

Forward Programming Megakaryocytes from Human Pluripotent Stem Cells

in vitro pathway to Transfusion Medicine

Thomas Moreau

Ghevaert lab

BBTS, Glasgow 14th September 2017

Race & Sanger Award... to a team!



Amanda Dalby



Amanda Evans



Moyra Lawrence



Annett Mueller



Roger Pedersen



Willem Ouwehand



Cedric Ghevaert

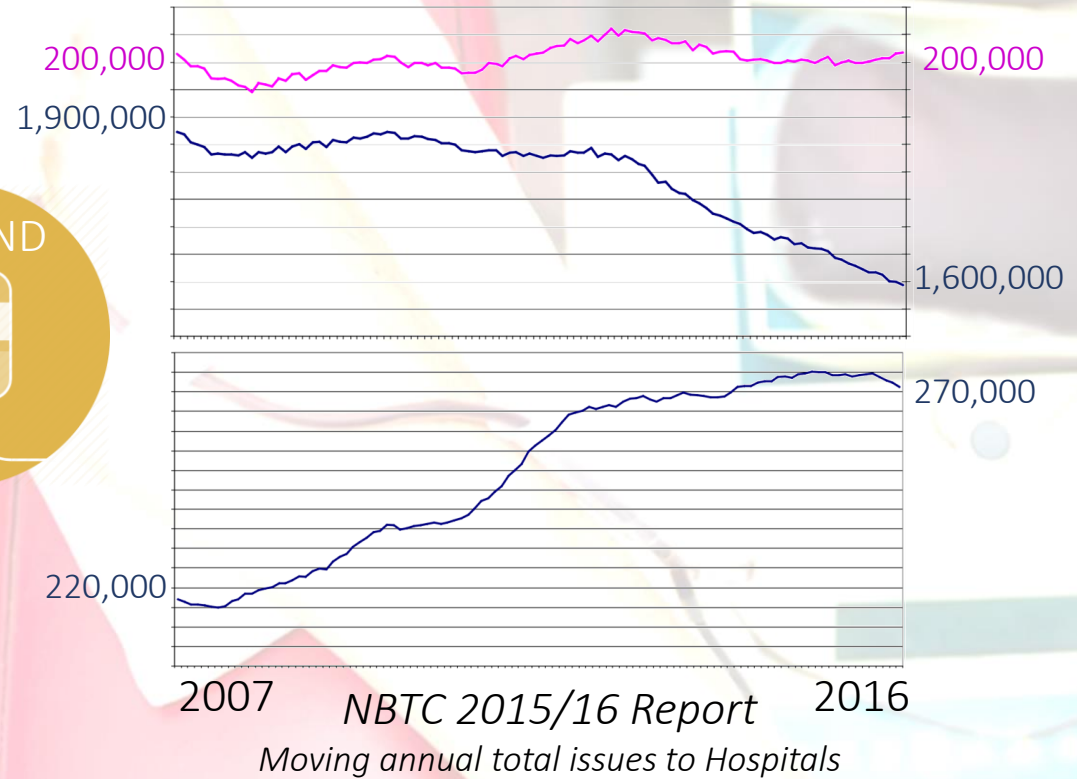
Manufactured “artificial” blood

STEM CELLS

RED BLOOD CELLS

PLATELETS

DEMAND



- Manufacture
- Controlled product
- Blood group specific
- Genetic engineering

- Availability
- Biosafety
- Rare groups
- Acquired immunity

| Manufactured “artificial” blood



**PUBLIC
PERCEPTION**

?



NIHR Blood and Transplant
Research Unit

Prof Julie Kent

Dr Rachel Hale

RESTORE


National Institute for
Health Research

2018

Red blood cells from
adult blood stem cells

Phase-I trial

*Recovery
Survival*

NOVO SANG



2018-20



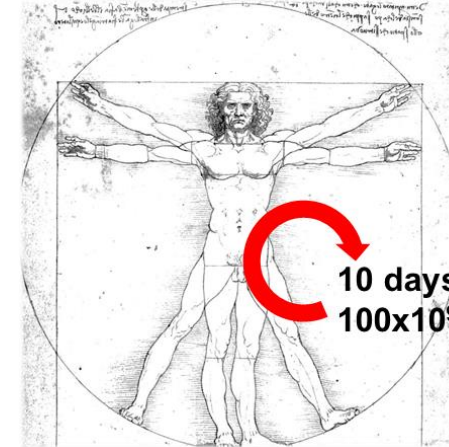
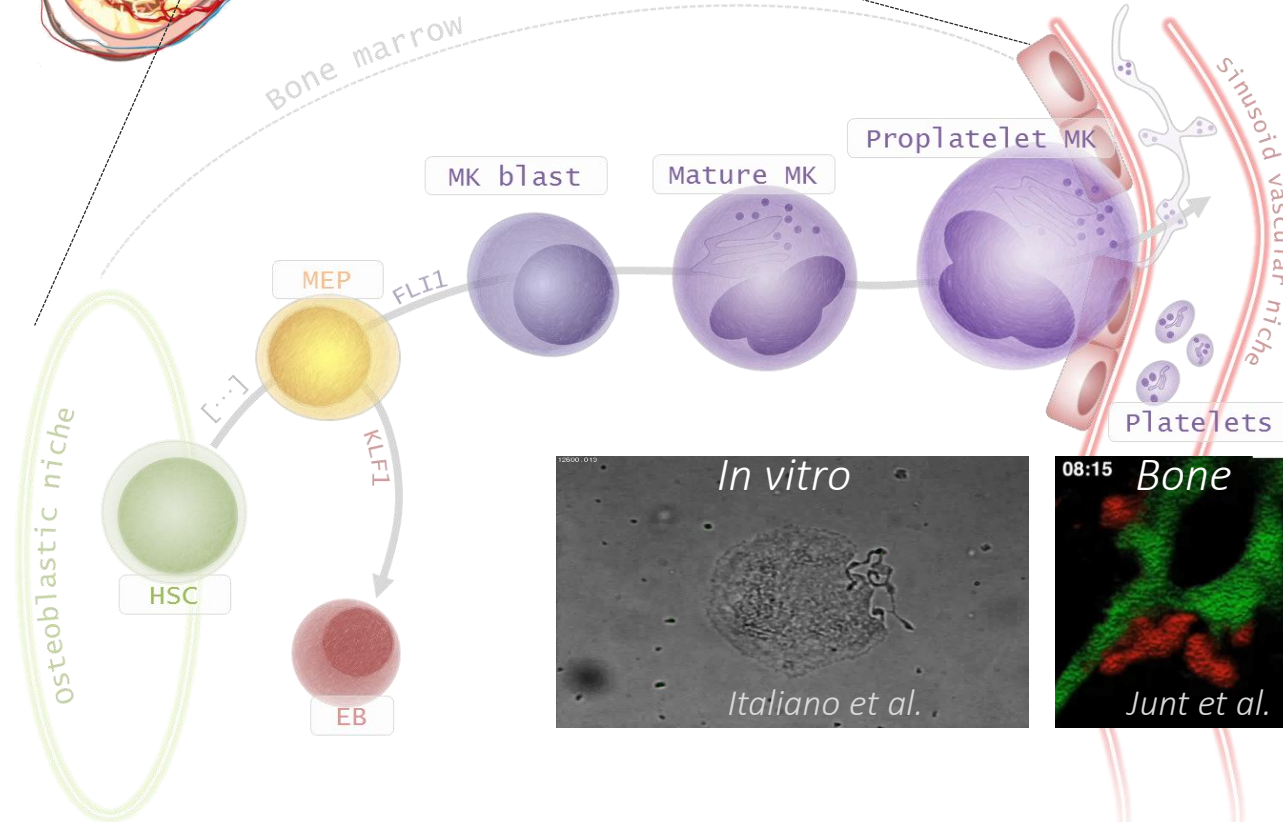
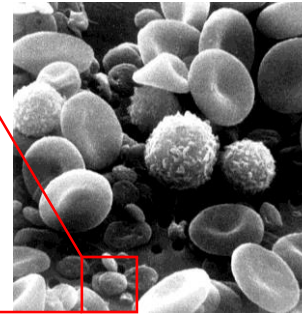
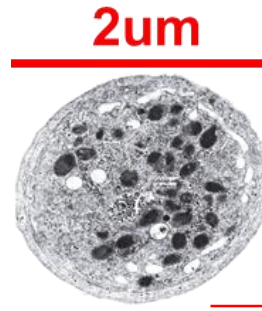
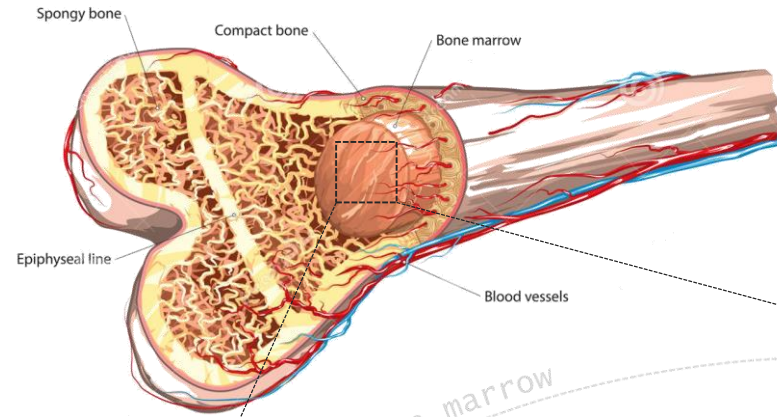
Red blood cells from
immortalised RBC line
(Bela) from adult HSC

Phase-I trial

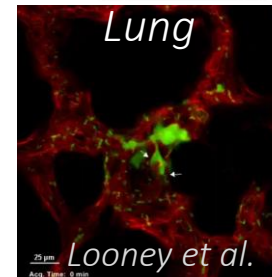
*Recovery
Survival*



Platelets, origin



10 days life-span
 100×10^9 produced daily

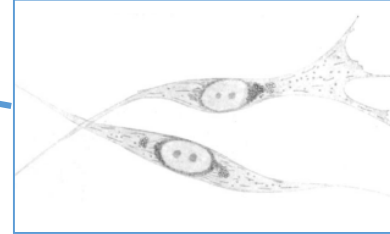


Platelets, stem cell options

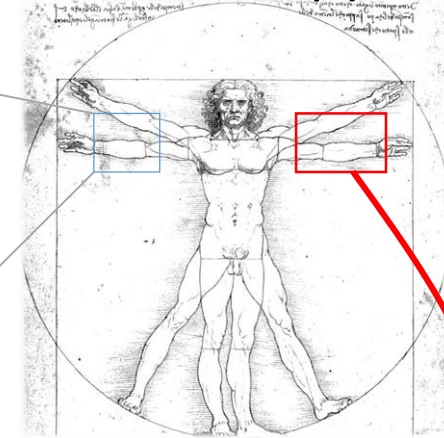
- ✓ Renewable
- ✓ Genetic engineering
- ✗ Genetic instability (but anucleate blood)
- ✗ Embryonic phenotype

Yamanaka factors
SOX2, OCT4, KLF4, MYC

DONOR(s)



Dermal fibroblasts



Cord blood

Pluripotent

STEM CELLS

Peripheral blood

Cedric GHEVAERT
NHS
Blood and Transplant

Koji ETO

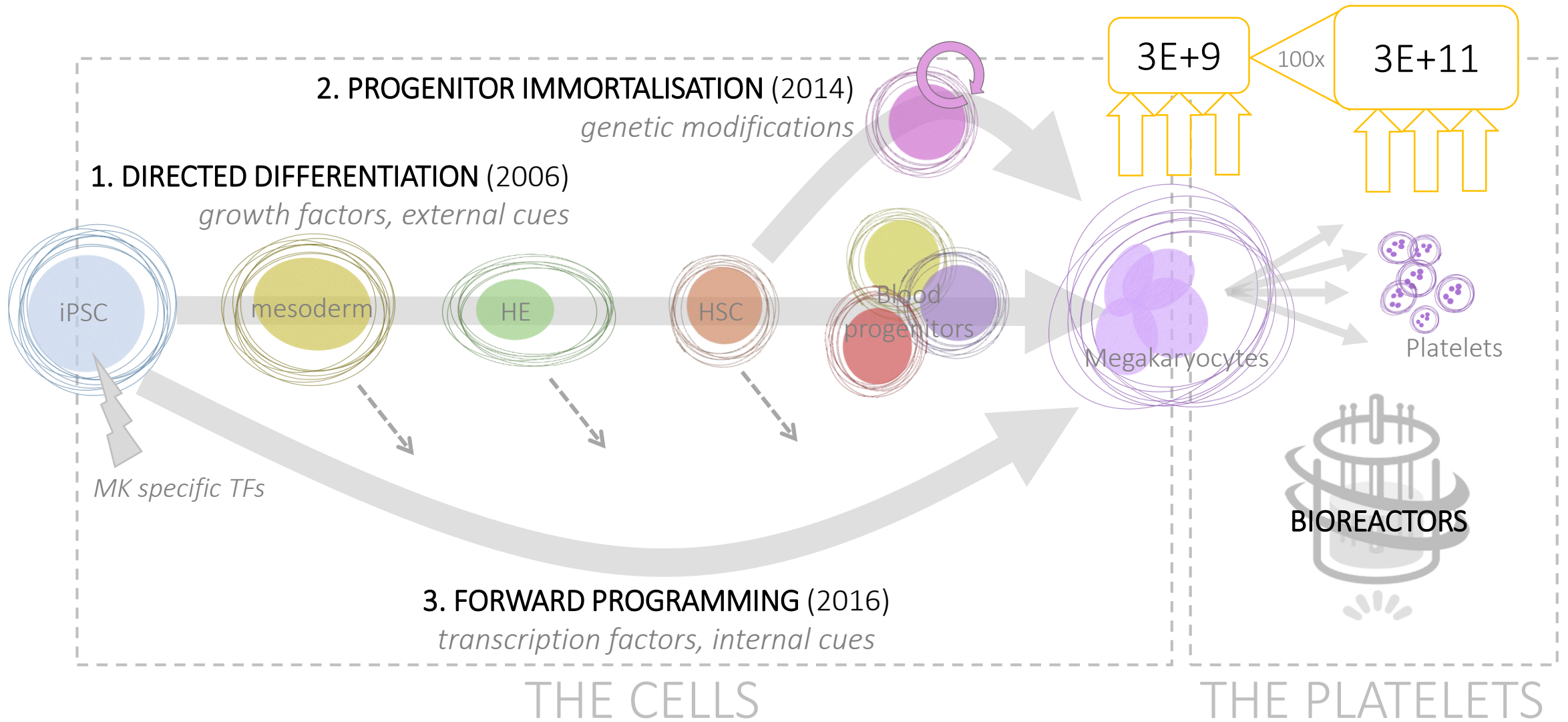
Mega
karyon
Megakaryon Corporation

 **PLATELET
BIOGENESIS**
Jonathan THON

 **PLATOD**
Production de plaquettes sanguines
Dominique BARUCH



Platelets, *in vitro* ways

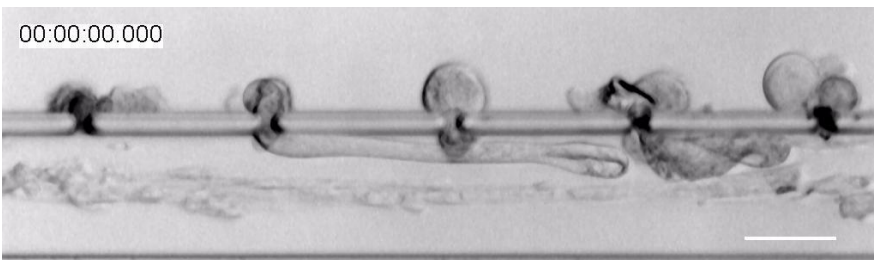
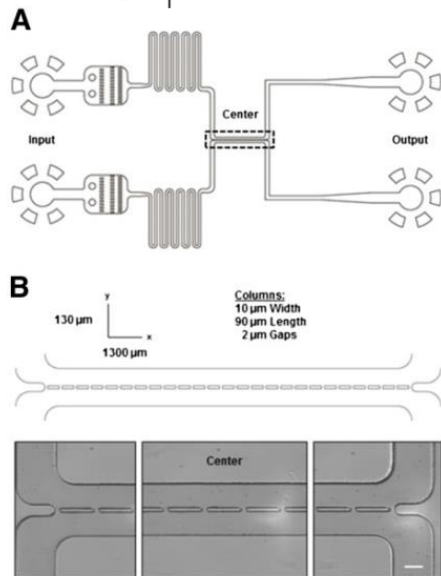


Improving ex-vivo platelet biogenesis

Microfluidics biochips

polydimethylsiloxane (PDMS, silicon-based organic polymer)

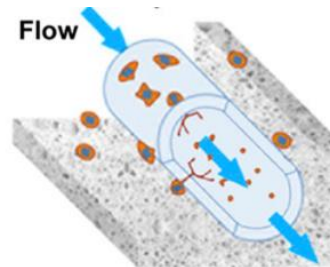
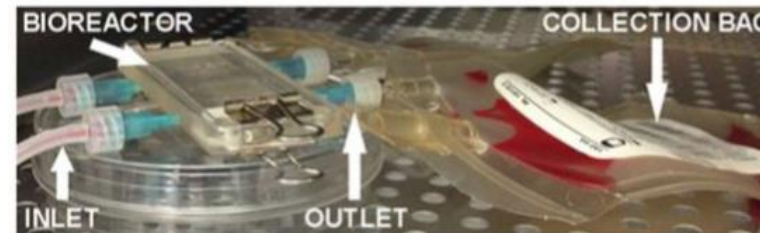
Jonathan THON, 2014



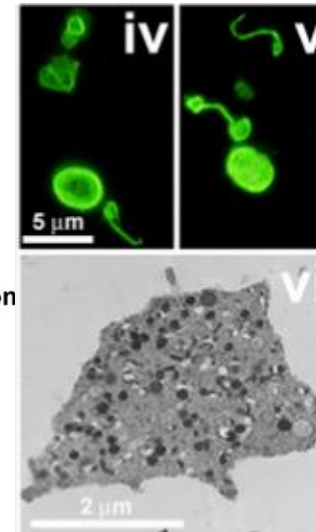
Biomimetic scaffolds

bioengineered silk micro-tubes

Alessandra BALDUINI,
2015



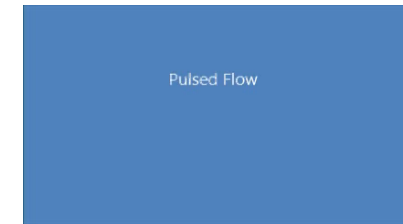
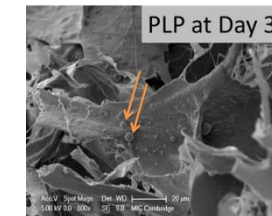
Platelet collection



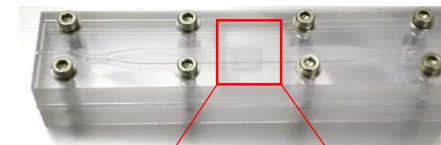
Biomimetic scaffolds

functionalised collagen scaffolds

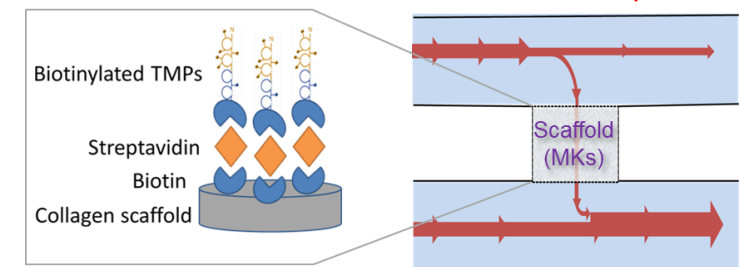
Cedric GHEVAERT, 2017



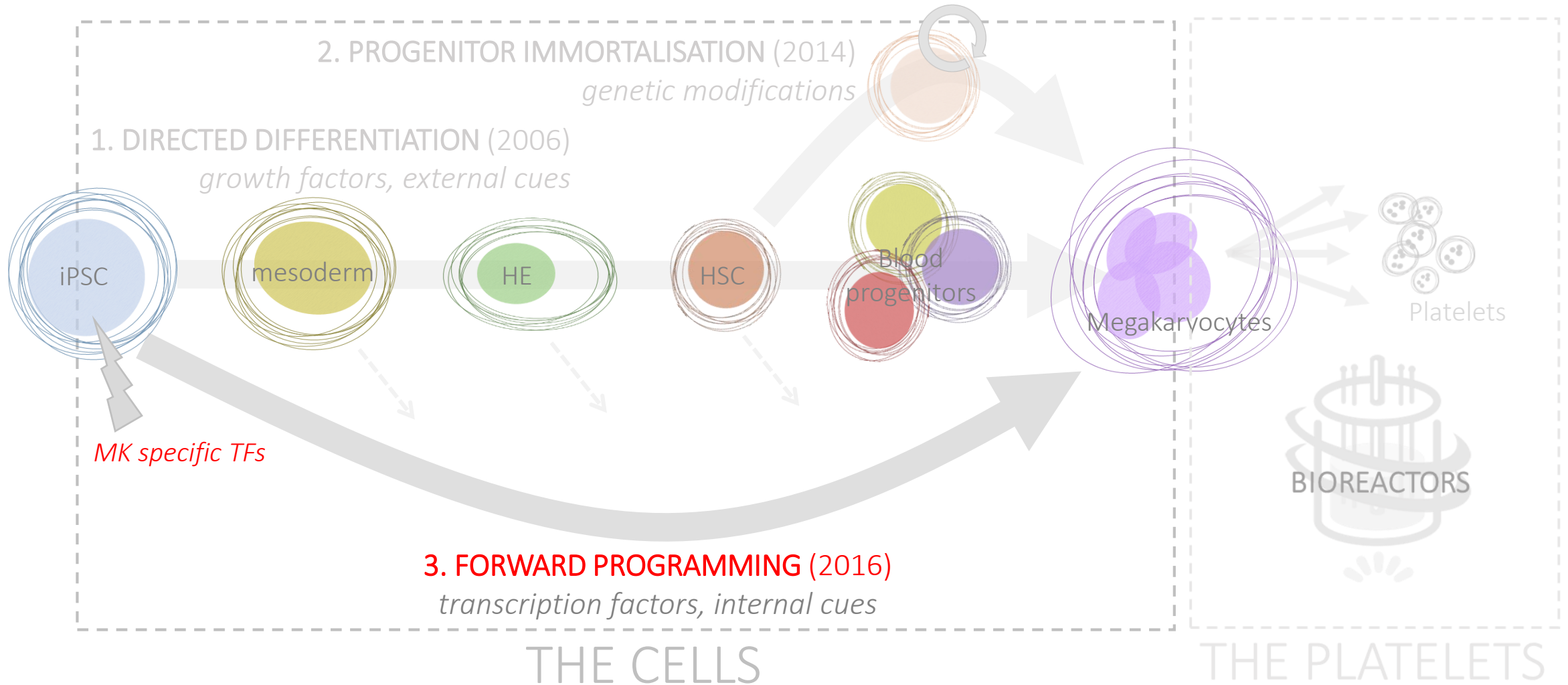
11.2 cm



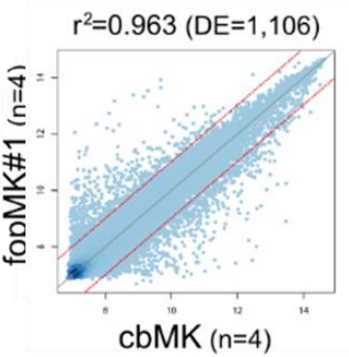
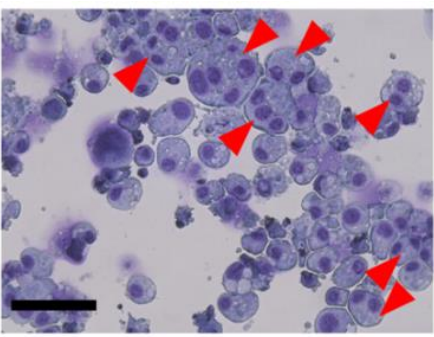
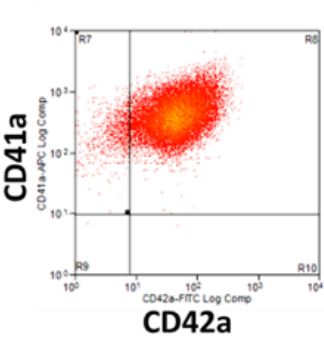
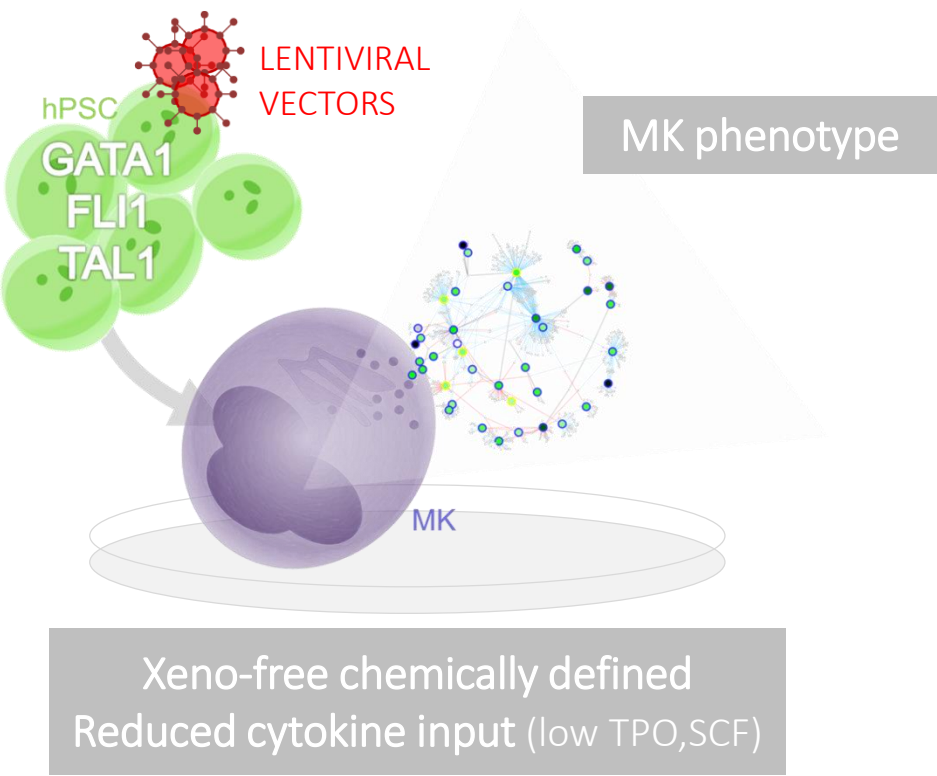
50E+6 MKs (0.2cm³)



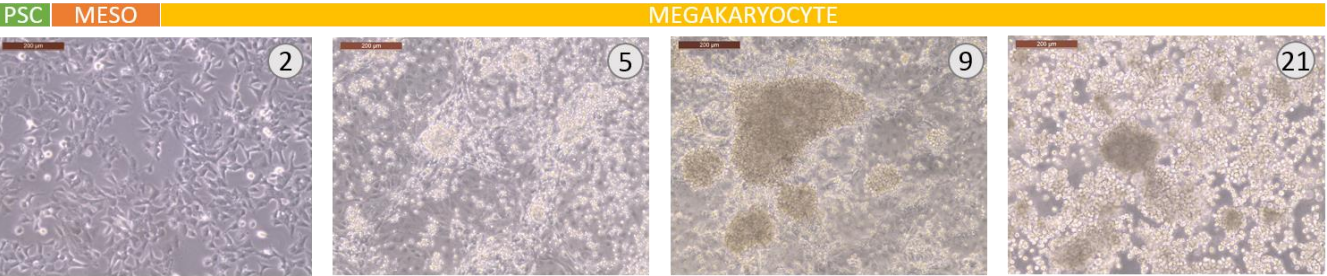
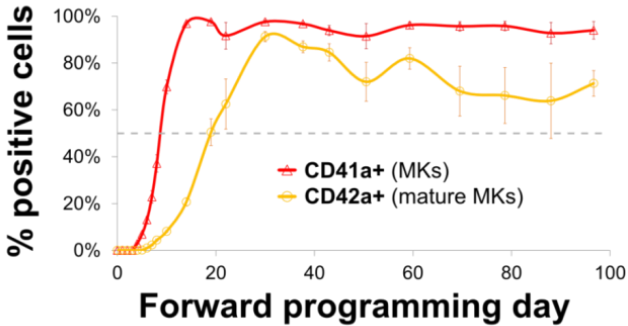
Platelets, *in vitro* ways



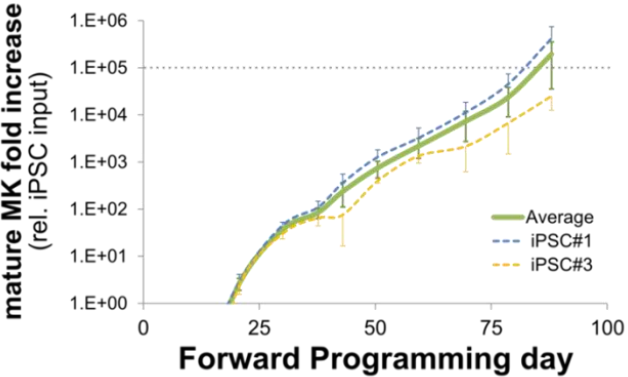
MK Forward Programming, the cells



Long-term purity
>90%MK, >60%maturity

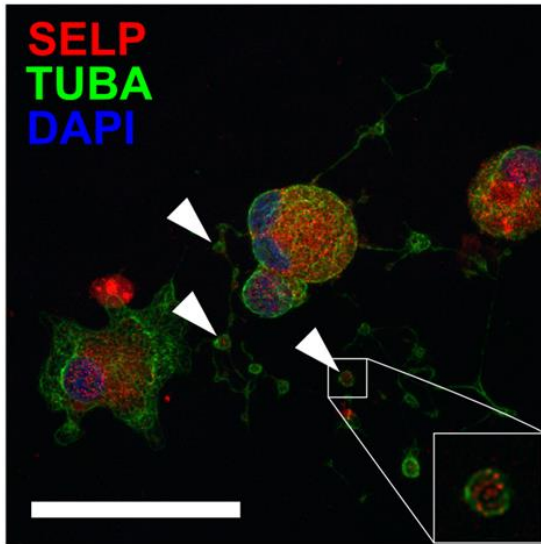


High cell yield
>200,000 MK yield

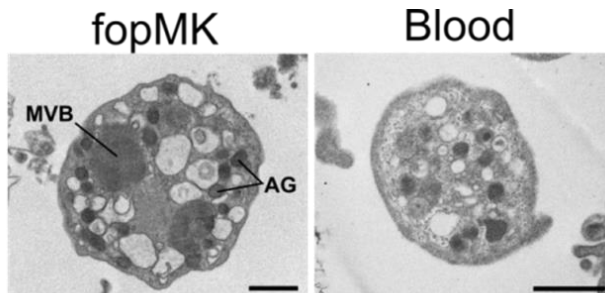


MK Forward Programming, the platelets

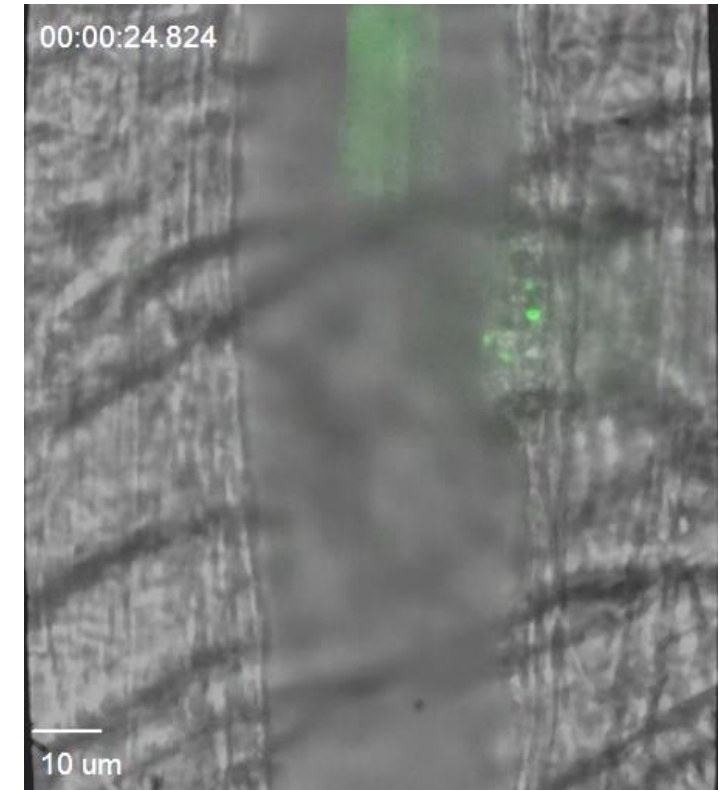
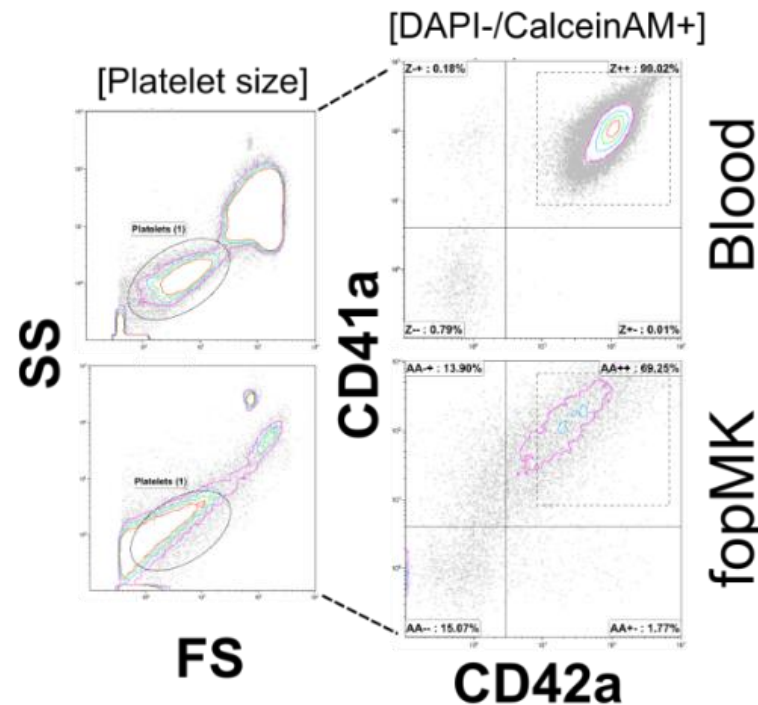
Proplatelets



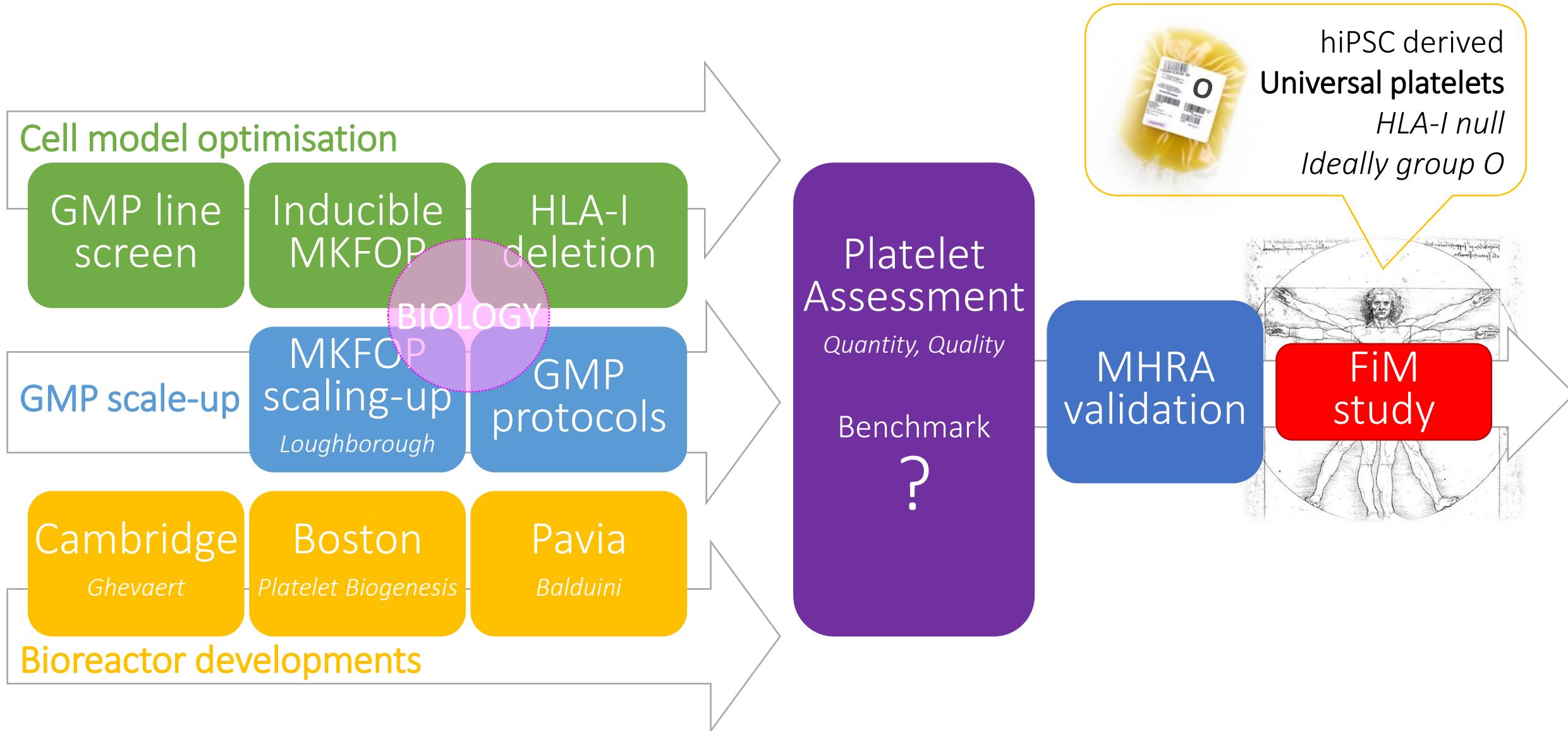
Platelet morphology



Platelet phenotype



Research programme towards FiM



Cell model optimisation

GMP line
screen

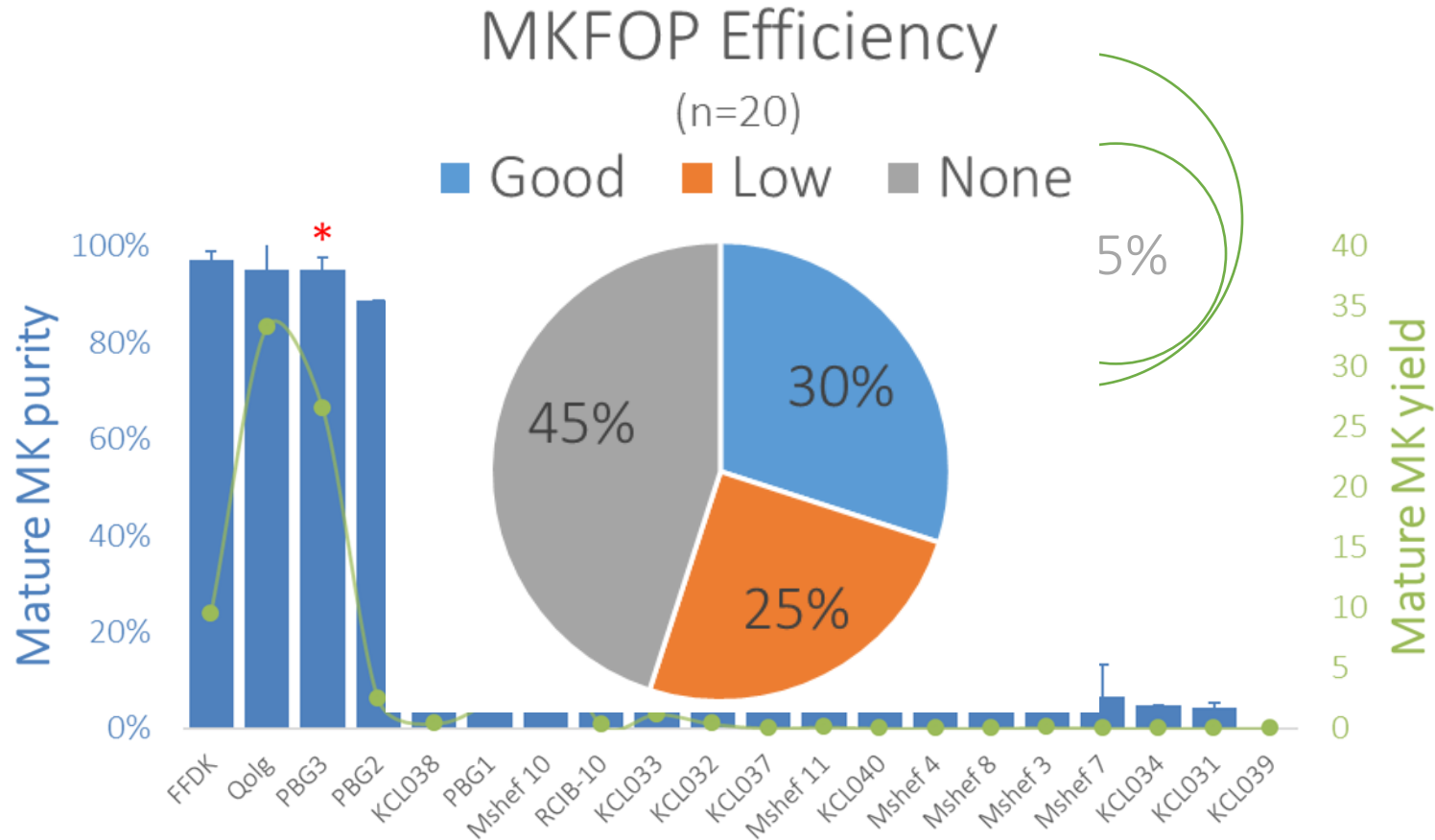
Inducible
MKFOP

HLA-I
deletion

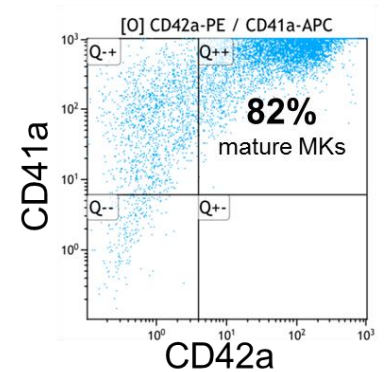
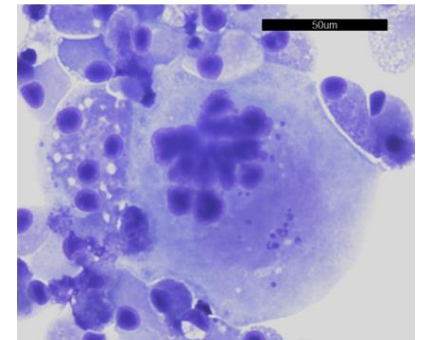
GMP stem cell screening

Identify GMP-grade hPSC lines with high MK programming potential

Amanda Evans



Mshef10



Cell model optimisation

GMP line screen

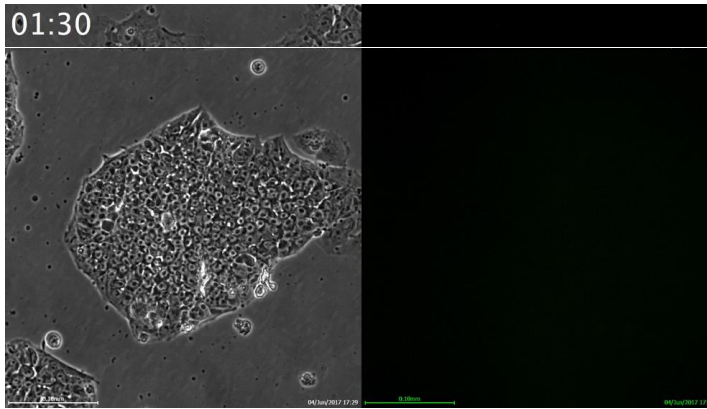
Inducible MKFOP

HLA-I deletion

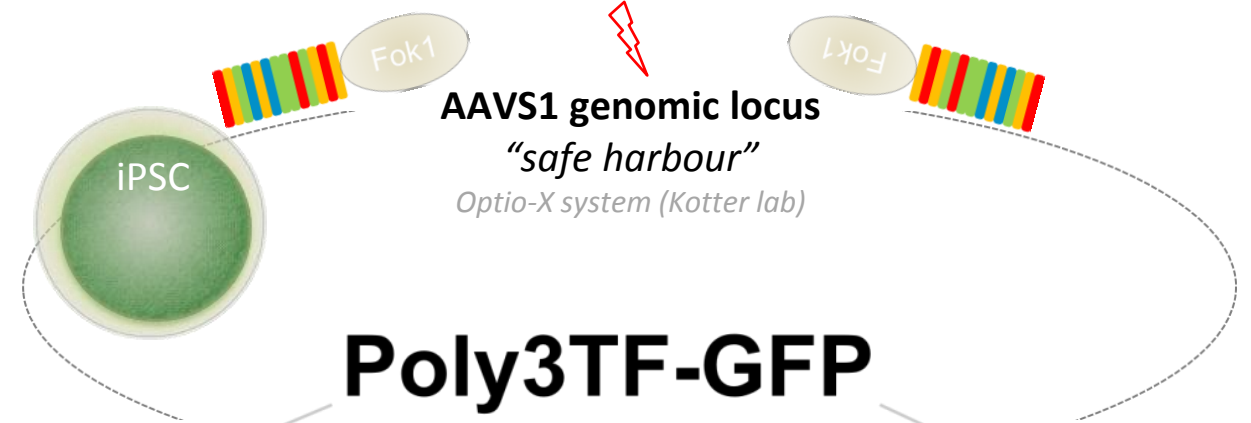
Inducible MK Programming

Replace lentiviral vectors for chemically inducible programming

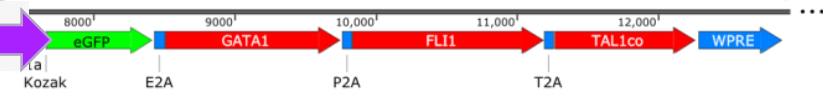
Amanda Dalby



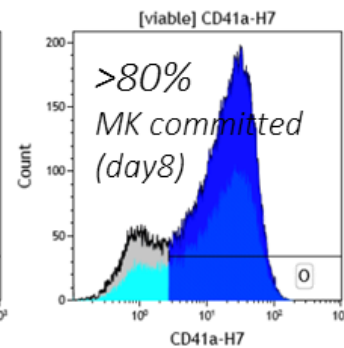
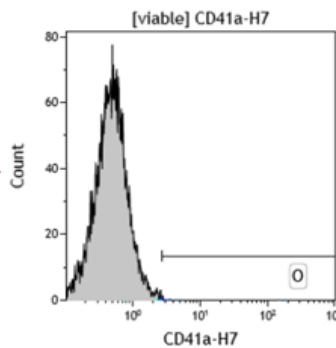
Zinc-finger Nucleases guided genetic modification



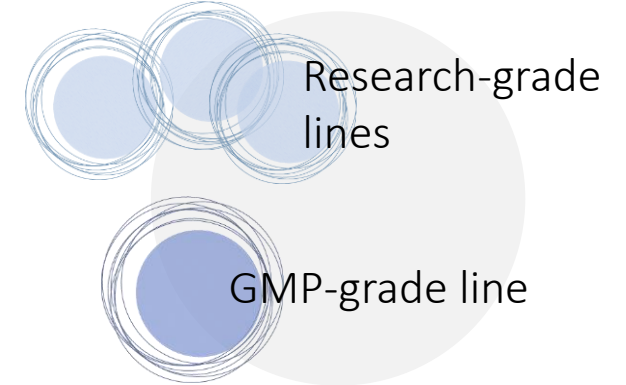
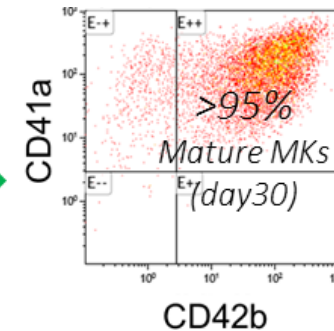
Doxycycline inducible promoter



-Dox



+Dox





HLA-null universal platelets

Prevent HLA class-I surface expression to create universal platelets

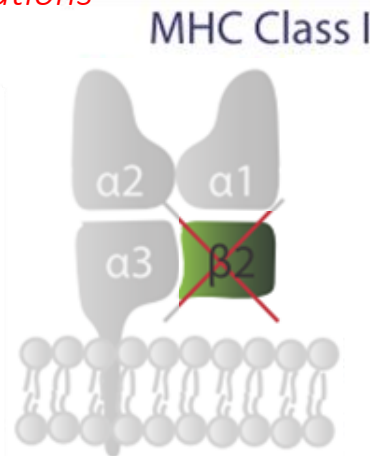
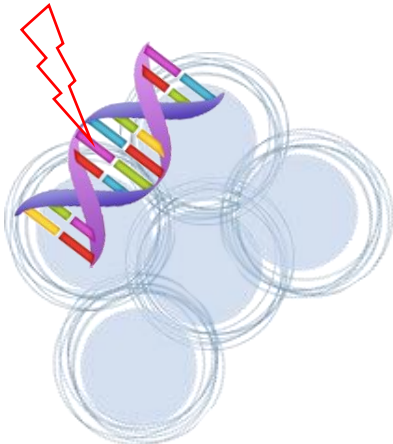
Annett Mueller



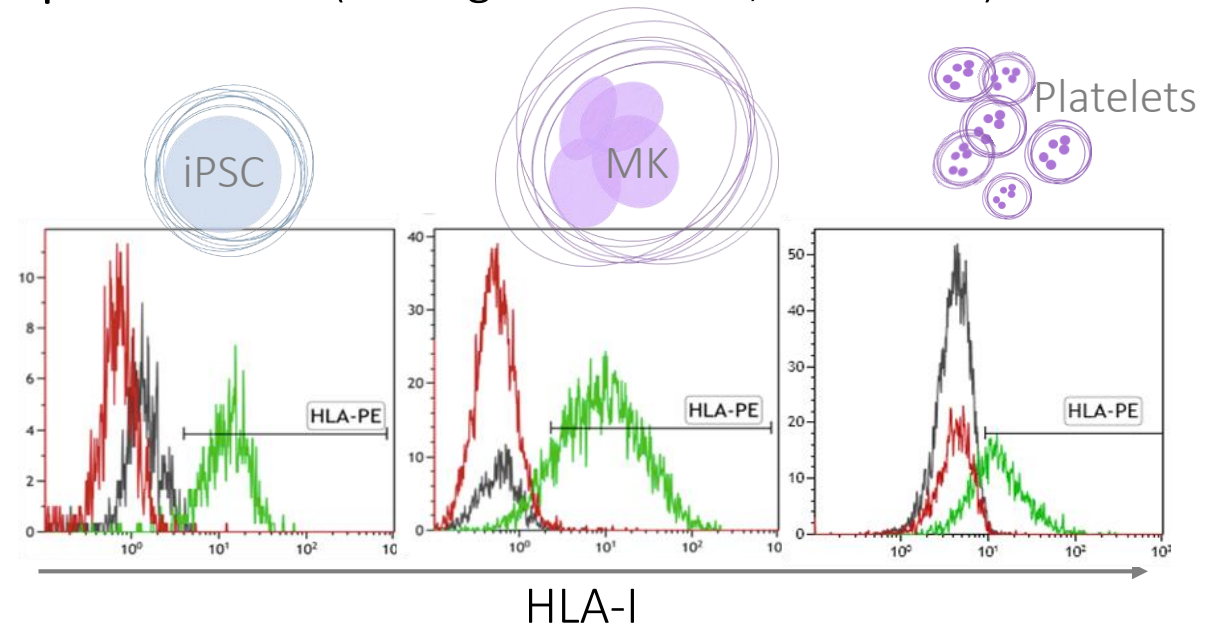
Platelet transfusion refractoriness:

- Chronically transfused patients, multiparous women may develop anti-HLA class-I alloantibodies
- The NHSBT provides 6% HLA-I matched platelet units (management issue, extra cost)

CRISPR/Cas9 guided genetic modifications
Beta2-microglobulin deletion



WT
KO



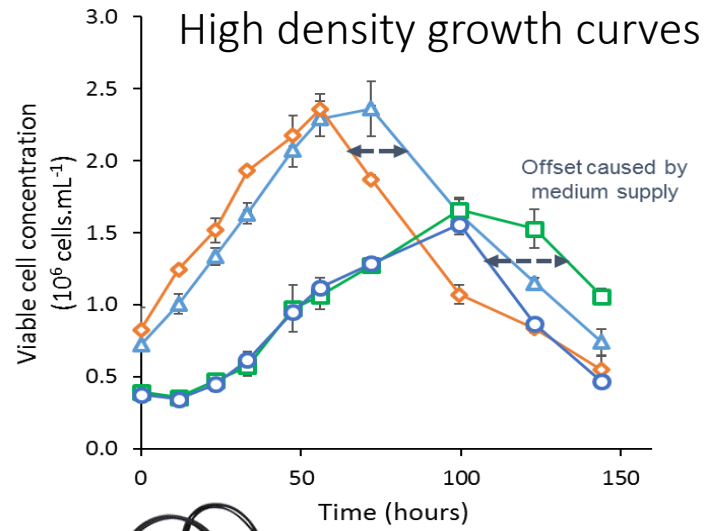
Stem cell MK culture scale-up

Optimising stem cell MK ex-vivo culture to scale-up production

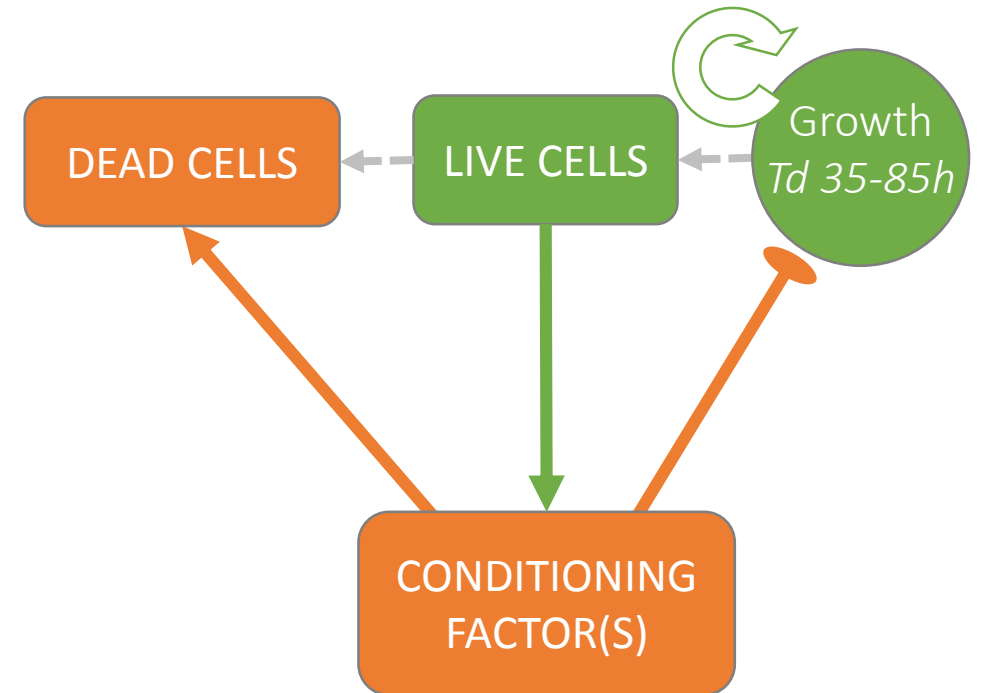
Elizabeth Cheeseman



Centre for Biological Engineering



Mechanistic hypothesis for cell growth dynamics
MODELLING



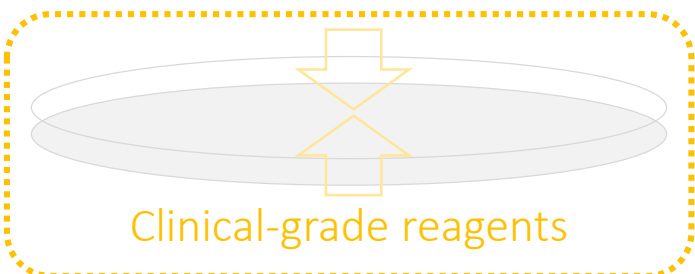
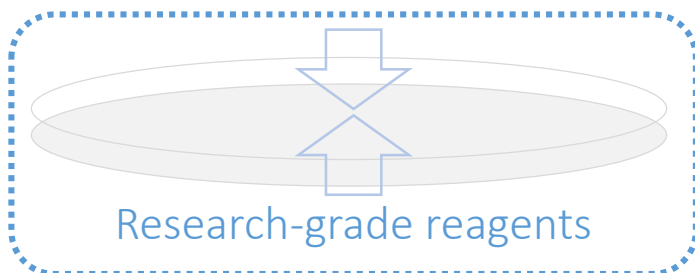
MK Programming GMP transition

Translation of the MK programming protocol to GMP procedures

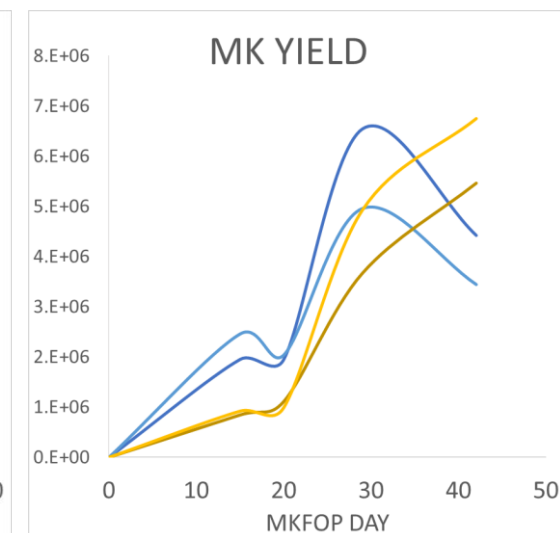
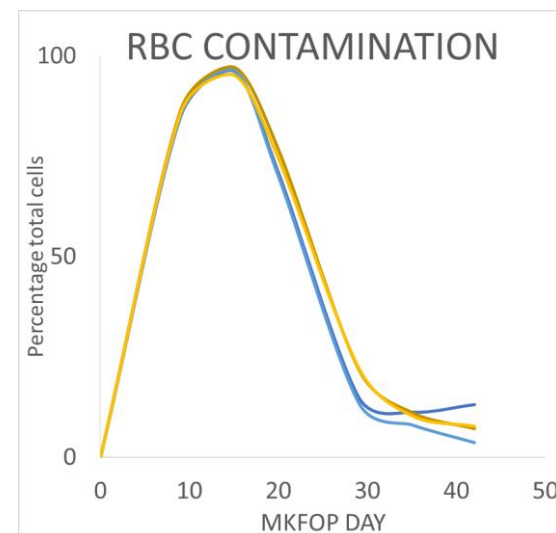
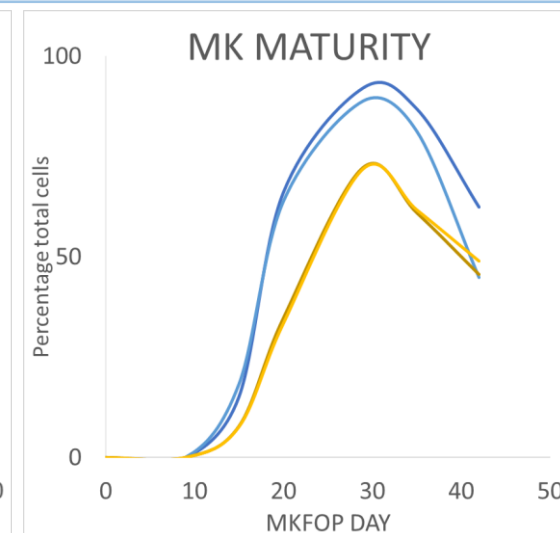
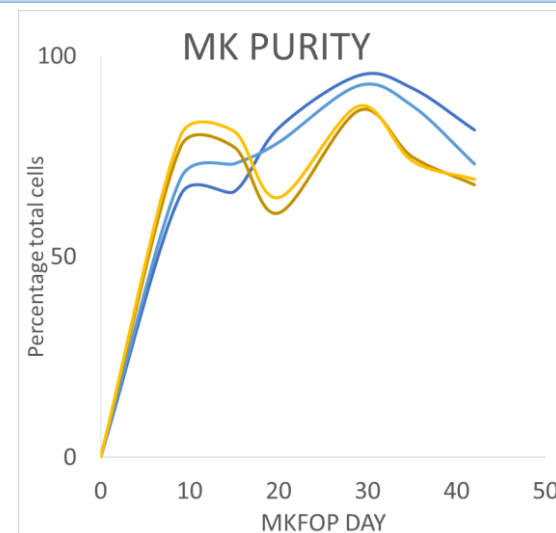
Moyra Lawrence



UK Regenerative
Medicine Platform



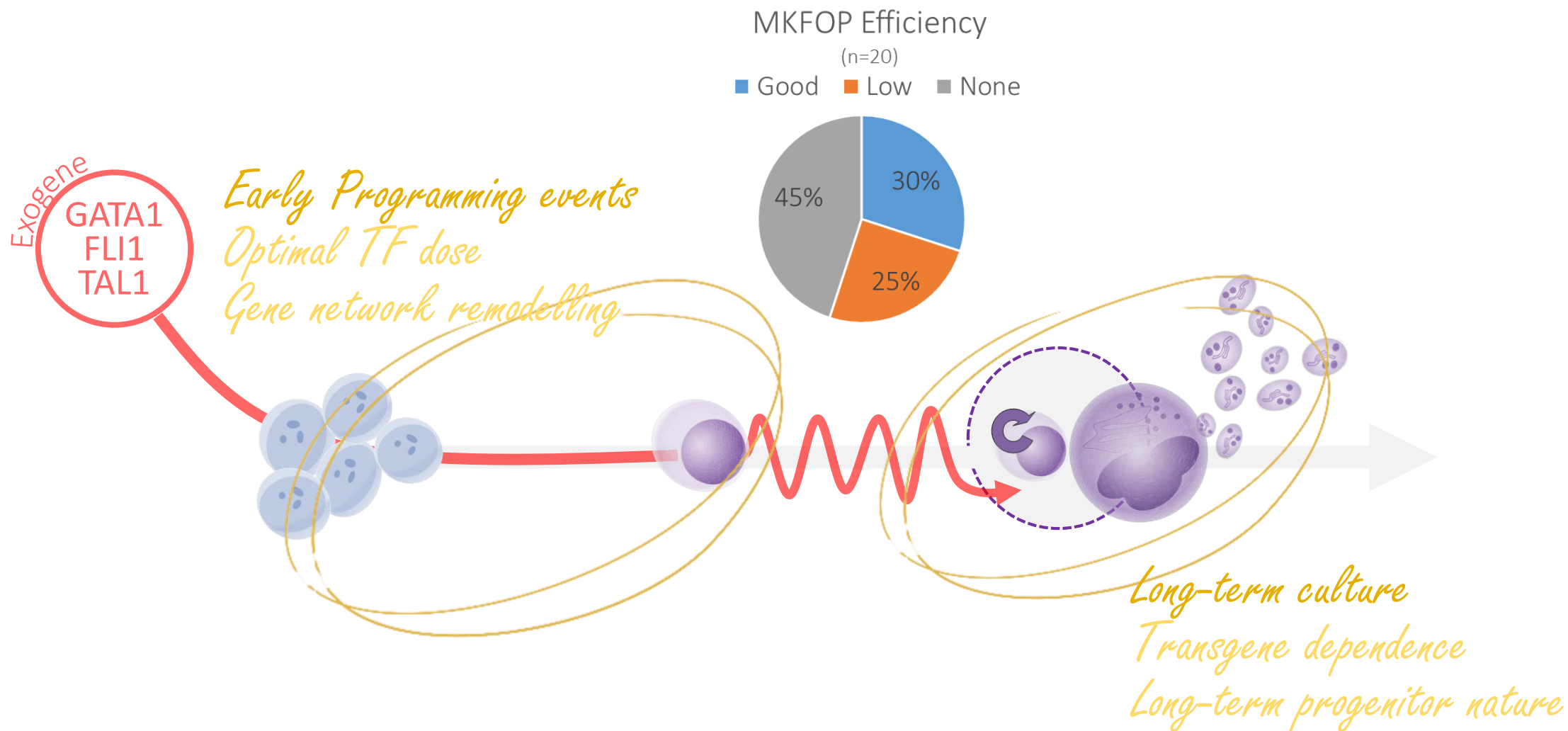
R&D
GMP





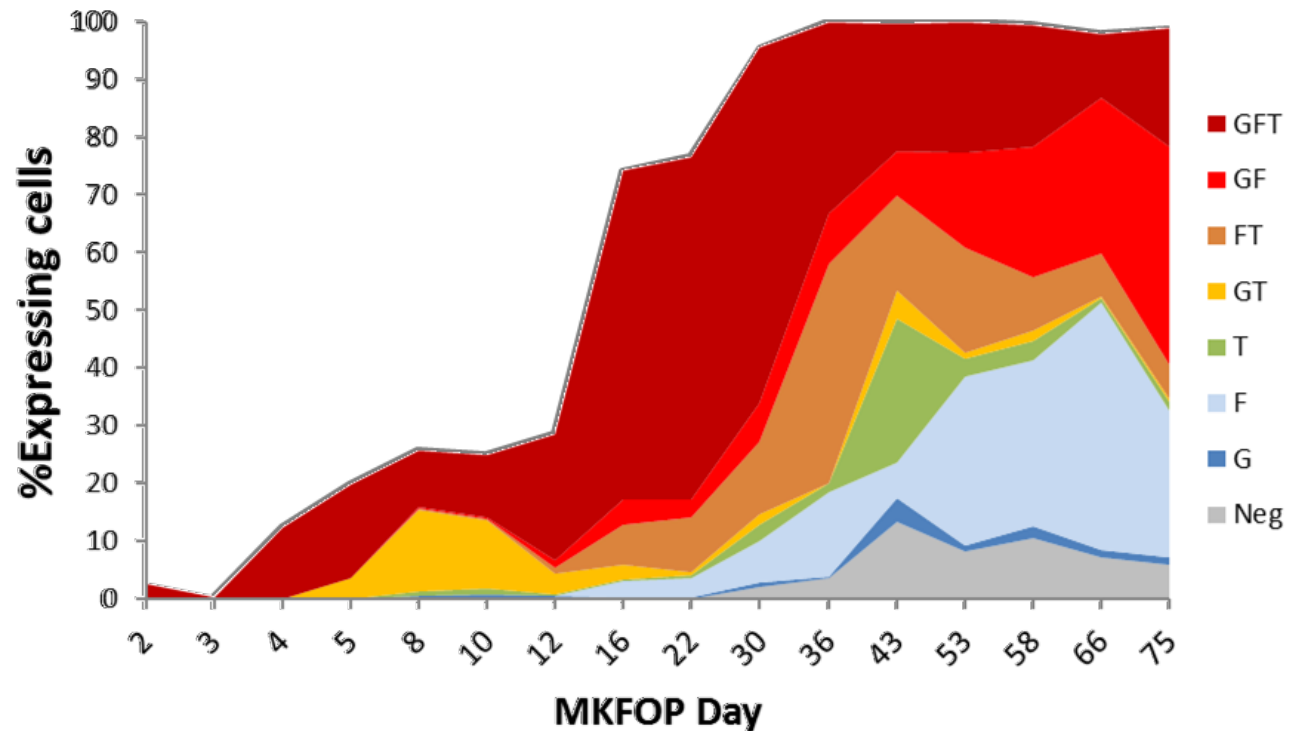
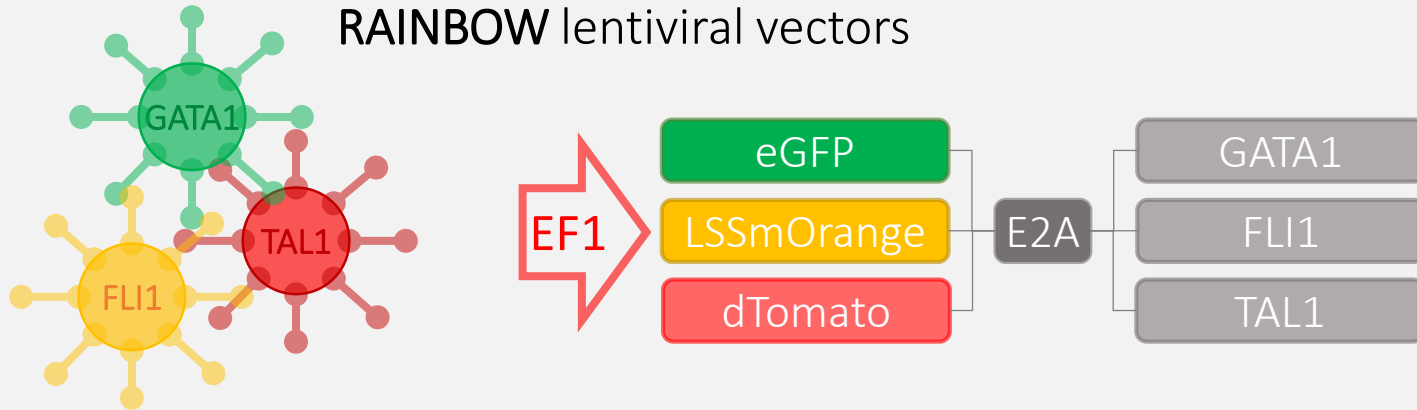
Biology of MK Programming

Understanding the **biology of MK programming** to improve outcome

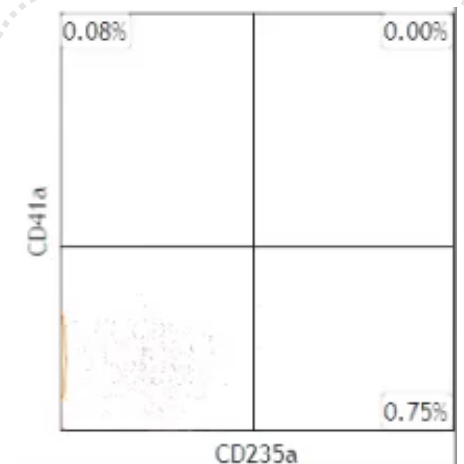
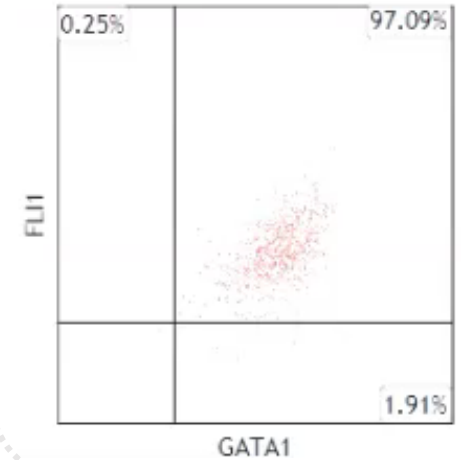


Biology of MK Programming

RAINBOW lentiviral vectors

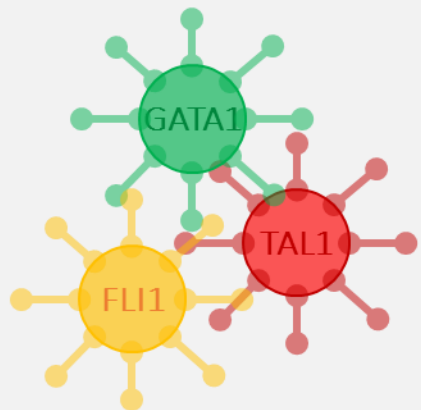


Programming TFs



Differentiation markers

RAINBOW lentiviral vectors

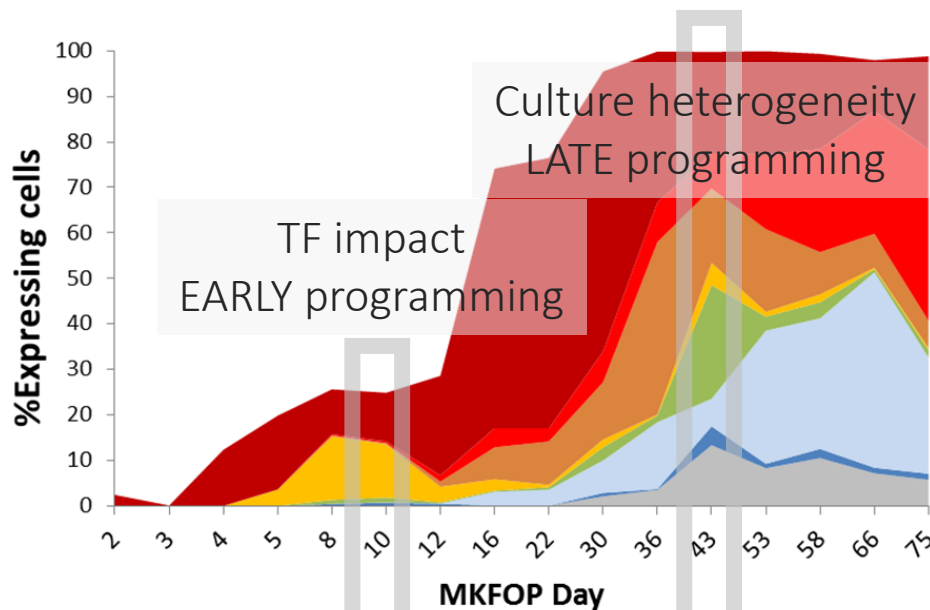


+



Single cell technologies

Biology of MK Programming



James Baye



Amanda Dalby

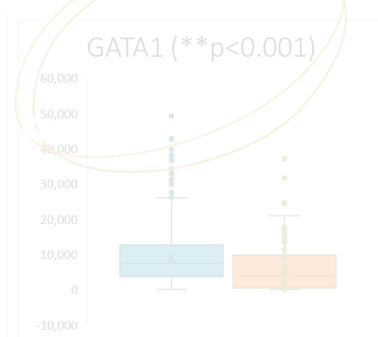
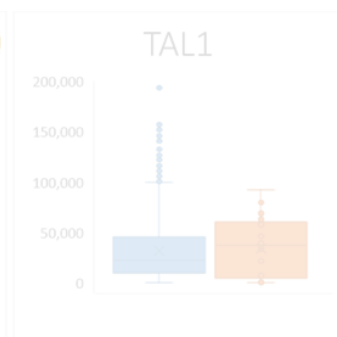
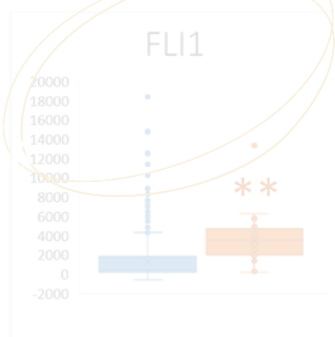


Productive MK programming

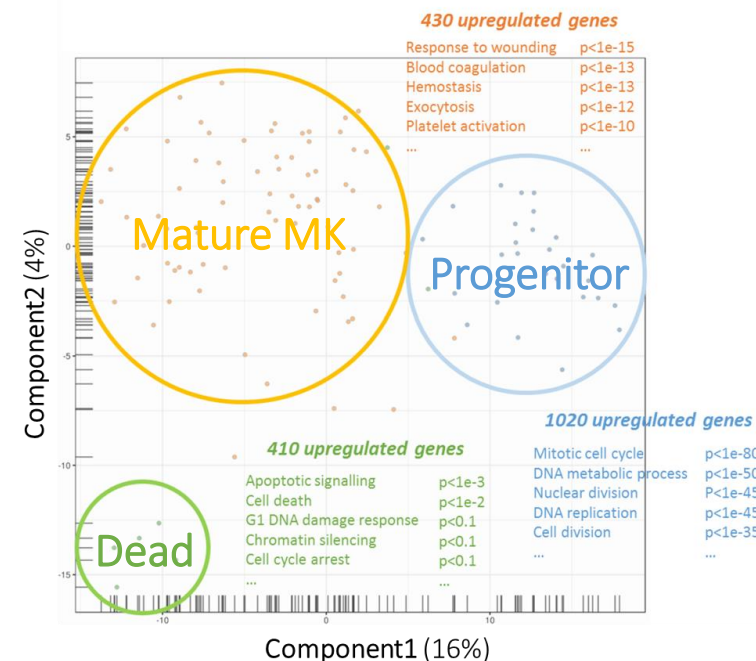
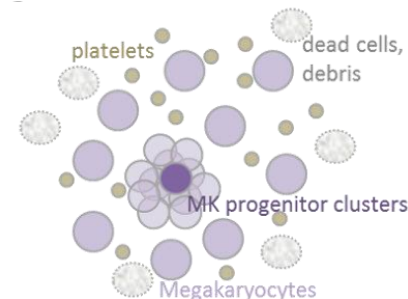


Rainbow Index FACS

Single-cell CFU assay



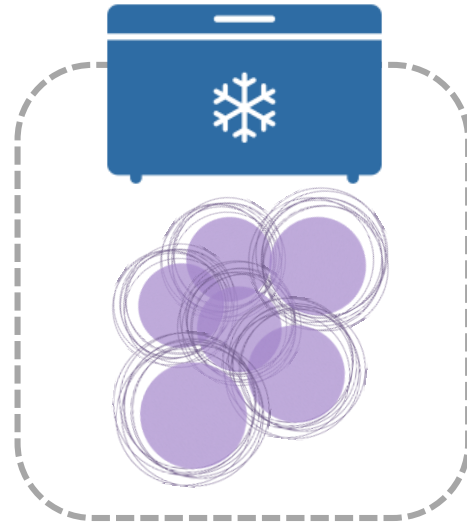
Single cell RNAseq



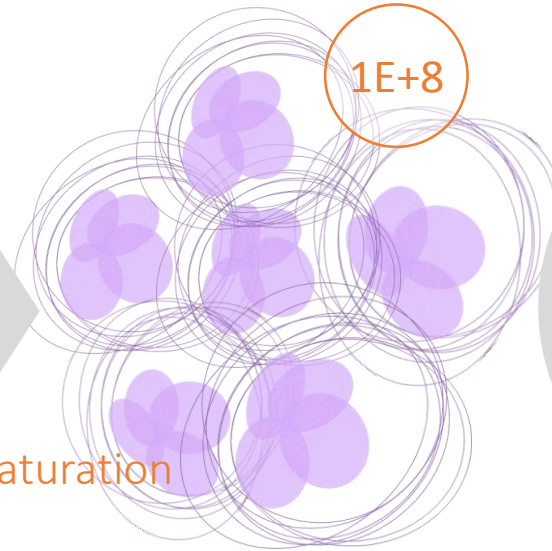
A FiM draft (2020-22)

MK progenitors
Cryobank

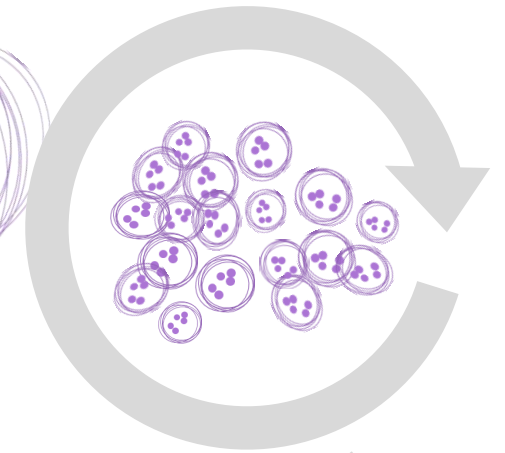
hiPSC line
GMP-grade
MKFOP inducible
HLA-null



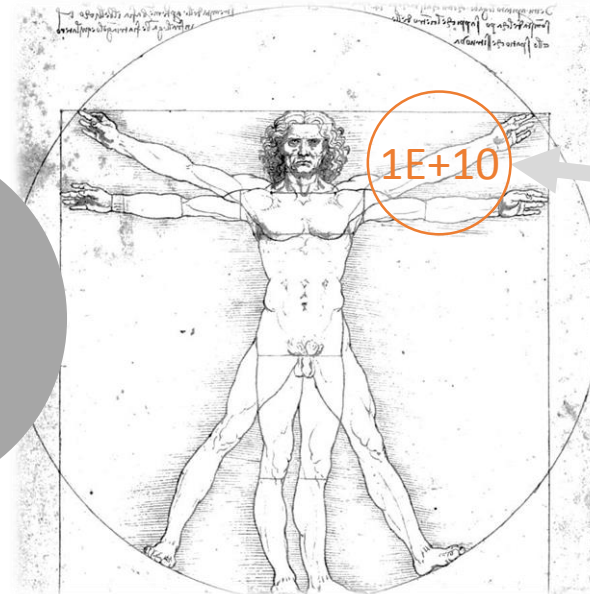
Controlled
expansion/maturation



Bioreactor



Labelling (Cr_{51})
Irradiation



STUDY
Recovery
Survival

People



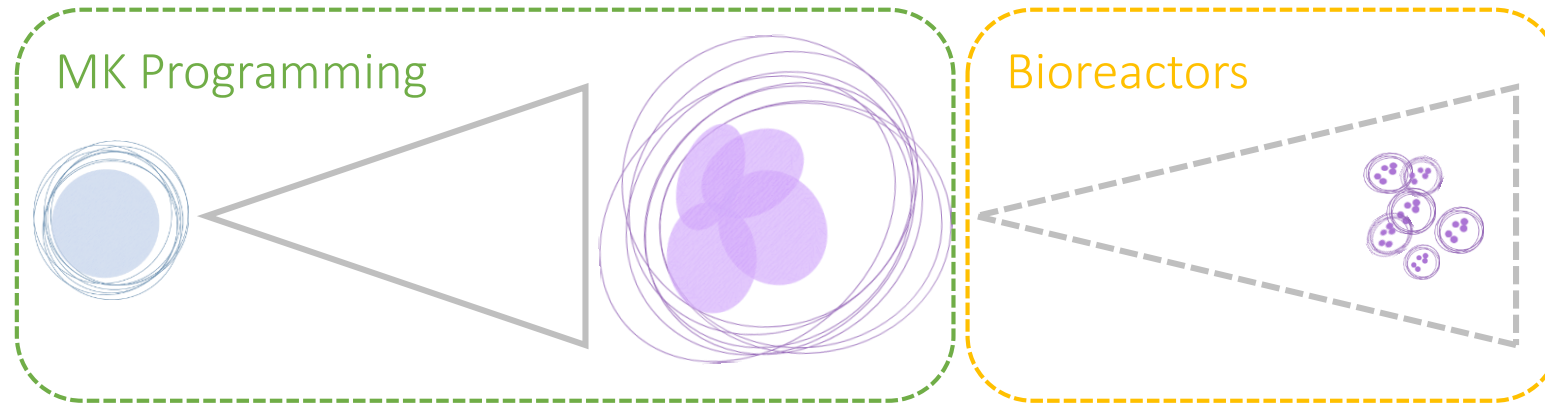
Funding



Environment



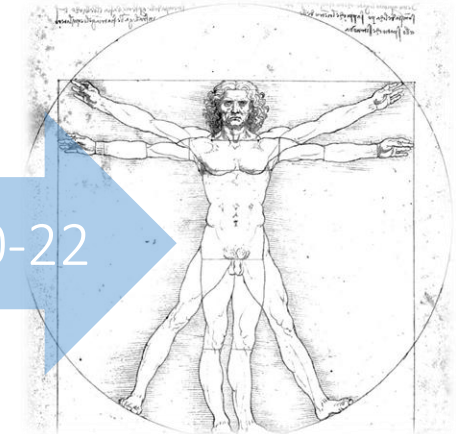
| Conclusions & Take-home



- MK Forward programming technology allows the **scalable generation of mature megakaryocytes *in vitro*** from human iPSCs.
- **Bioreactor systems under development (*in house* and collaborations)** to increase platelet biogenesis *ex vivo* (Quantity & Quality)
- MKFOP technology **progresses towards clinically compliant material and methods** (GMP lines, virus-free inducible programming, HLA-I deletion)



FiM 2020-22



Acknowledgements

NHSBT Cambridge, Haematology

Cedric GHEVAERT
Meera ARUMUGAM
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Daniel HOWARD
Moyra LAWRENCE
Thomas MOREAU
Annett MUELLER
Momal TAIMOOR
Amie WALLER
Wing Han WU
Rotations
James Baye
Carmen DIAZ
Riina LAMPELA
Jane PATRICK



NHS
National Institute for
Health Research

 **The Leukemia &
Lymphoma Society.**
Fighting Blood Cancers

NHS
Blood and Transplant



MRC | Medical
Research
Council



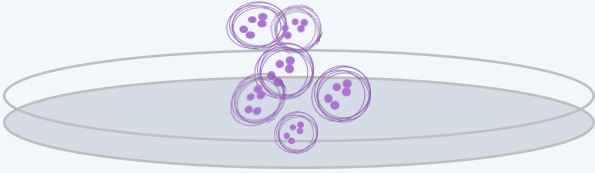
NHS
Blood and Transplant

 **UNIVERSITY OF
CAMBRIDGE**

Wellcome Trust - Medical Research Council
 Cambridge Stem Cell Institute

(...)

Assessing *ex-vivo* platelets



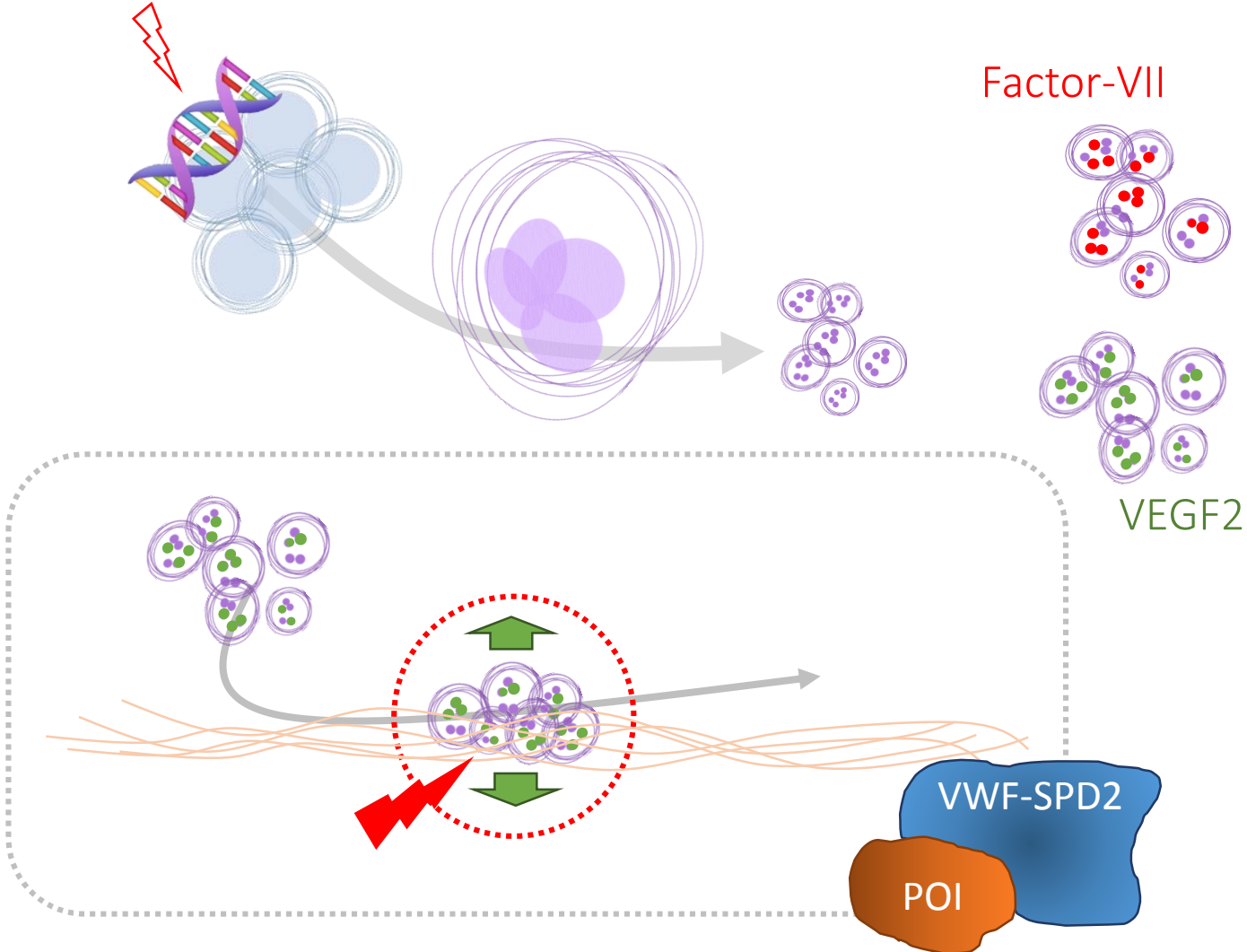
- Morphology (Sysmex, TEM)
- Function:
 1. Agonist activation (Flow)
 2. Aggregation
 3. Thrombus under flow



- Recovery, survival post transfusion
- Thrombotic dose
- Function:
 1. Thrombus formation
 2. Haemorrhages assay
 3. Immunogenicity
- Improved mouse models (hVWF,...)

Futuristic Platelets

CRISPR guided genetic modifications
 α -granule targeting of therapeutic proteins



Wilcox, 2013

Enhanced haemostasis
cardiac surgery

Tissue repair
myocardial infarction

