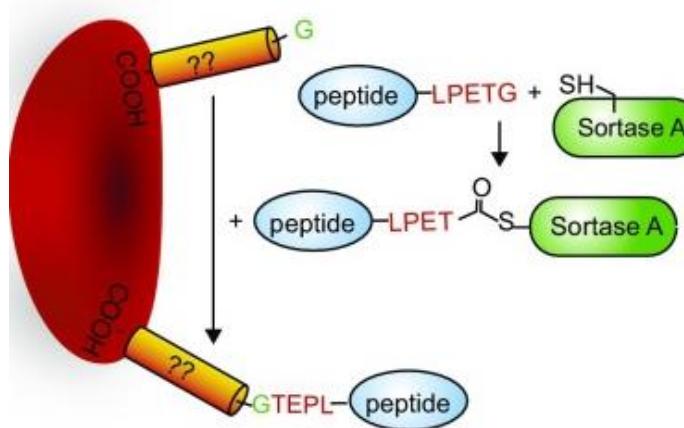
A

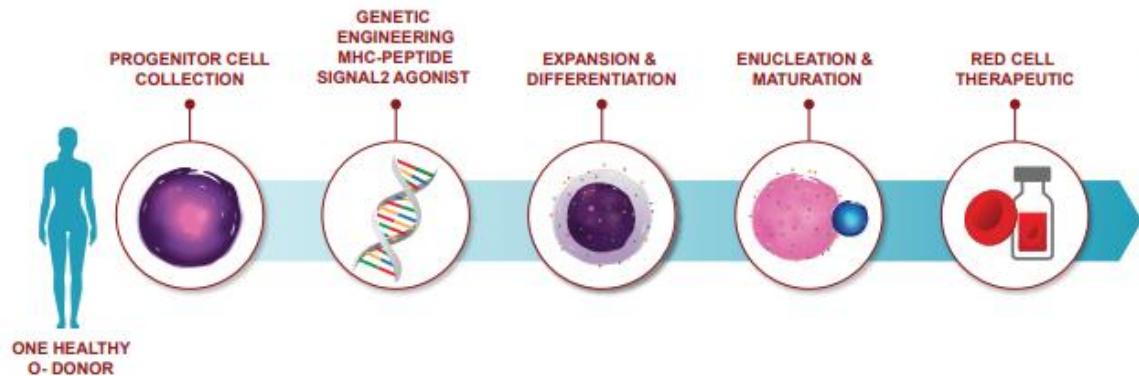
Superior survival of ex vivo cultured human reticulocytes following transfusion into mice
Sabine Kupzig, Stephen F. Parsons, Elinor Curnow, David J. Anstee, and Allison Blair
Haematologica. 2017 Mar; 102(3): 476–483.



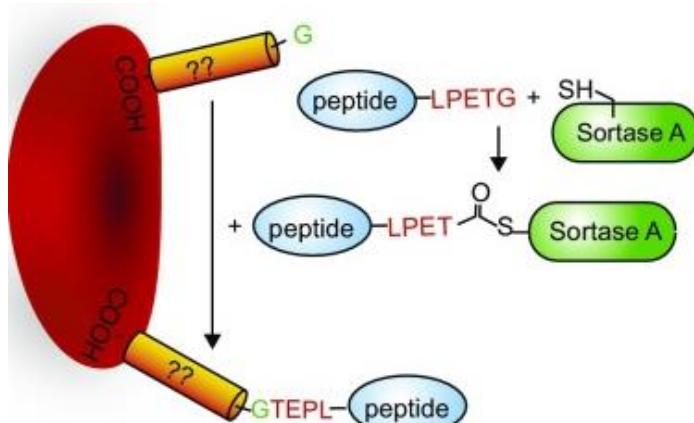
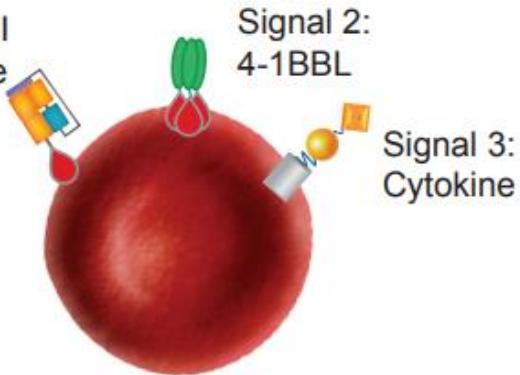
Company	Technology	Target disease/application
Erydel	Hypotonic swelling and resealing for loading of dexamethasone sodium phosphate	Ataxia telangiectasia
Erytech	RBC encapsulated: 1. L-asparaginase 2. Methionine-γ-lyase, Arginine deiminase 3. Therapeutic enzymes 4. Tumor antigens	1. Various leukemias including acute lymphoblastic leukemia and acute myeloblastic leukemia and solid tumors including pancreatic cancer 2. TBD cancer target 3. Enzyme replacement 4. Tumor vaccination
Orphan Technologies	Encapsulation of thymidine phosphorylase	Mitochondrial neurogastrointestinal encephalomyopathy
Rubius	Genetically modified hematopoietic stem cells are turned into RBCs in culture that eventually express therapeutic proteins	Treat metabolic diseases, autoimmune diseases, and cancer
Anokion	Engineered proteins are attached to circulating RBCs following IV injection, and via natural cell death the RBCs and the attached engineering proteins are processed by immune cells for enhanced antigen processing	Inducing protein specific immune tolerance without immunosuppression



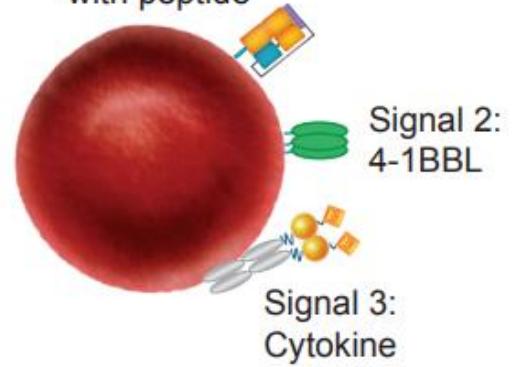


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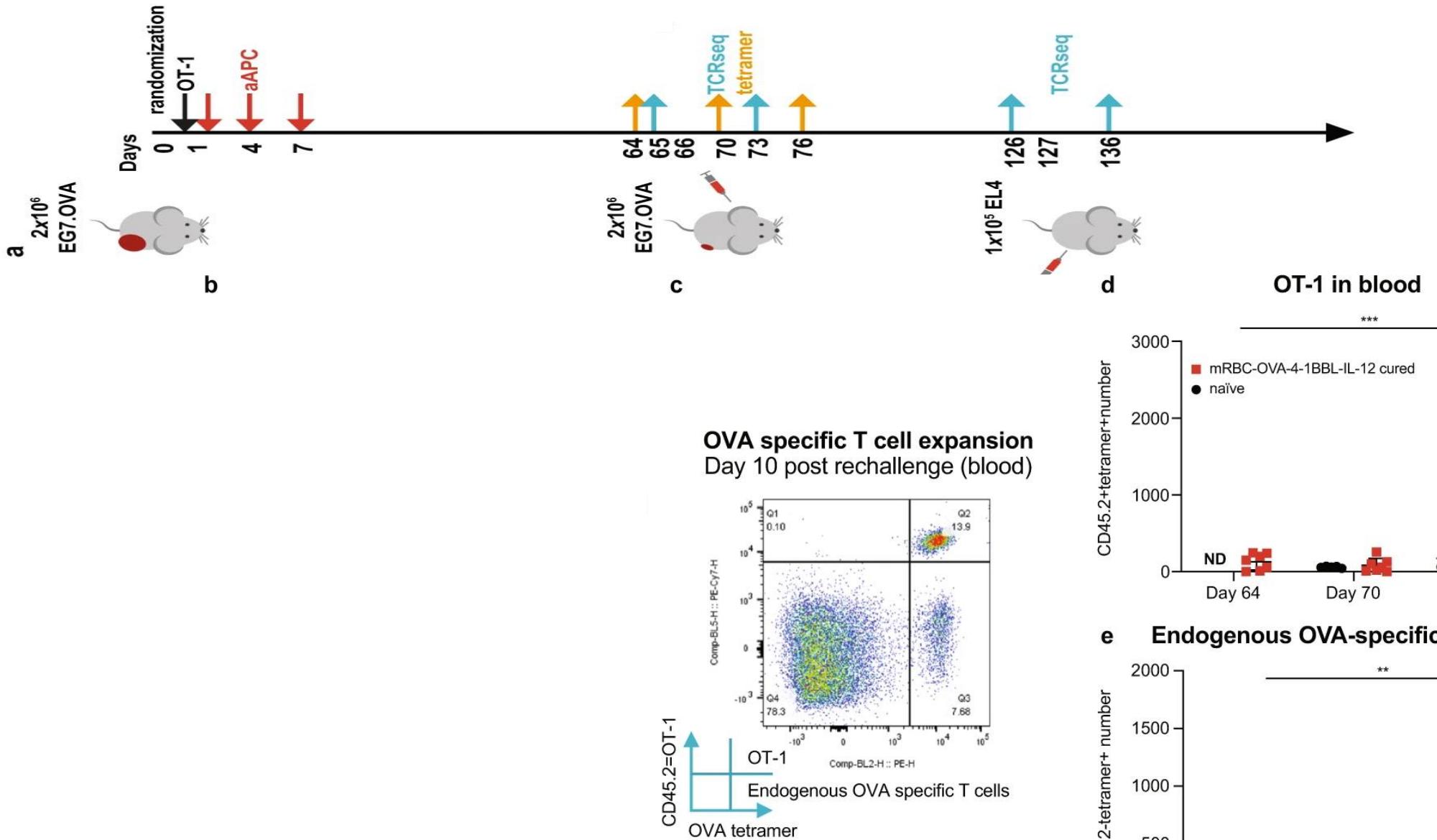
Signal 1:
MHC class I
with peptide

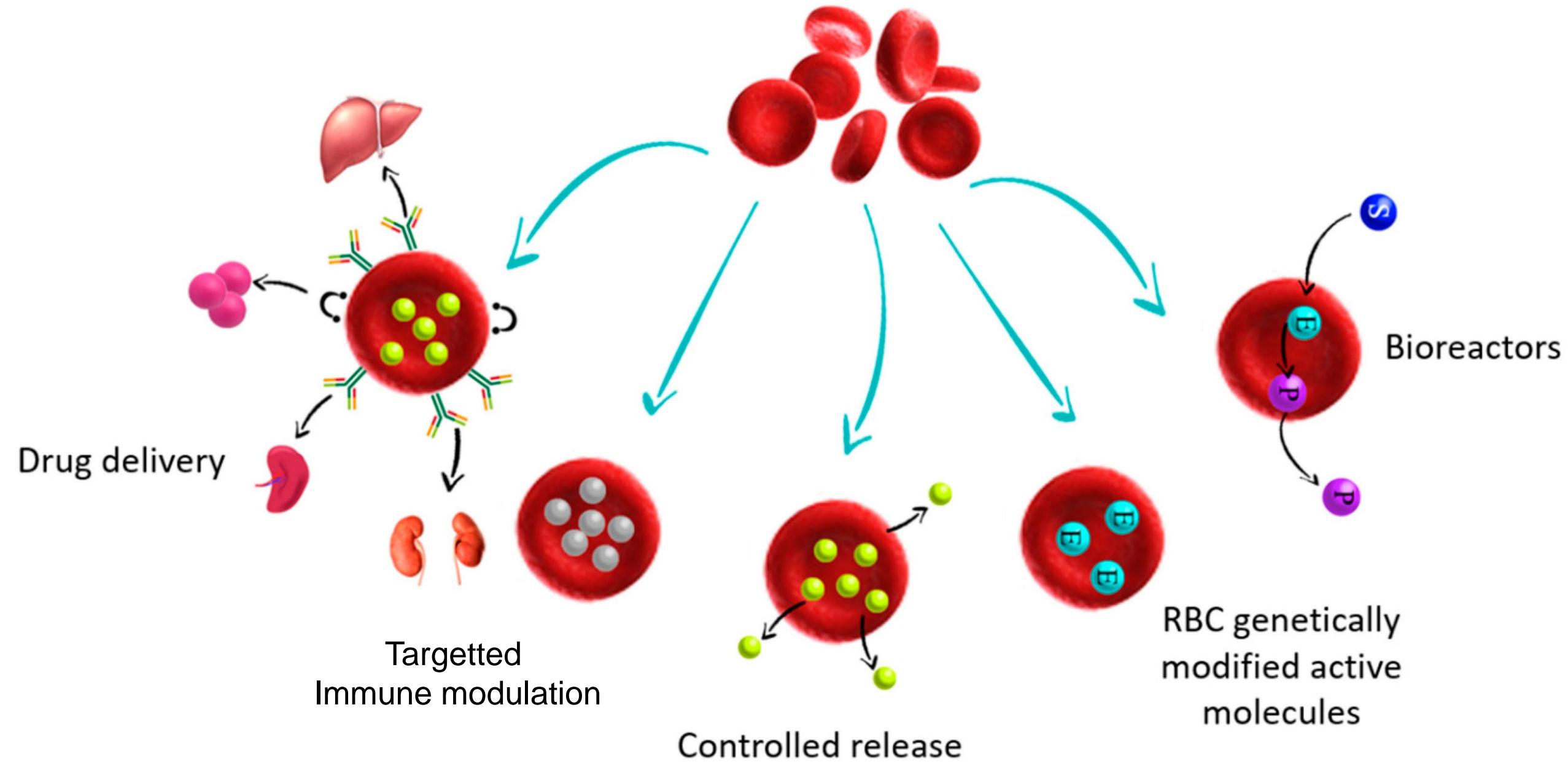


Signal 1:
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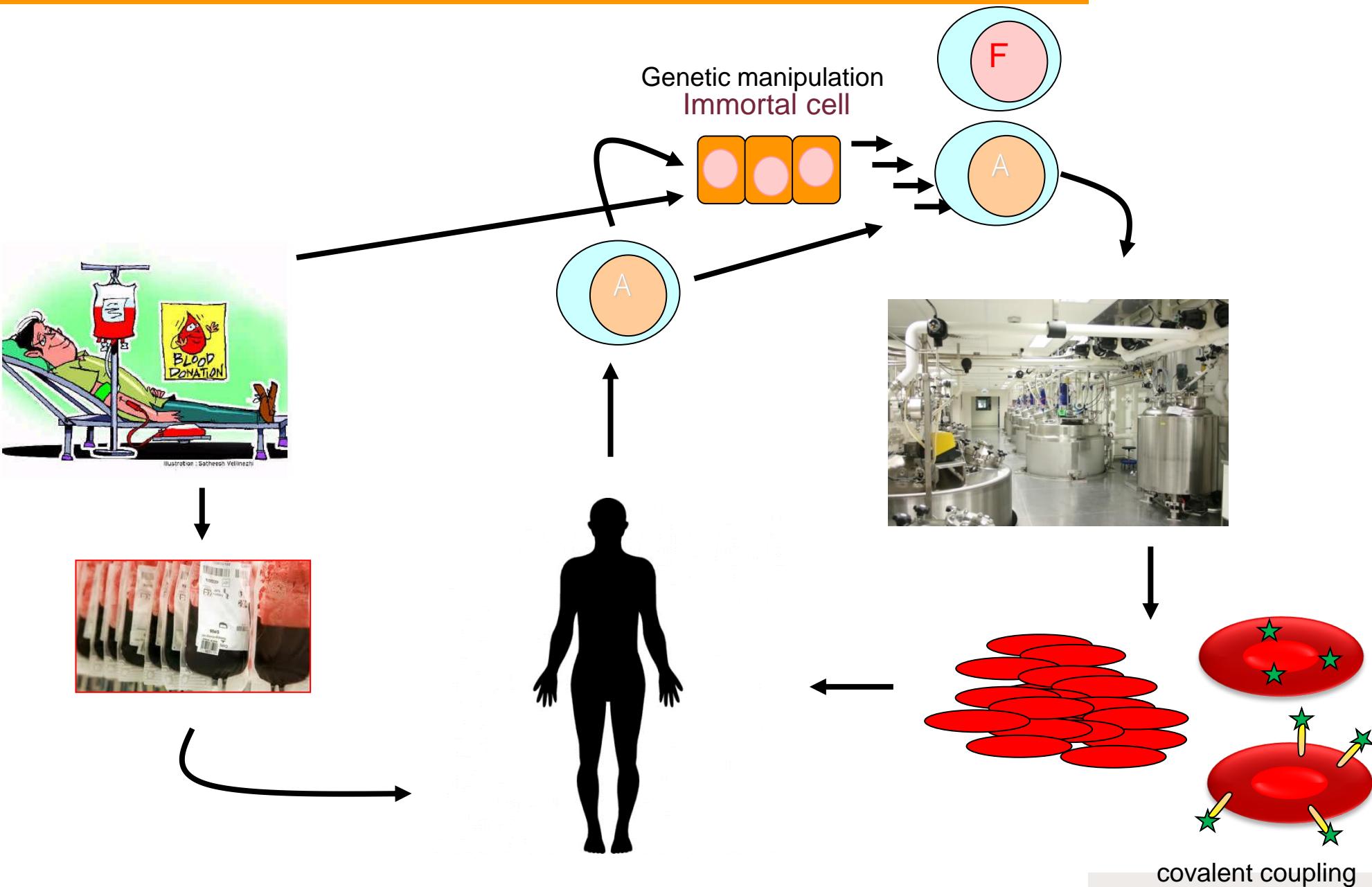


Clicked RBCs

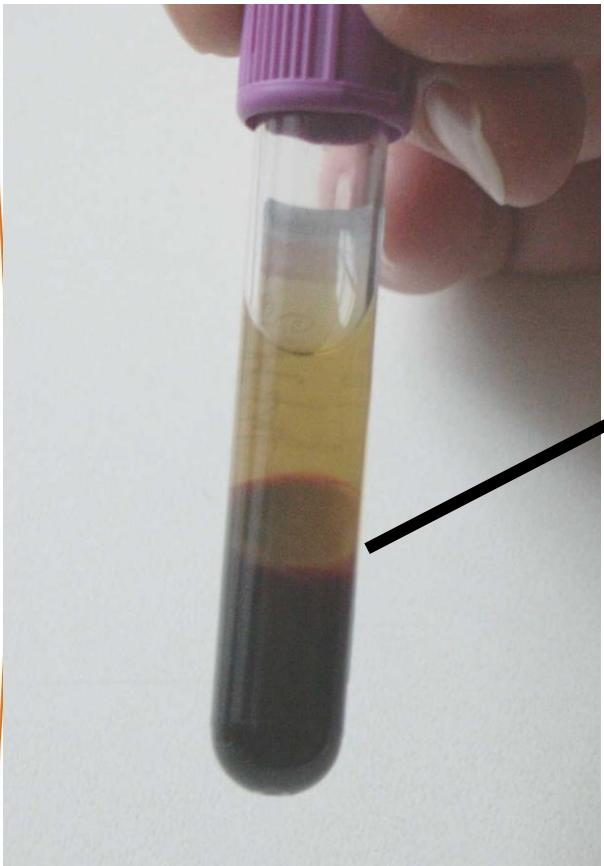




In vitro produced erythrocytes: the future!?



Erythropoiesis and erythrocytes: the daunting numbers



Between 40-48% of blood is erythrocytes
(~ 20-30 trillion ($2-3 \cdot 10^{13}$))

Blood concentration: 5 million red blood cells/ μl

Average life span: 120 days; travels 450km

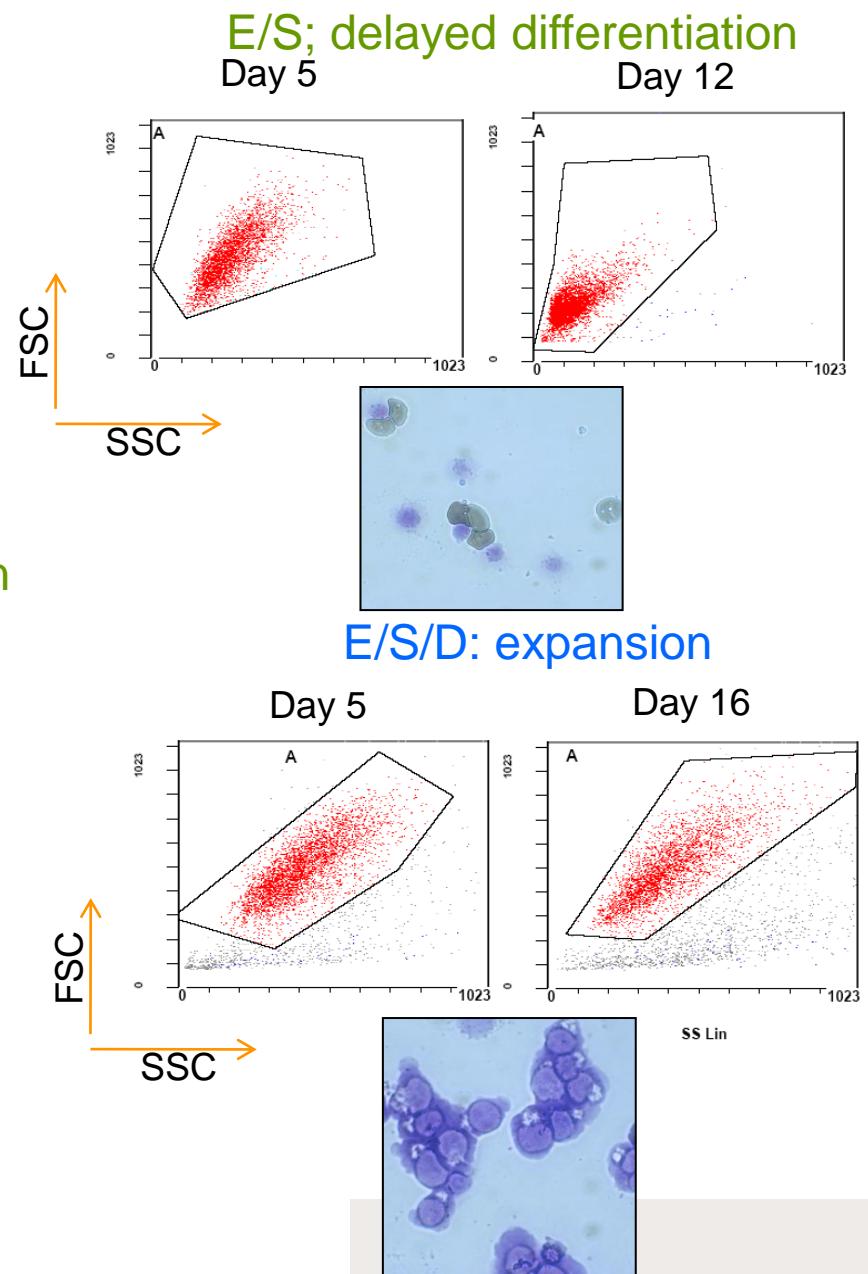
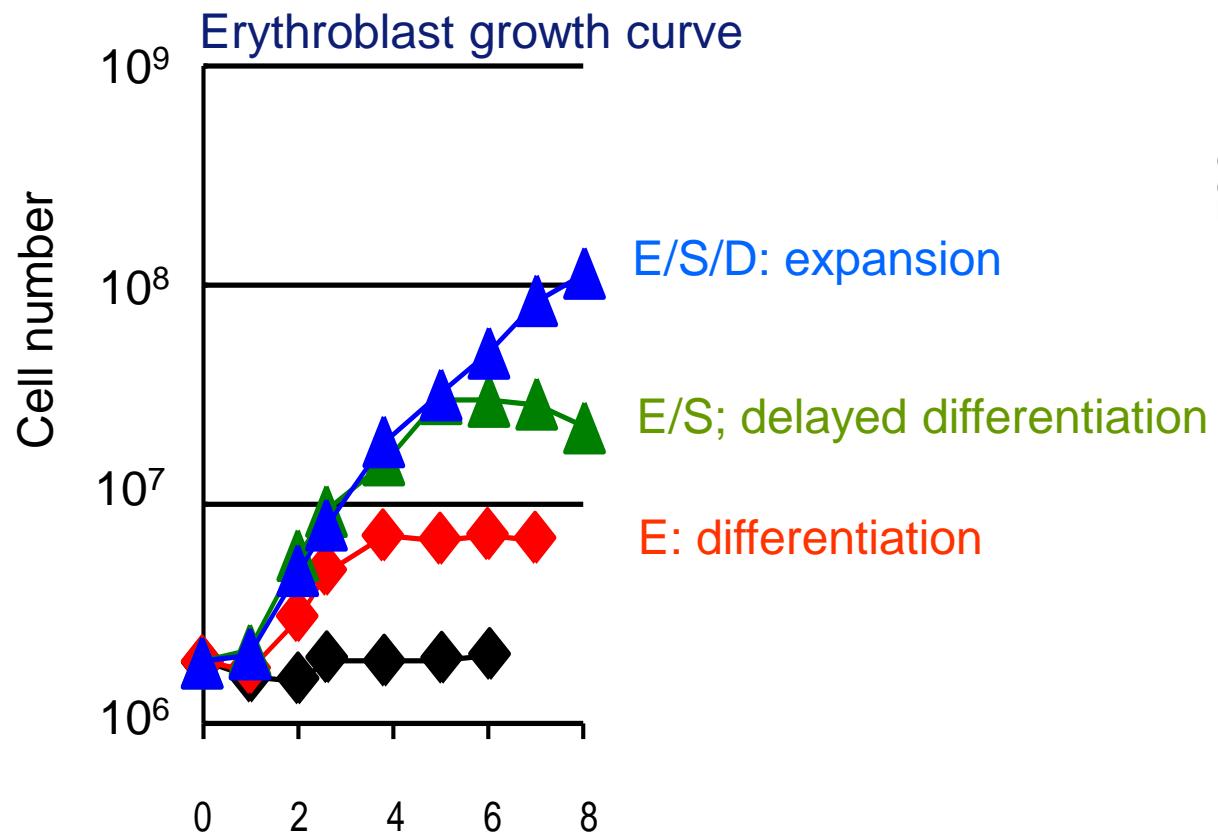
Production: 2.4 million red blood cells/second

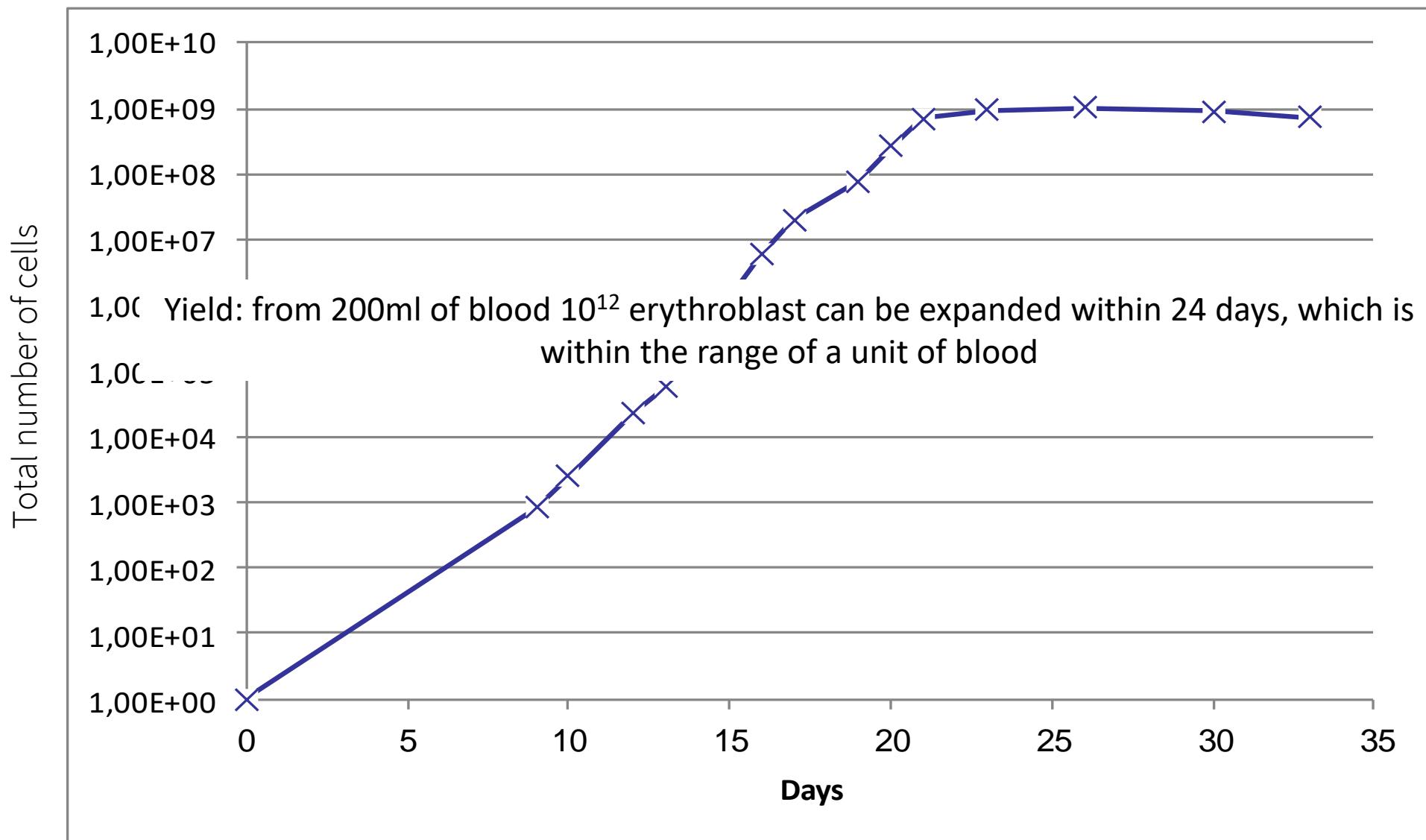
~270 million hemoglobin molecules in 1 red blood cell

2.5 grams of Iron is stored in total

1- $2 \cdot 10^{12}$ erythrocytes in one transfusion bag or unit of blood (around 400ml blood or 150-200ml packed)

Stress erythropoiesis model system





Static cultivation is inadequate for large scale production of cRBCs



BIOTECHNOLOGY
and
BIOENGINEERING**Expansion and differentiation of ex vivo cultured erythroblasts in scalable stirred bioreactors**

✉ Joan Sebastián Gallego-Murillo, Giulia Iacono, ✉ Luuk A.M. van der Wielen, ✉ Emile van den Akker,
✉ Marieke von Lindern, ✉ Sebastian Aljoscha Wahl

doi: <https://doi.org/10.1101/2022.02.11.480112>



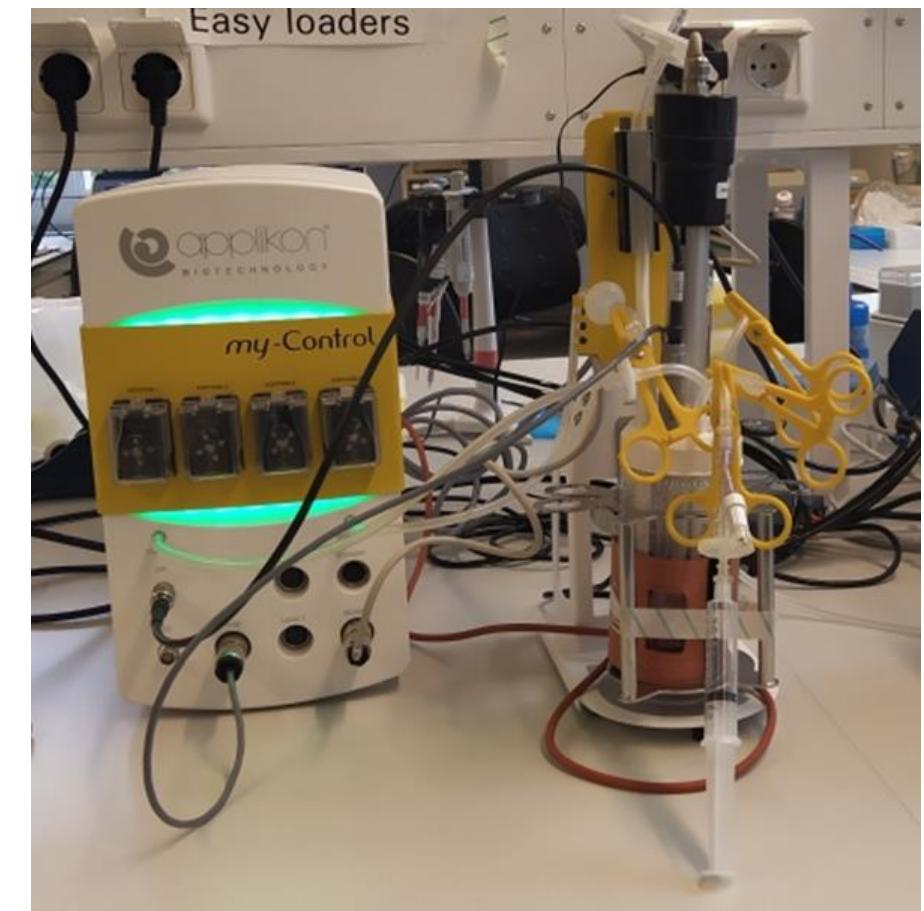
Cell density limit

Media requirements

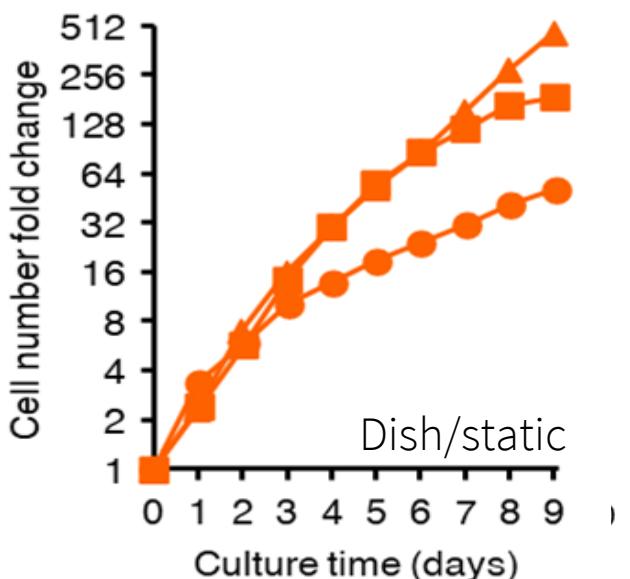
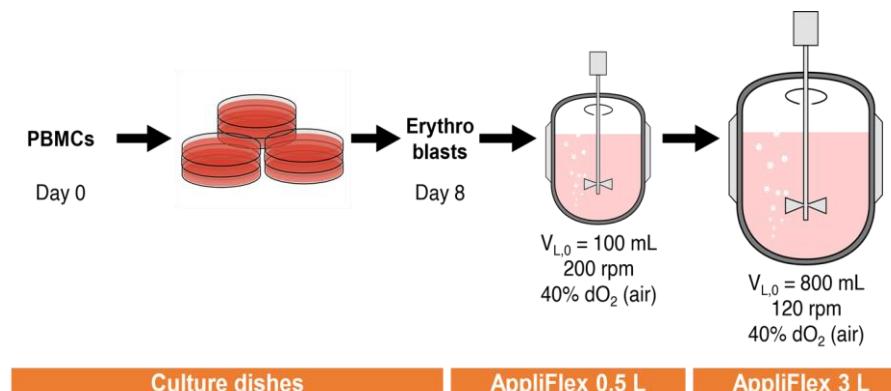
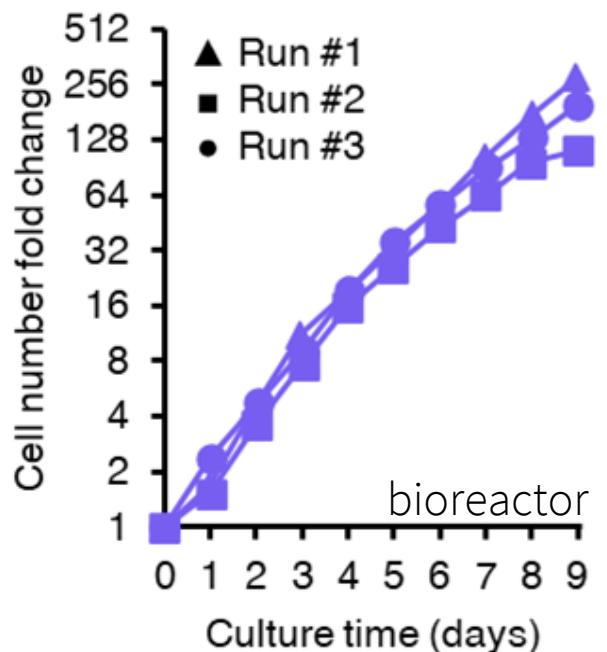
Oxygen requirements

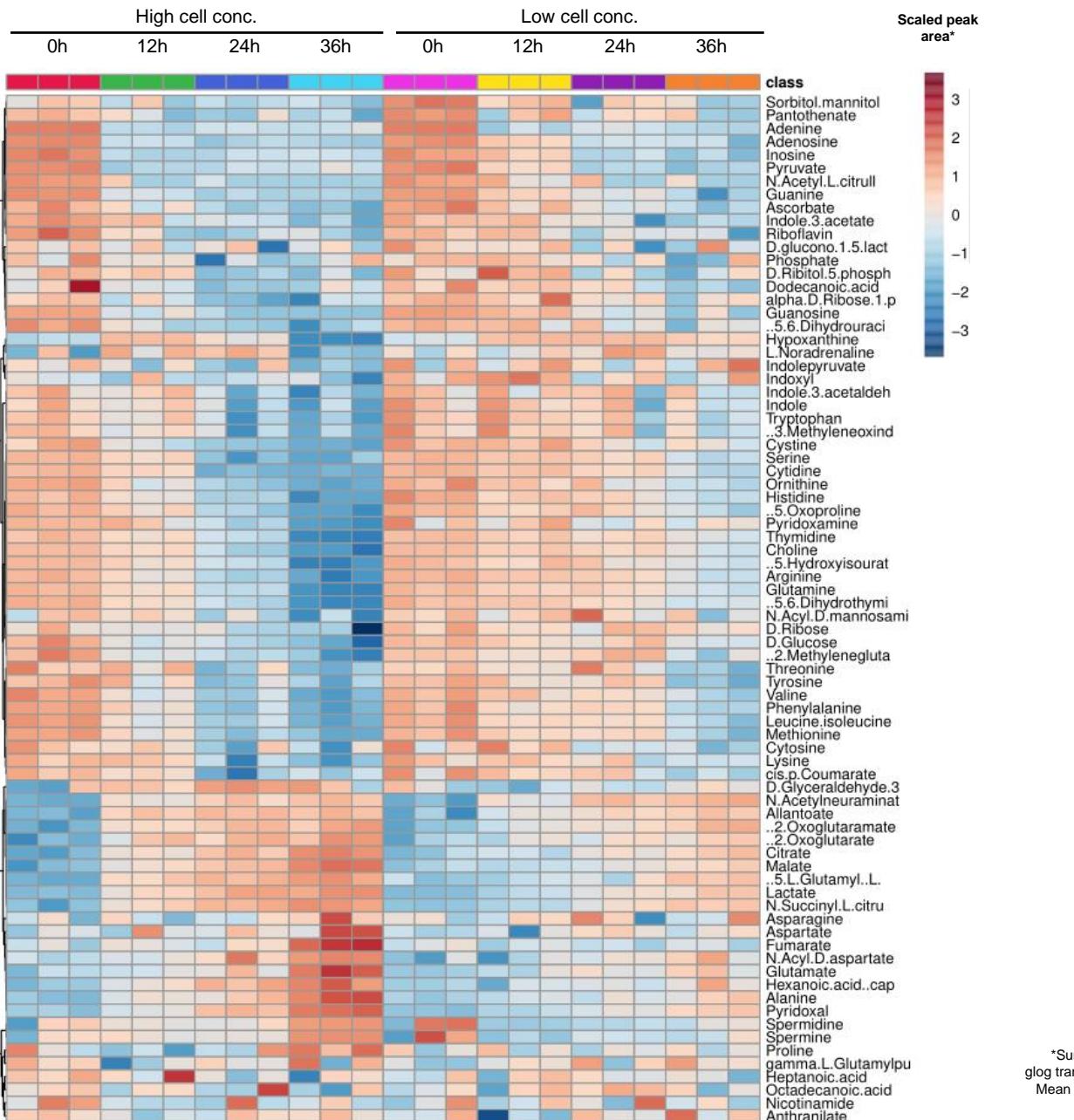


Growth factors



Similar expansion in dish and bioreactor



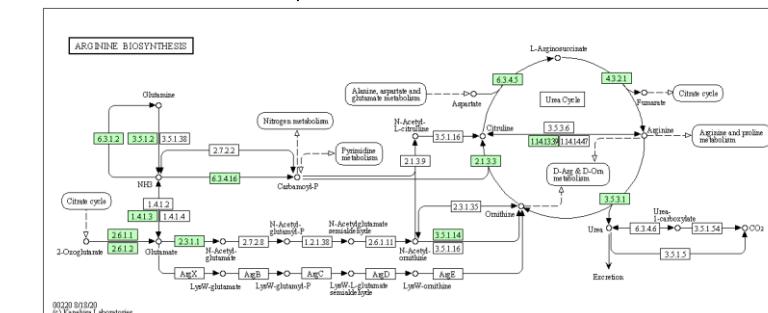


Metabolomics / multiomics to improve medium conditions

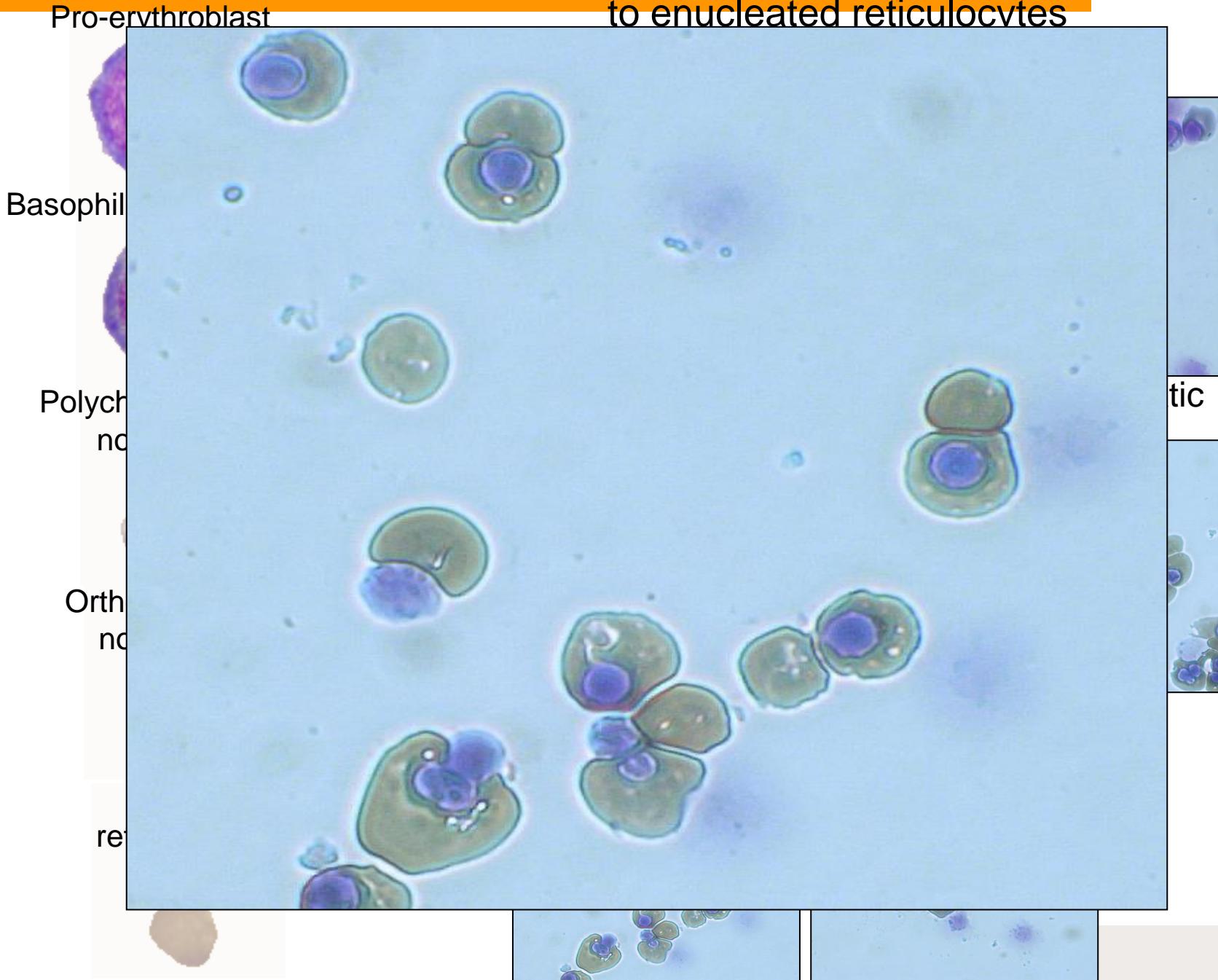
proteomics + metabolomics

available pathways

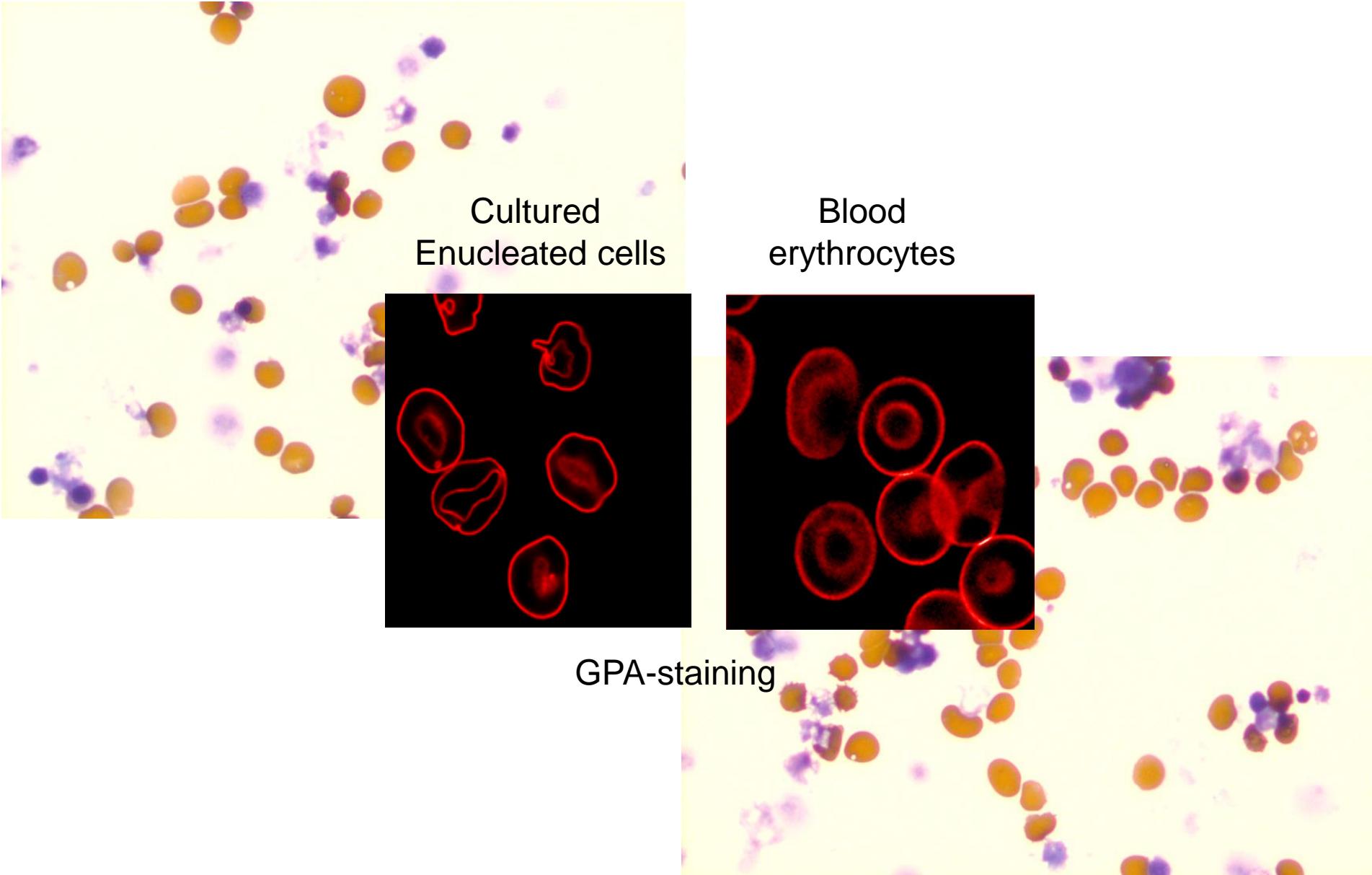
Improved perfusion



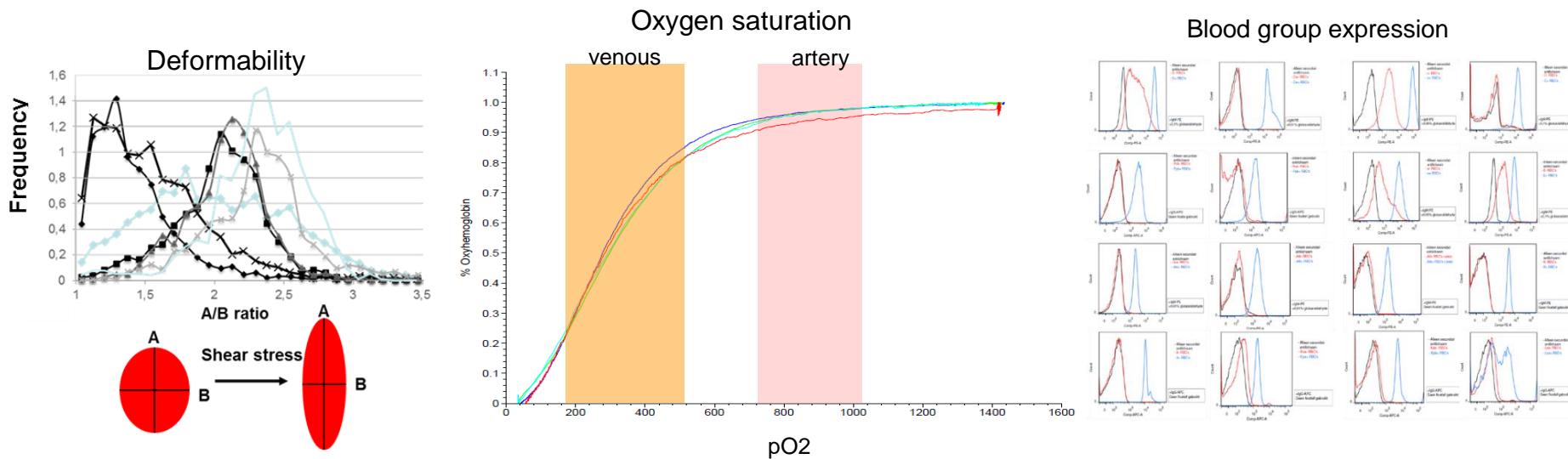
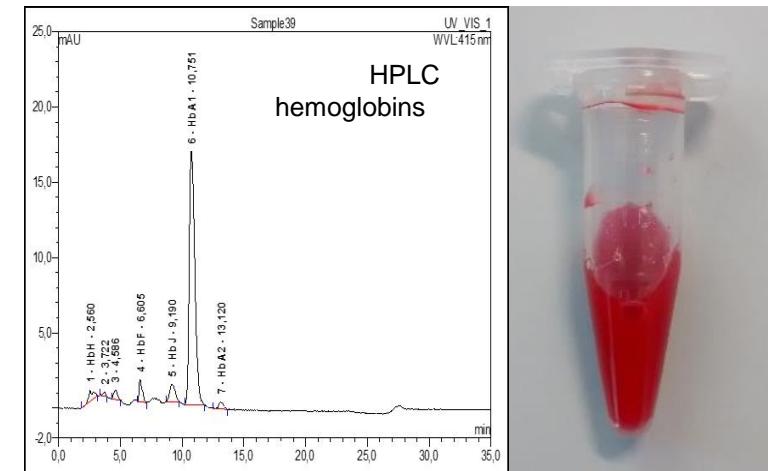
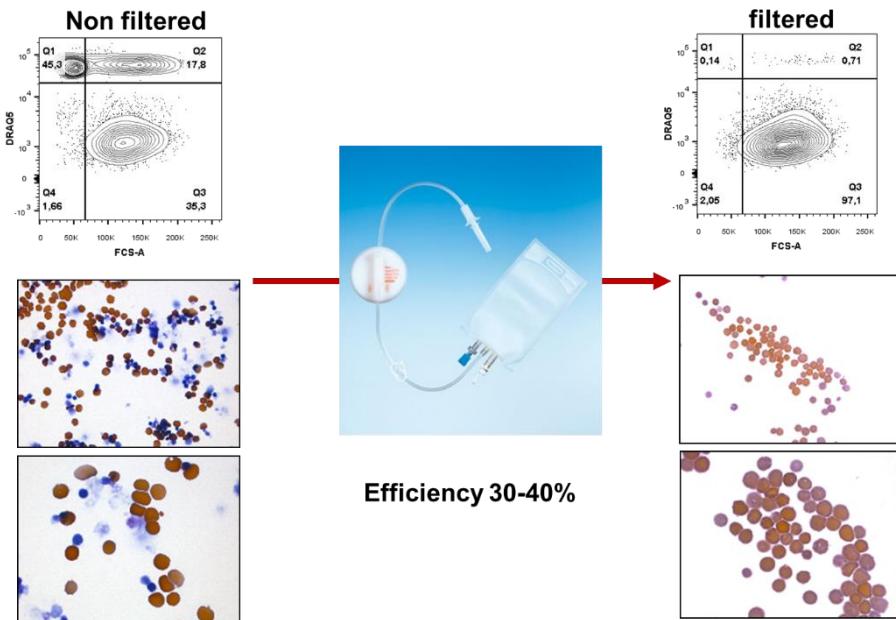
Synchronised terminal differentiation of erythroblasts to enucleated reticulocytes



Terminally differentiated cells are primarily enucleated reticulocytes

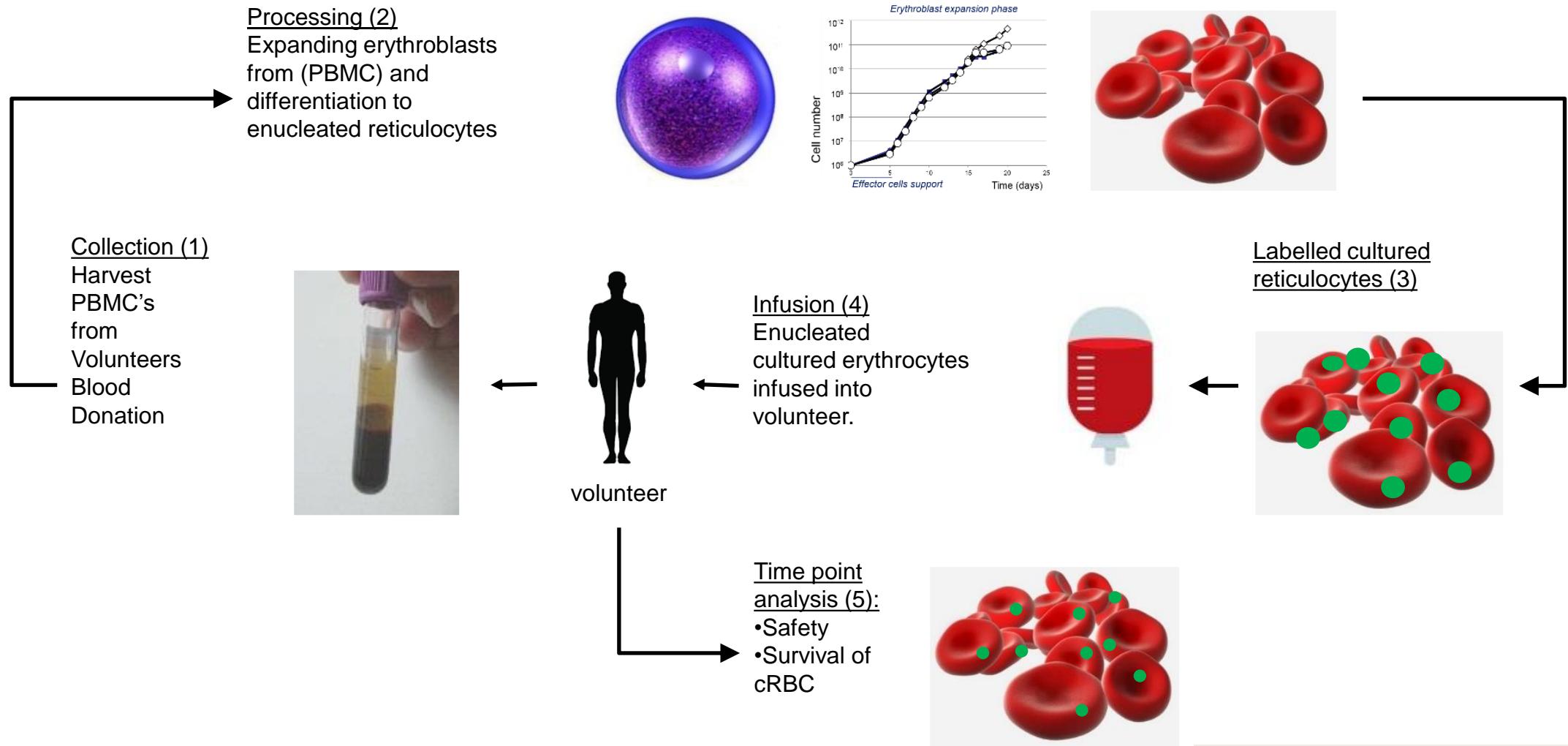


Cultured erythrocyte parameters

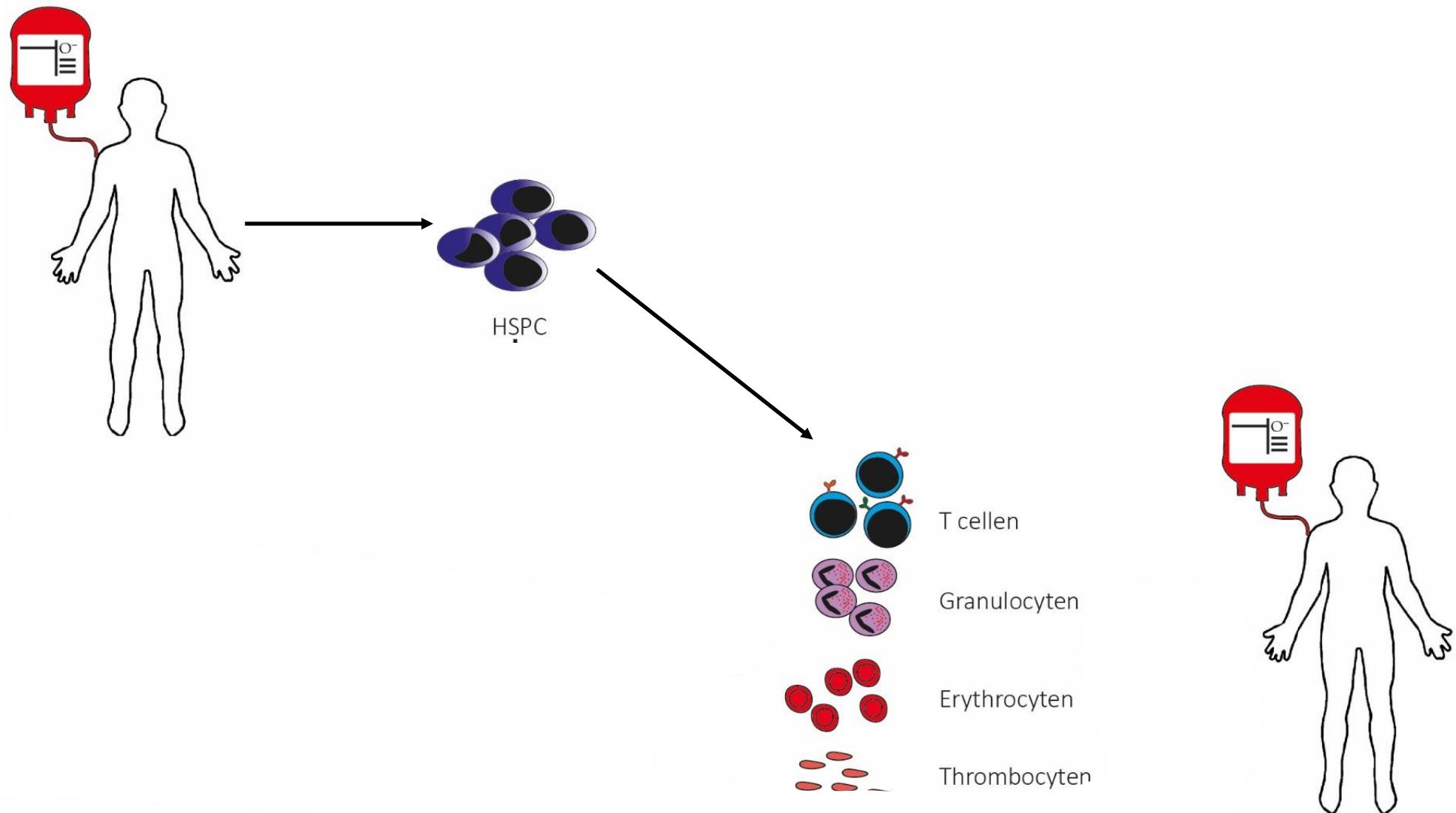


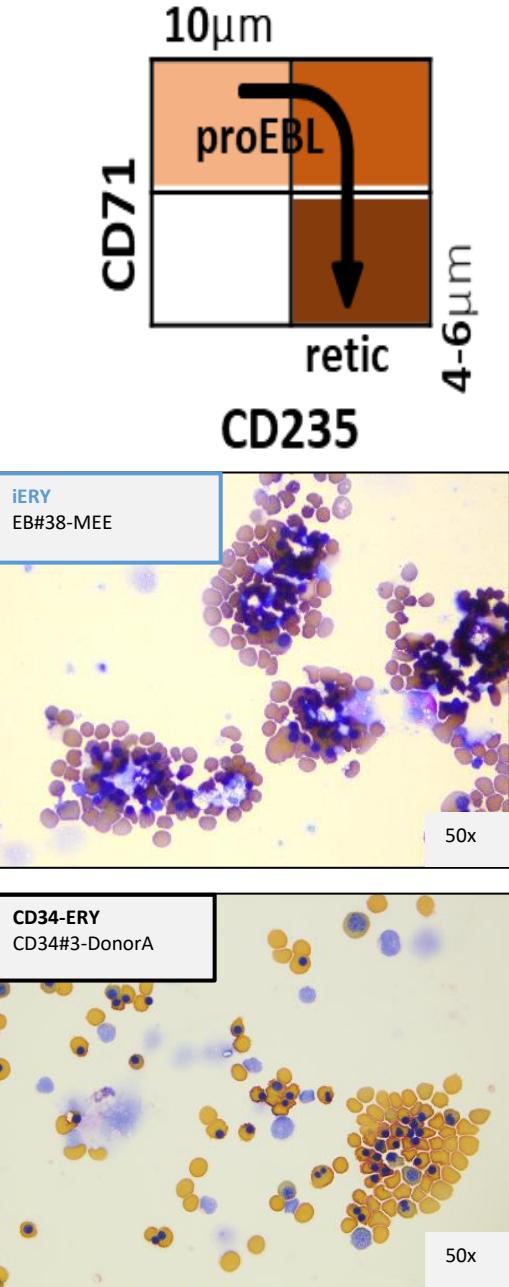
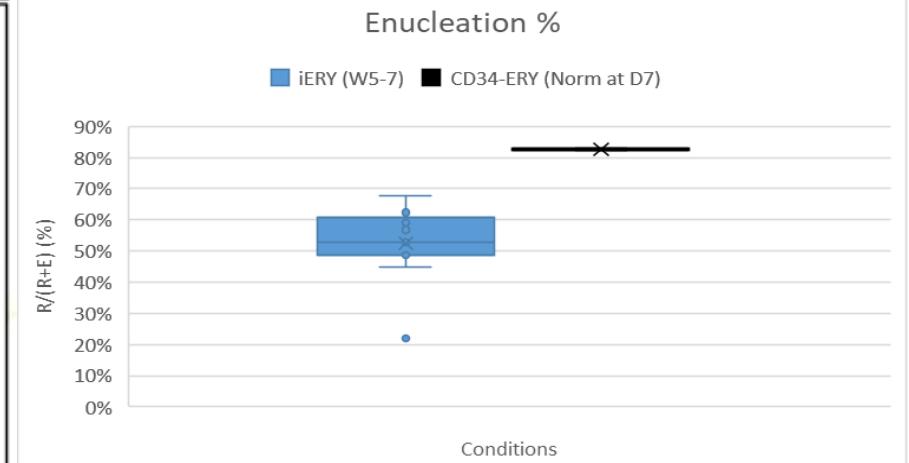
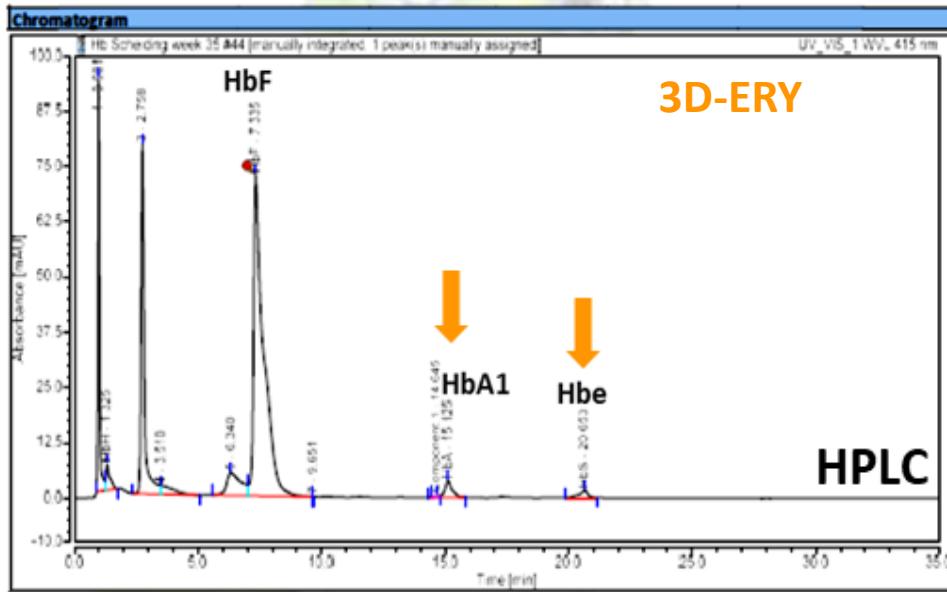
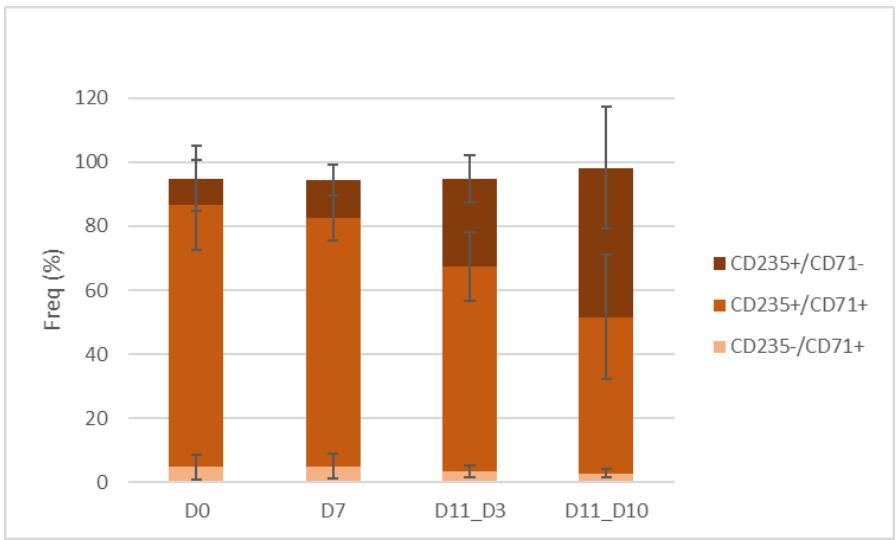
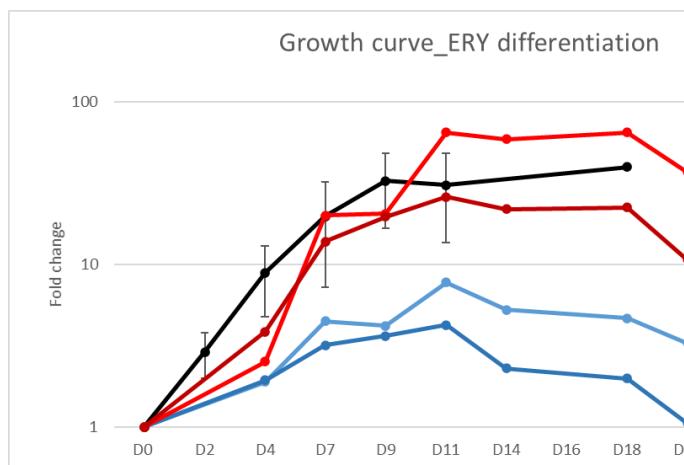
SCEVAT

Survival of Cultured Erythrocytes in healthy volunteers after Autologous Transfusion.



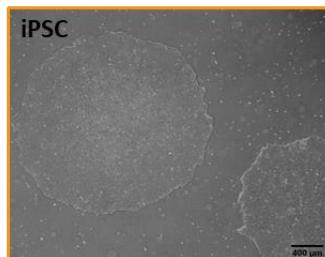
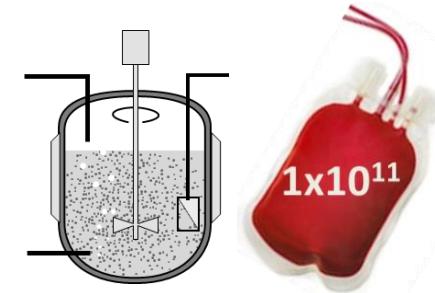
From iPSC to Cellular products





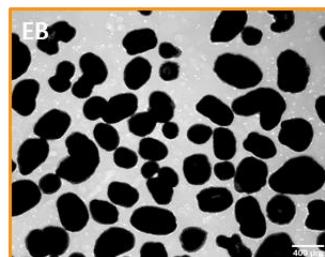


Optimization / Upscaling



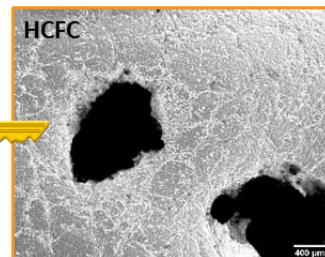
Immortal source

1) iPSC maintenance



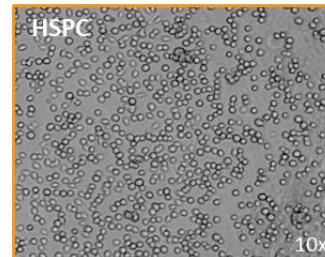
Spontaneous differentiation
5 days

2) EB formation

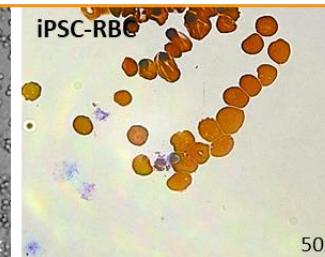


Hematopoietic specification
3-4 weeks

3) HCFC formation



Repeated harvest
4-8 weeks



Terminal differentiation
18 days

4) erythroid diff.

Culture conditions



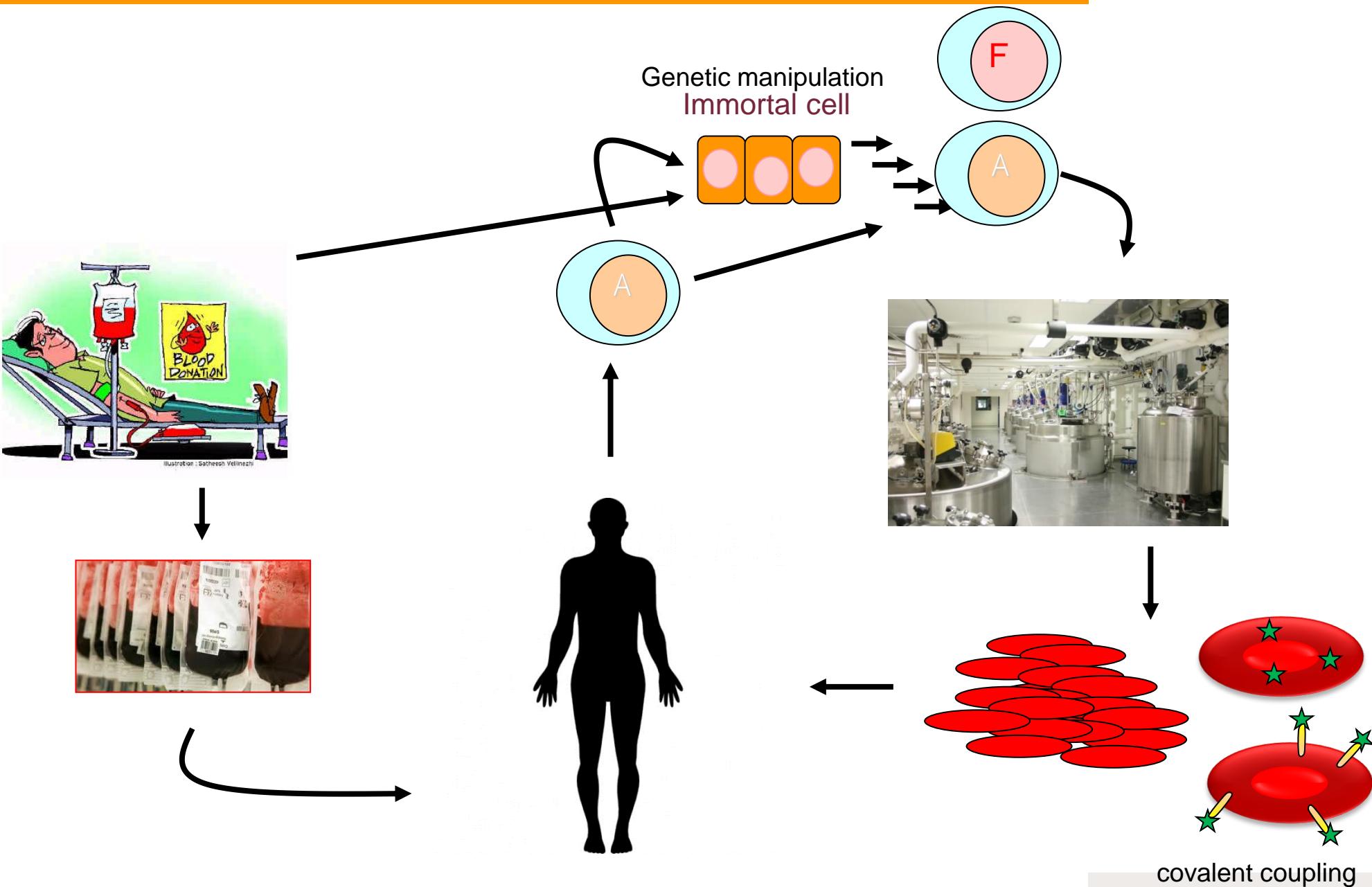
Adherent

Suspension

Static

Dynamic

In vitro produced erythrocytes: the future!?



Acknowledgements

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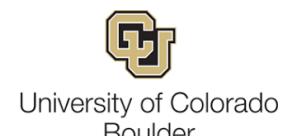
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