NHS Blood and Transplant

BBTS Harrogate September 28th 2012

'Assessing the therapeutic potential of induced pluripotent stem cells'

Dr Lee Carpenter

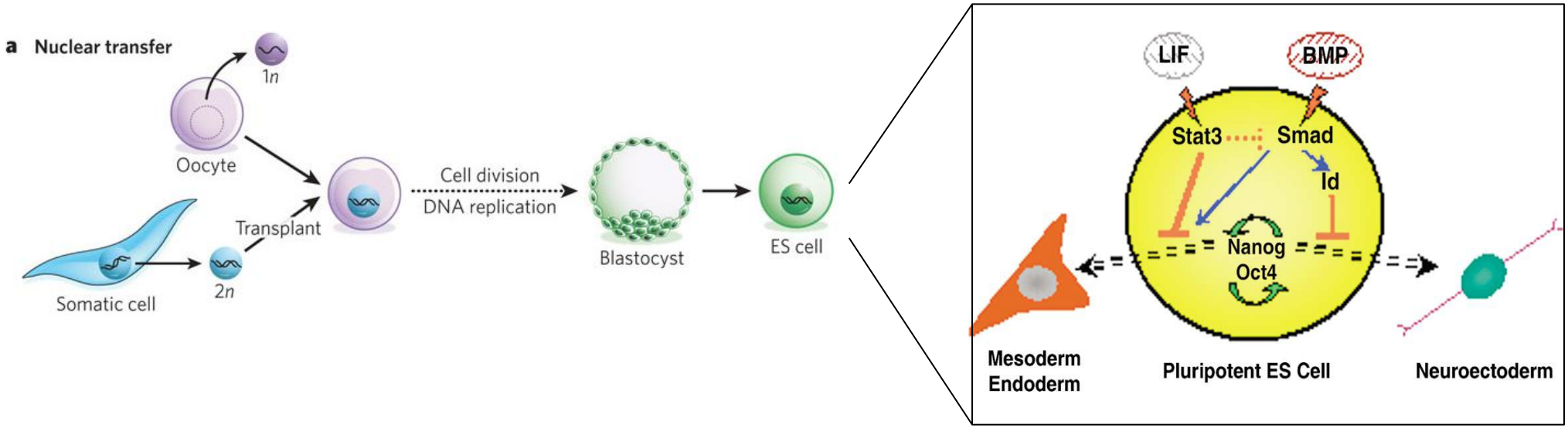
Research and Development- Oxford
NDCLS- University of Oxford

OXFORDMARTIN SCHOOL

UNIVERSITY OF OXFORD

Understanding and Inducing Pluripotency

NHS Blood and Transplant



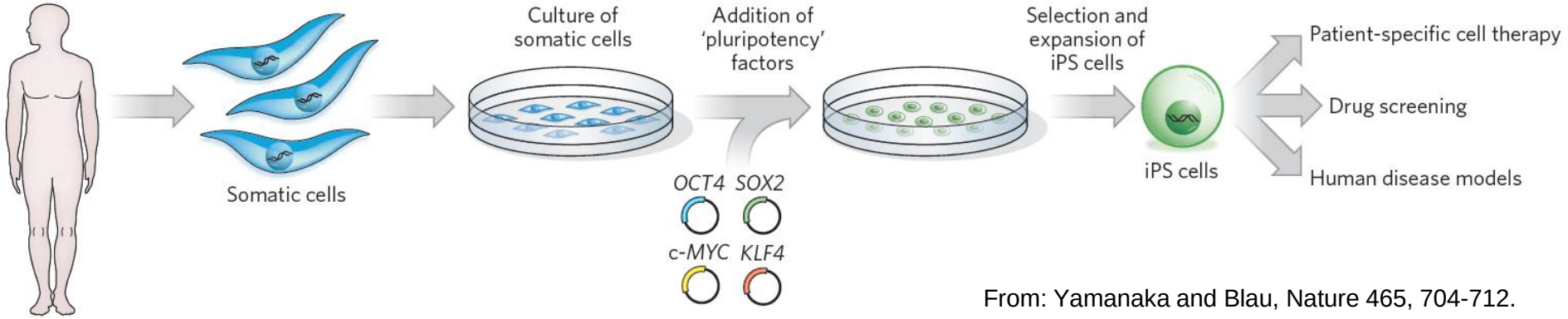
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

NHS Blood and Transplant

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

Cell *131*, 1–12, November 30, 2007 ©2007 Elsevier Inc. 1



From: Yamanaka and Blau, Nature 465, 704-712.

NHS Blood and Transplant

NIHR Programme A: Cellular and Molecular Engineering

Aim 3: Defining best practice for reprogramming

'to define safe and GMP compliant approaches for clinical trials'

iPSC Reprogramming systems

NHS Blood and Transplant

REPROGRAMMING SYSTEMS			
Integrating vectors			
Delivery system	**Reprogramming vector**	**Genomic disposition**	**Reference**
Retroviral	DNA	Integrated	Takahashi et al[2]
Lentiviral	DNA	Integrated	Yu et al[1]
Lentiviral/Cre-lox	DNA	Excisable	Soldner et al[6]
Transposon/Transposase	DNA	Excisable	Woltjen et al[7]
Episomal vectors			
Delivery system	**Reprogramming vector**	**Vector disposition**	**Reference**
Co-culture	Small molecules	Transient (replacing one or more transcription factors)	Lyssiotis et al[49]
Episomal Vectors	Plasmid DNA	Degraded / kicked out	Yu et al[8]
Recombinant Protein	Protein	Degraded	Kim et al[9]
Sendai Virus	RNA	Degraded / kicked out	Fusaki et al[10]
Synthetic nucleotides	Synthetic mRNA	Degraded / kicked out	Warren et al[11]

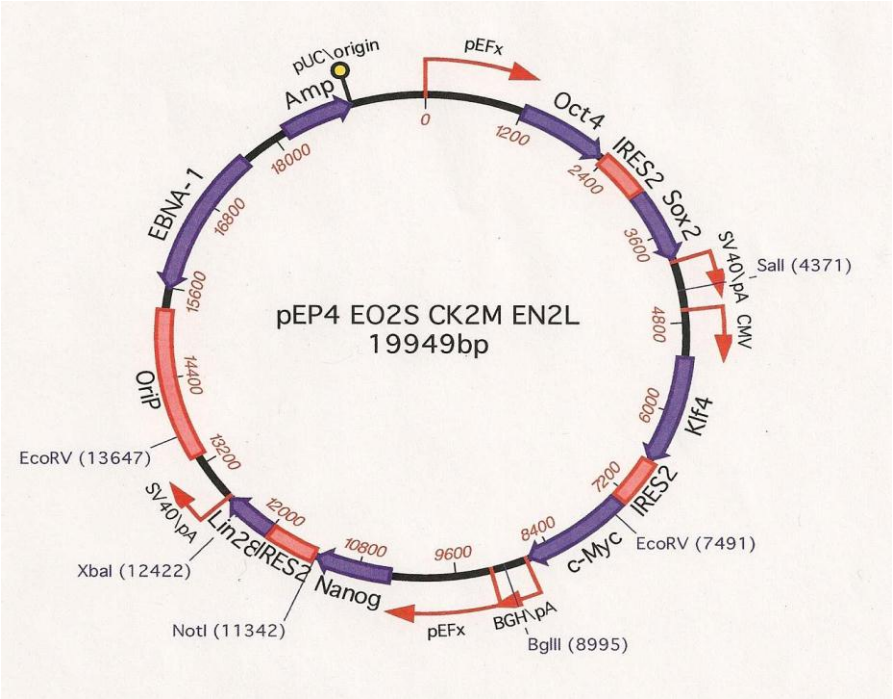
Drug Discovery World Winter 2010/11 p34

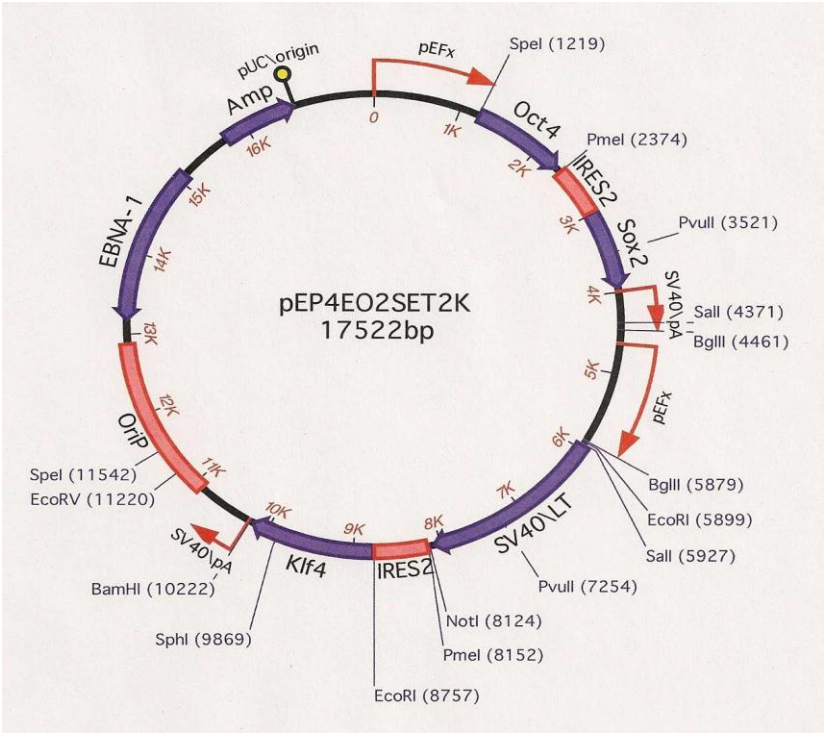
Problems

Genomic Insertion: the risk of mutations being inserted into the target cell's genome

Output: 0.067% of reprogramming efficiency.

Thomson's episomal plasmids

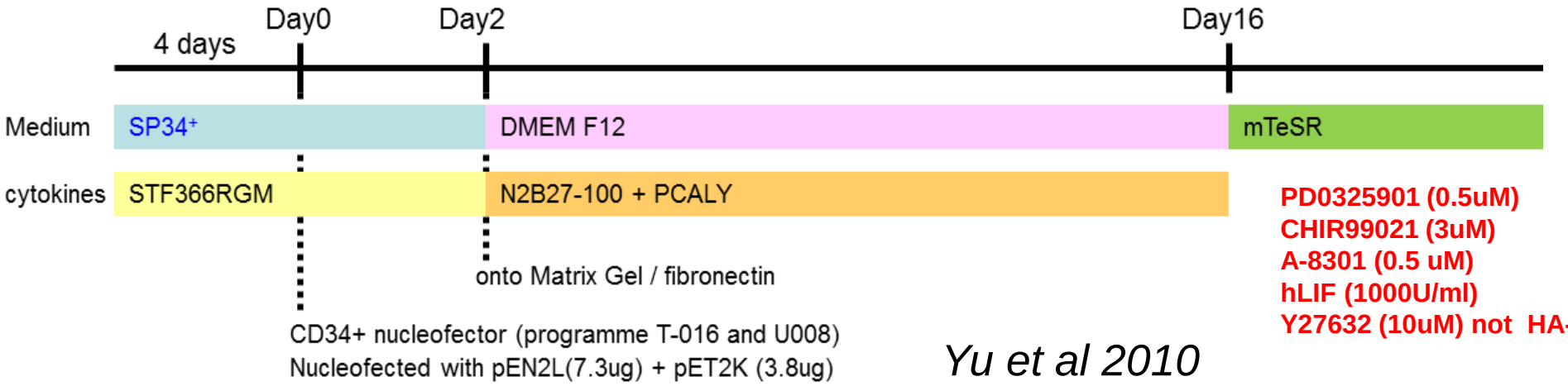




- 6 Factor (OSKMLN) + SVT
- SVT large antigen added to counteract the toxic effects seen with high levels of c-Myc expression
- Transfection with 2 plasmids each with Oct4 and Sox2 to produce higher levels of these 2 factors
- Only single nucleofection required.

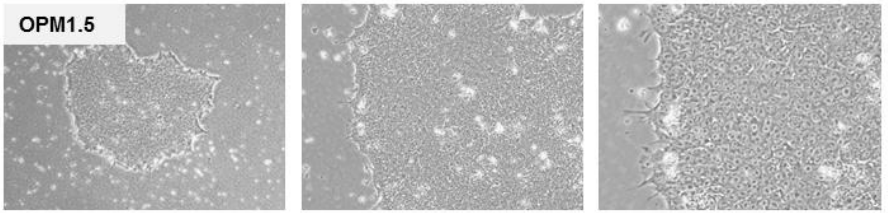
Reprogramming from peripheral blood.

NHS Blood and Transplant

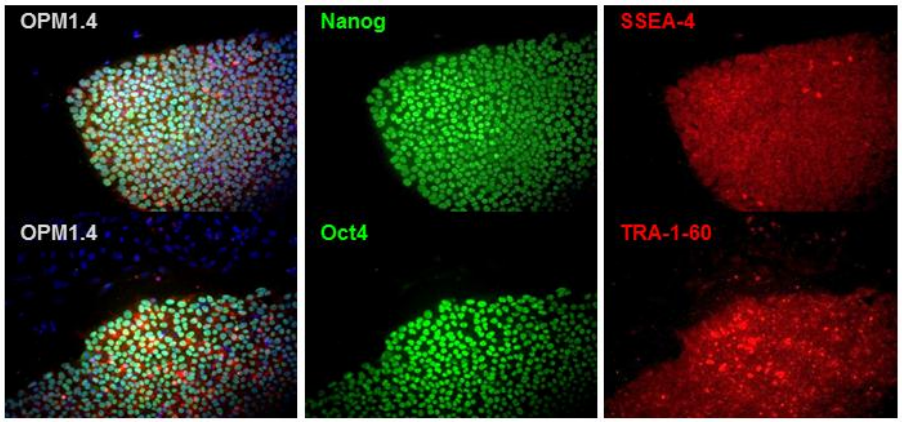


Yu et al 2010

PD0325901 (0.5uM)
CHIR99021 (3uM)
A-8301 (0.5 uM)
hLIF (1000U/ml)
Y27632 (10uM) not HA-100



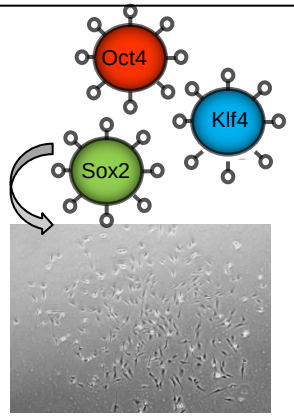
iPSCs adapt to
mTeSR system



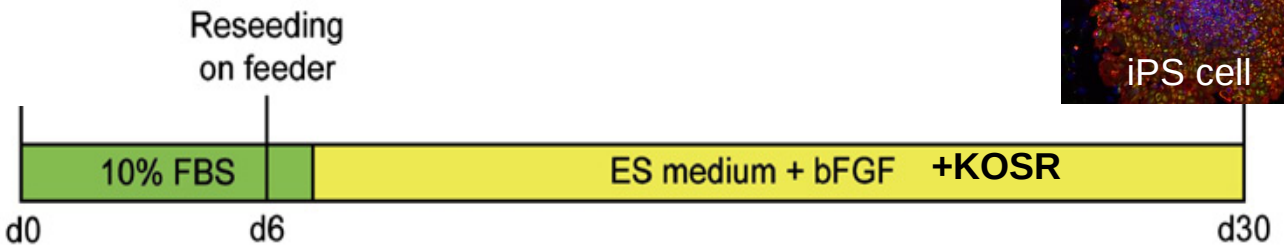
3 colonies from MNC (PBMNC)

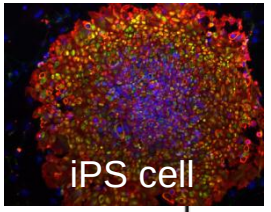
Reprogramming strategies in Oxford

Retrovirus

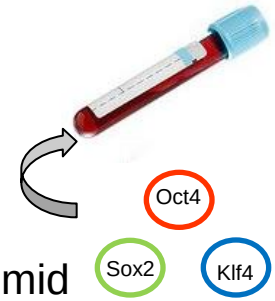


2008

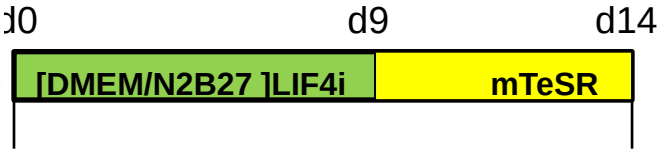


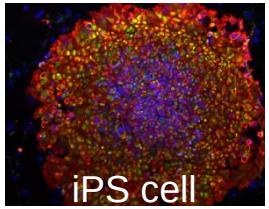


2012



DNA Plasmid





Reprogramming is now:
Quicker, Safer
GMP compliant
And from Blood

NHS Blood and Transplant

NIHR Programme D: Erythropoiesis in Health and Disease

Aim 2: Novels sources of Red Blood Cells

'to define novel sources for ex vivo production of red blood cells'

Background

NHS Blood and Transplant

- Need for alternative sources of blood
  -Diagnostics

FORM FRM833/1.1 Effective: 25/08/09

3 Cell Screen Profile Product PR121 & PR122

CE 0843

IVD

NHS Blood and Transplant

NBS REAGENTS

Product	Lot No	Product	Lot No.	Expiry Date
Alsevers	R121 3287	CellStab	R122 3287	2009.10.08

Unless otherwise indicated, all cells are positive for Kp^b and Lu^b and negative for Wr^a, Lu^a and Co^b
Instructions for use can be found at http://www.blood.co.uk/hospitals/diagnostic_services/reagents/index.asp#Pro

	Rh	C	D	E	c	e	C^w	M	N	S	s	P1	K	k	Kp^a	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Other
1	$R_1^wR_1$	+	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	
2	R_2R_2	0	+	+	+	0	0	+	0	+	0	0	+	+	0	0	+	0	+	+	+	
3	rr	0	0	0	+	+	0	0	+	0	+	+	0	+	+	0	+	+	0	0	+	

- Transfusion of iRBCs;

  -Hard to transfuse groups (Thalassemias/Sickle cell) due to allo-antibodies, with complications including iron overload.

  (ORh^{null} would cover 73% of Sickle population)

- First in Man study conducted by Giarratana/Douay 2011
  - (from peripheral blood CD34^{+} cells)

Workplan

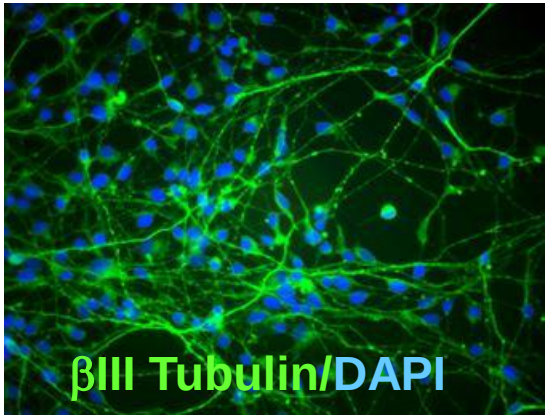
- Determine most appropriate erythroid differentiation protocol
  - EB formation
  - Directed
  - OP9 Stroma
- Undertake comparative studies with cord/adult CD34 derived RBCs (array profiling).

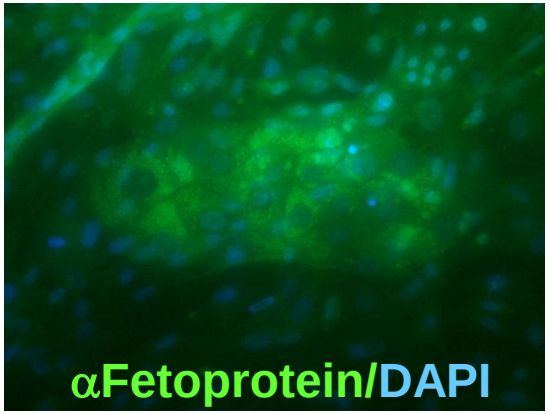
- Consider further ‘reprogramming’ to improve outcome.

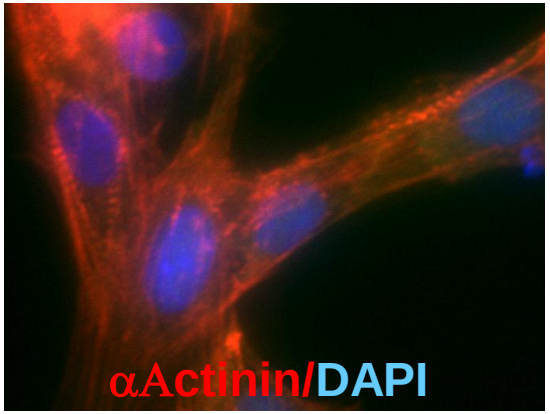
Demonstrating Pluripotency

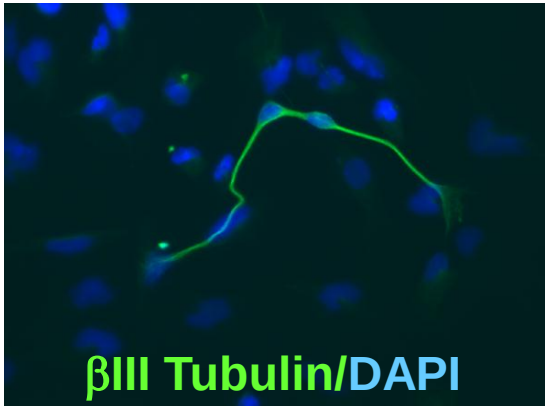
NHS Blood and Transplant

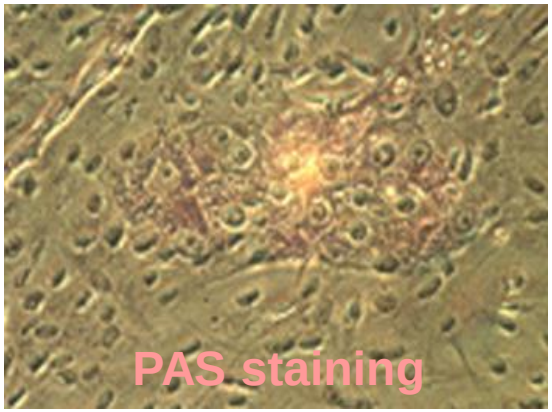
neurons | hepatocytes | cardiomyocytes

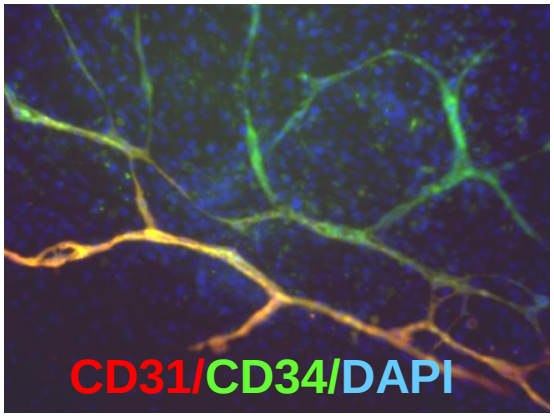




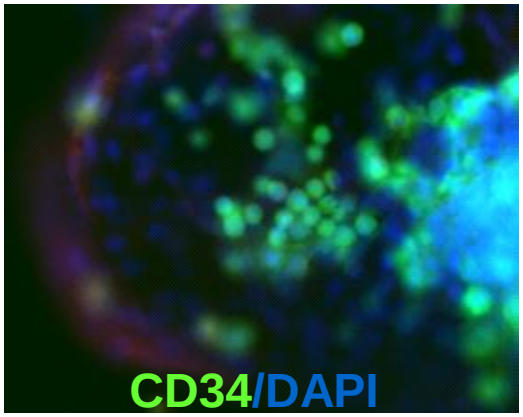


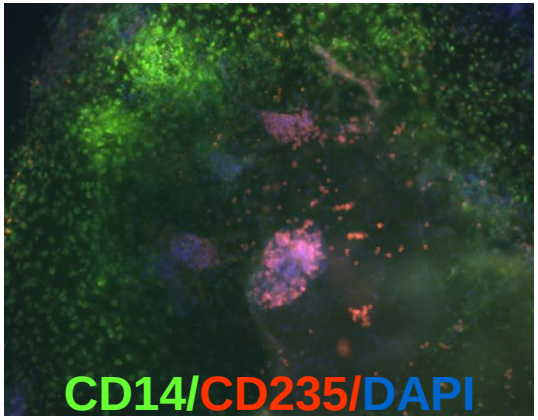


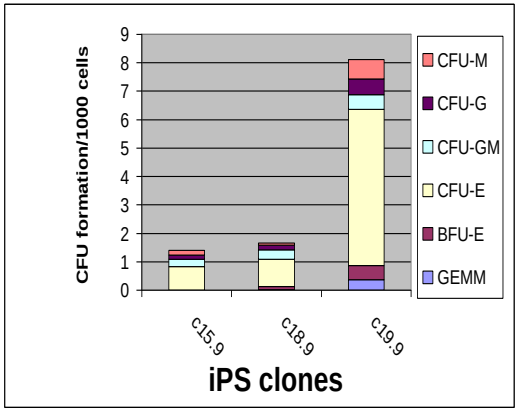


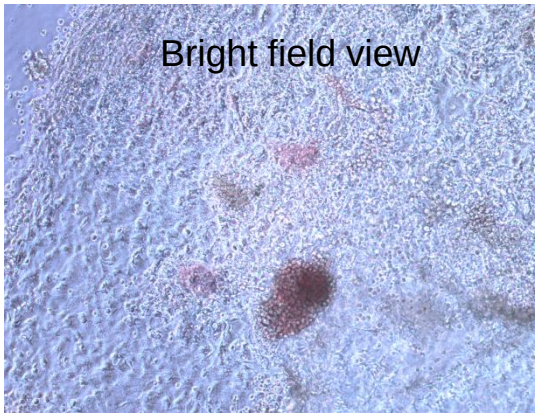


i) Erythropoiesis by EB formation

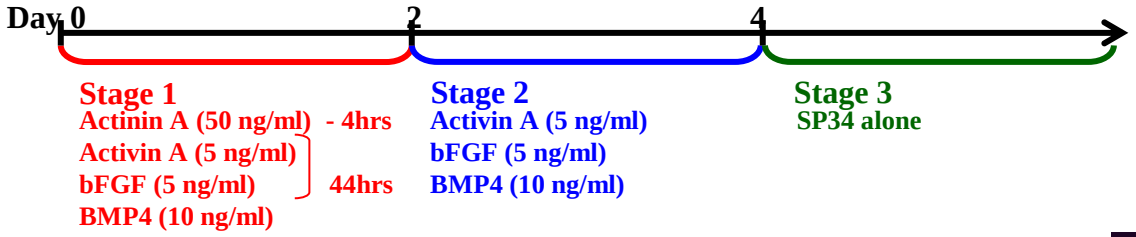


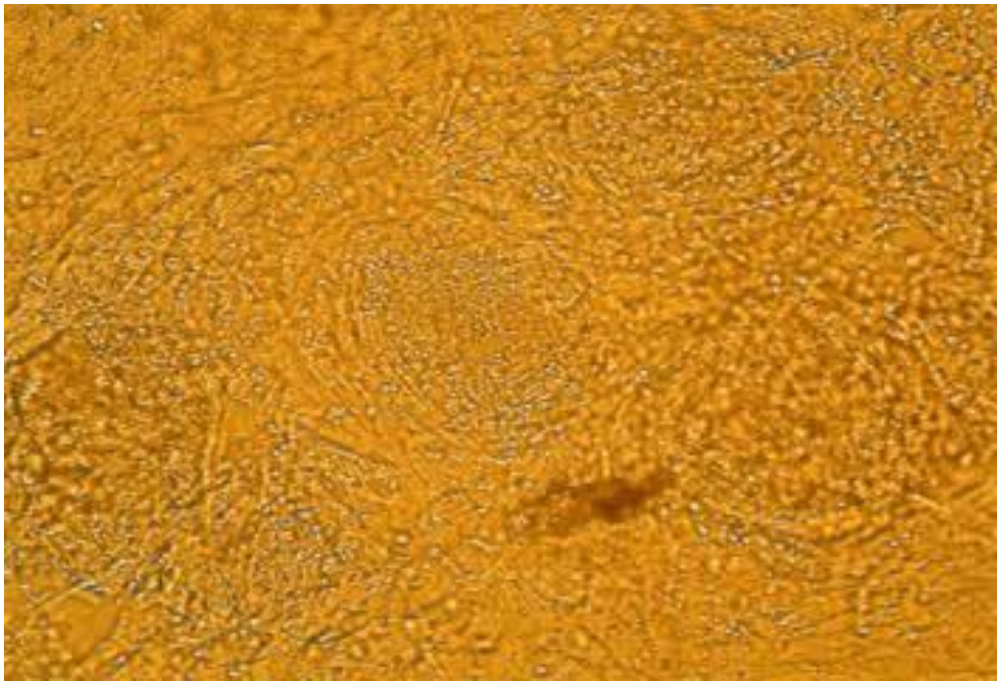


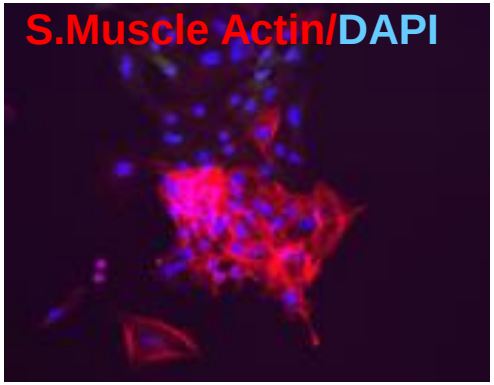


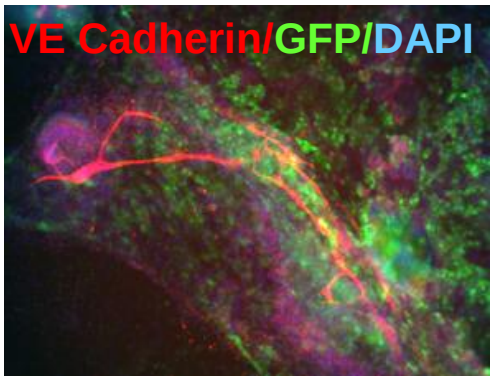


ii) Erythropoiesis by directed differentiation









Carpenter et al 2012

iPS cells contribute efficiently to endothelium and venules

NHS Blood and Transplant

Directed differentiation

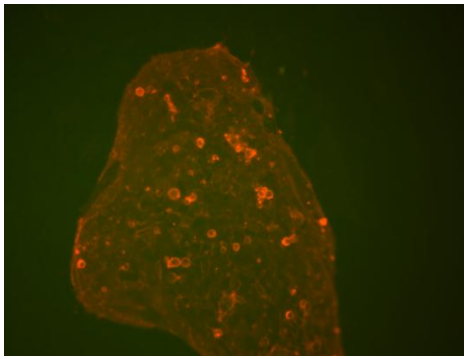
Cardiac differentiation for 28 days

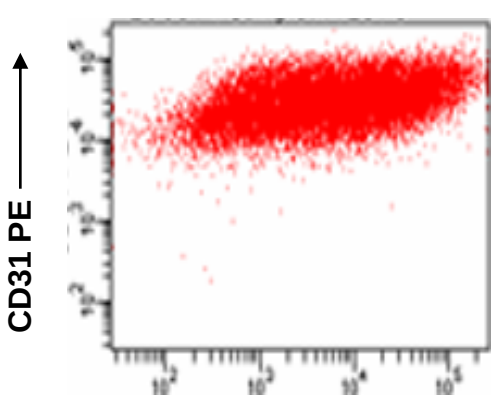
↓

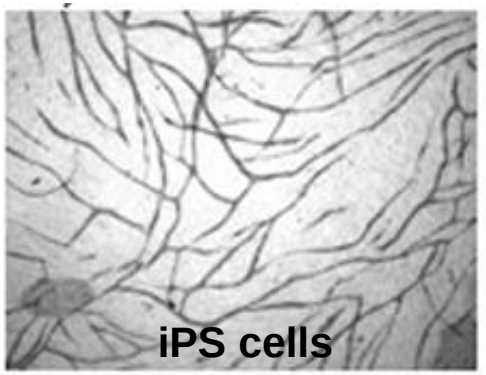
MACS selection on CD31+

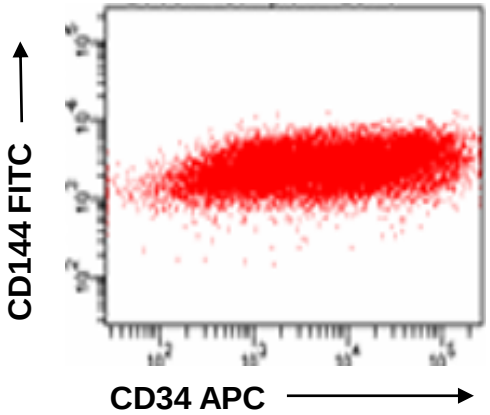
↓

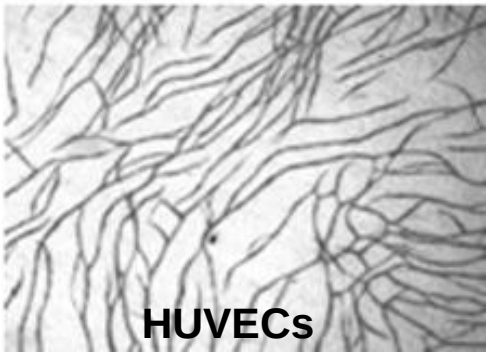
Expansion in EGM2 (Lonza)







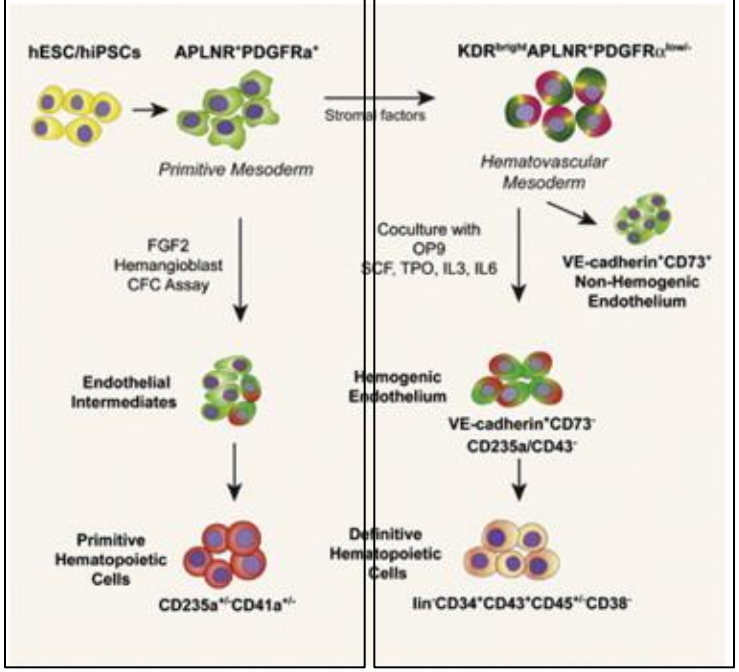




Lessons from development

Terminology	Primitive	1st Definitive	2nd Definitive
Time	E7.5	E8.5	E12.5
Origin	Yolk Sac	YS and Embryo	Embryo
Progenitor	Haemangioblast	Haemogenic Endothelium	
Output	EryP	EryD/lymphocytes	HSC

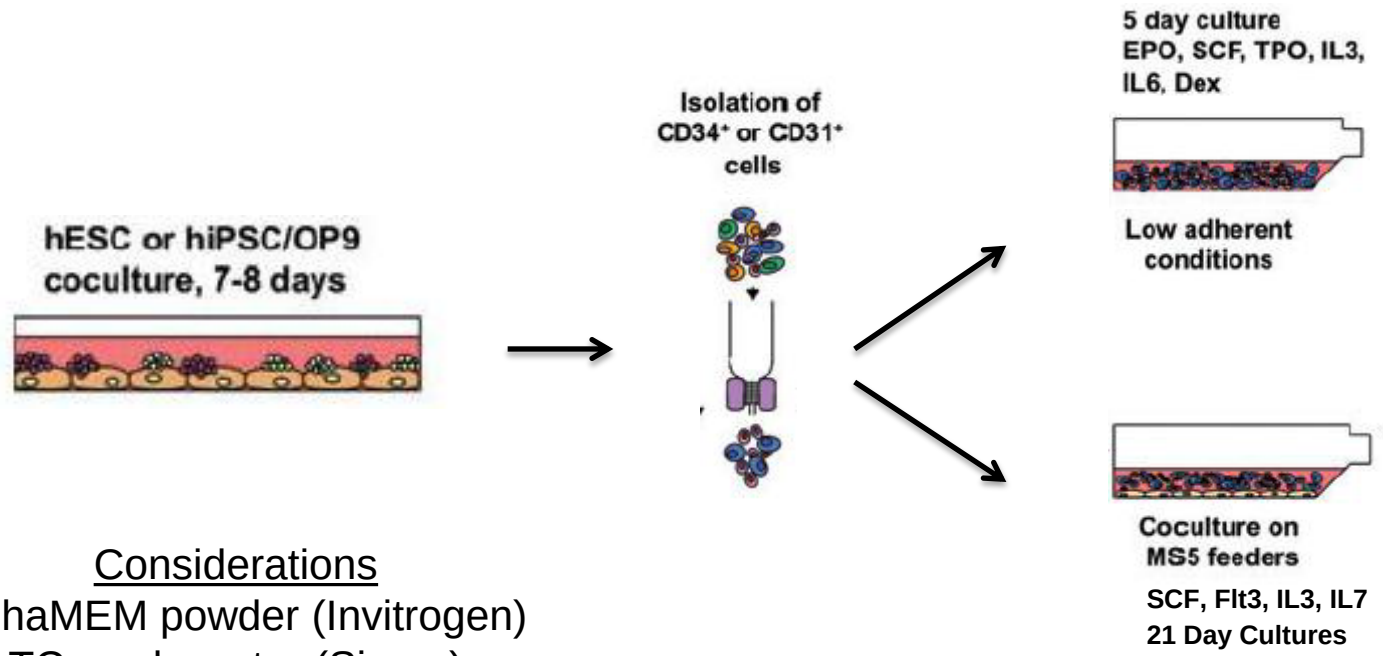
Haematopoiesis in-vitro from ESC/iPSCs



Choi *et al*, Cell Reports 2:1-15, Sept 2012

Lymphoid and erythroid differentiation from iPSC clones

Dias/Slukvin 2011- iPSC co-culture
Lapillonne/Douay 2010- iPSC EBs
Lu/Lanza 2008- hES/HoxB4/feeders



Erythroid Cells

B Lymphoid Cells

Considerations
alphaMEM powder (Invitrogen)
TC grade water (Sigma)
Defined serum (Hyclone)
Permissive OP9 (Slukvin)

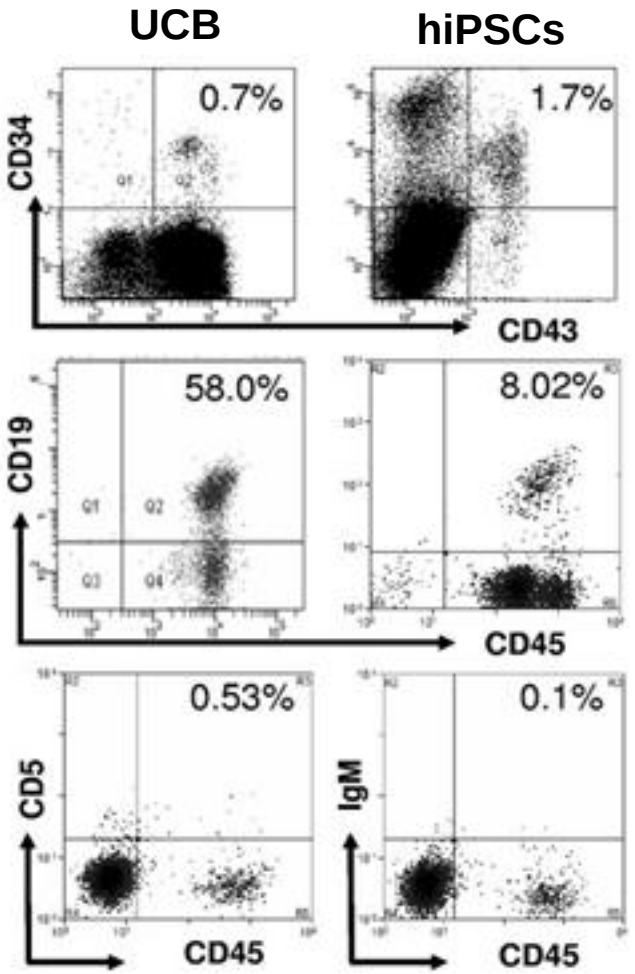
Vodyanik and Slukvin 2008

Brief report

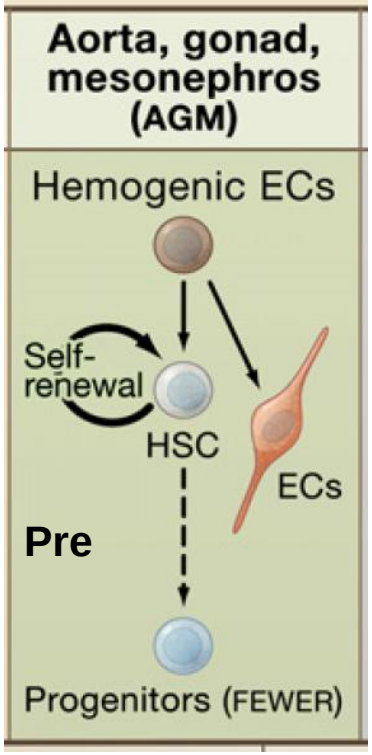
Human induced pluripotent stem cells are capable of B-cell lymphopoiesis

Lee Carpenter,[1,2] Ram Malladi,[3] Cheng-Tao Yang,[1,4] Anna French,[1,5] Katherine J. Pilkington,[1] Richard W. Forsey,[1] Jackie Sloane-Stanley,[3] Kathryn M. Silk,[6] Timothy J. Davies,[6] Paul J. Fairchild,[6] Tariq Enver,[5] and Suzanne M. Watt[1,2]

NHS Blood and Transplant



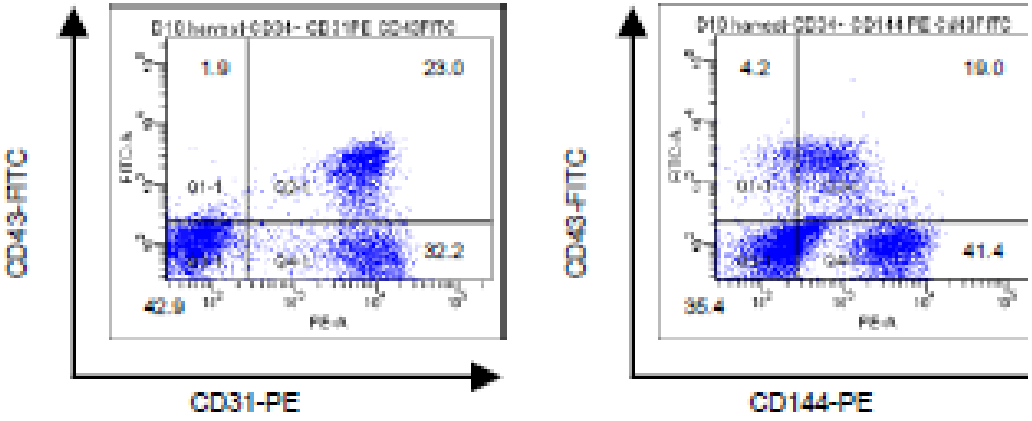
Orkin and Zon 2008

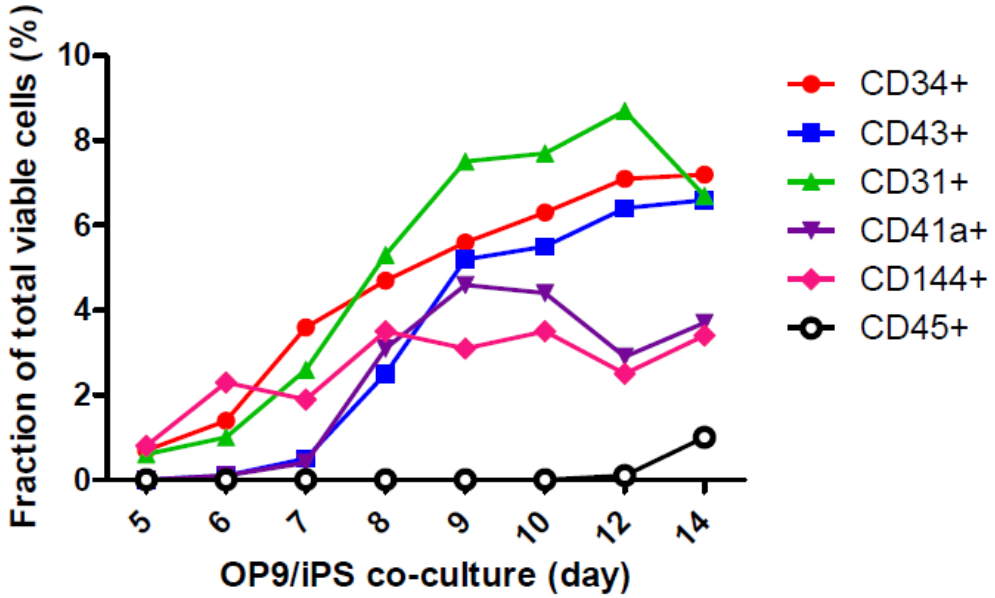


B cells now shown to be IgM Positive and fully differentiated

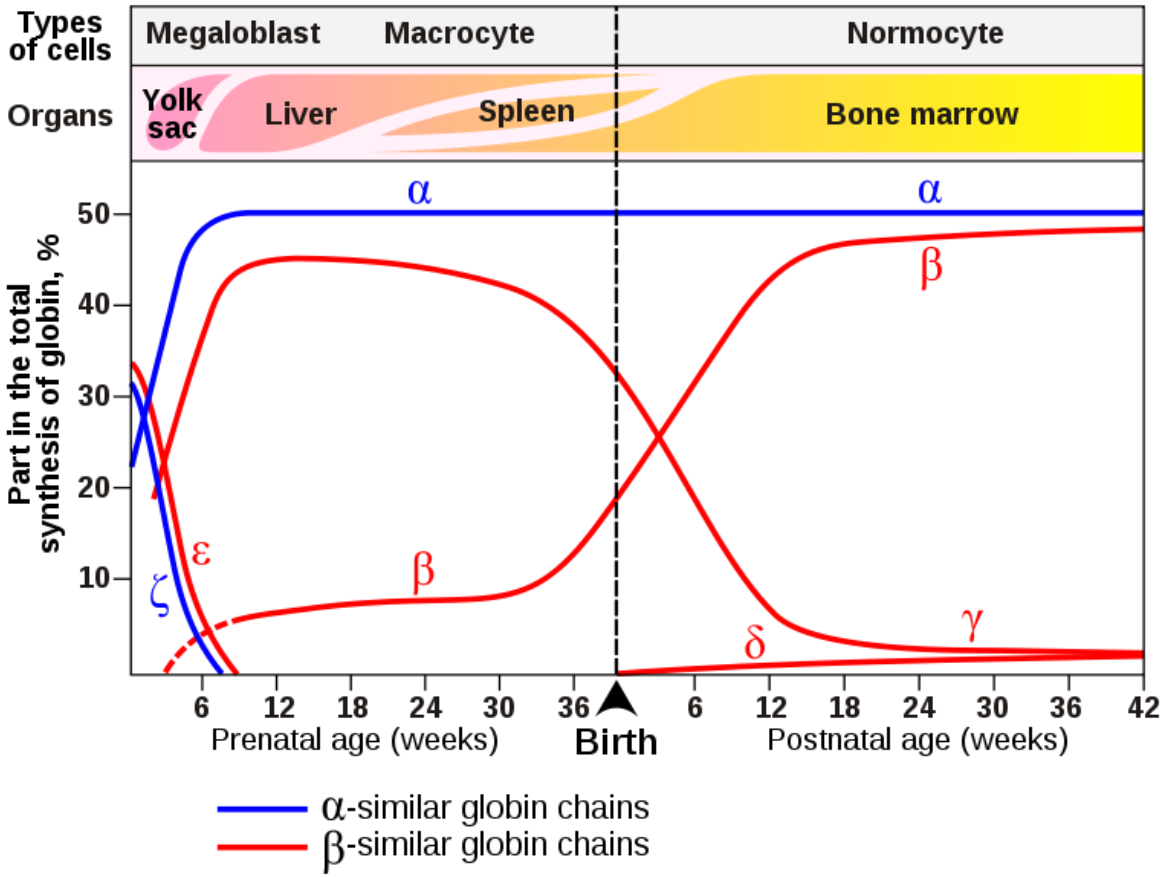
Towards isolating haemogenic endothelium: to provide progenitors for erythropoiesis

- CD45-PFV phenotype
- Or $CD144^{+}CD43^{-}$





- Beta-globin switching



data on *Wood W.G.*, (1976). Br. Med. Bull. 32, 282
http://en.wikipedia.org/wiki/File:Postnatal_genetics_en.svg

Major challenges:

- Globin expression profile typically embryonic and fetal (ε/γ not β)

- Enucleation; observed for adult CD34 but not from cord or pluripotent erythroblasts (can be overcome with stroma/macrophages).

- Scale-up/manufacture ($2x10^{12}$/unit)

Lessons from developmental haematopoiesis will continue to help us overcome these challenges.

Progress

NHS Blood and Transplant

i) New generation iPSCs have been derived from blood, using safe and GMP 'ready' approaches…. We are now ready for rare blood !!

ii) Haemogenic endotheliun has been identified, which indicates definitive haematopoiesis.

iii) B lymphocytes can fully differentiate.

iv) Several protocols have been assessed for red cell production.

v) Comparative studies with cord and adult RBCs underway.

NHS
Blood and Transplant

Thankyou

- Dave Roberts, Suzanne Watt, Amit Nathwani

- Cheng-Tao Yang (NHSBT) red cells
- Anna French (MRC DPhil student) B cells
- Pollyanna Tat (UCL) iPS cells

Conclusions

NHS Blood and Transplant

hiPSCs may not as good as cord or peripheral blood (but how can they be)

hiPSCs are at least as good as hESCs. (similar advantages and drawbacks)

hiPSCs do offer major advantages over hESCs (ethics/IP) And cord/adult sources (long term provision of any blood rare group)