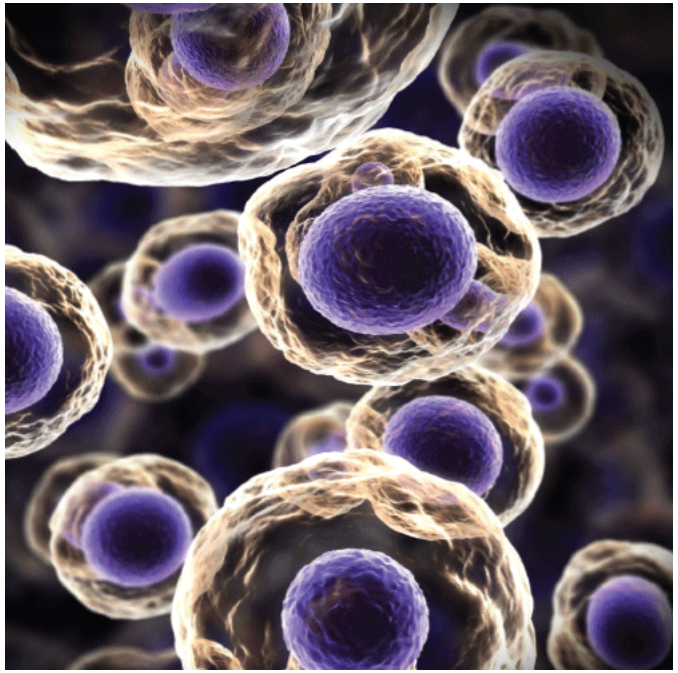
(Haematopoietic) Stem Cell Technologies



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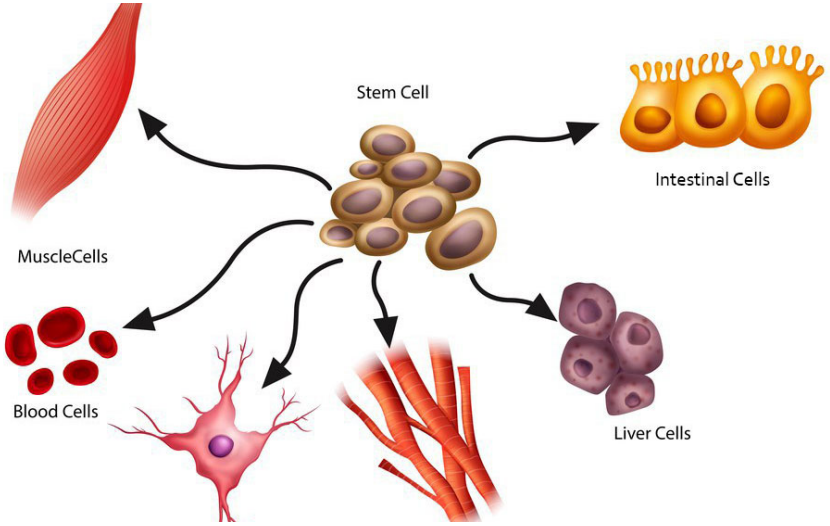
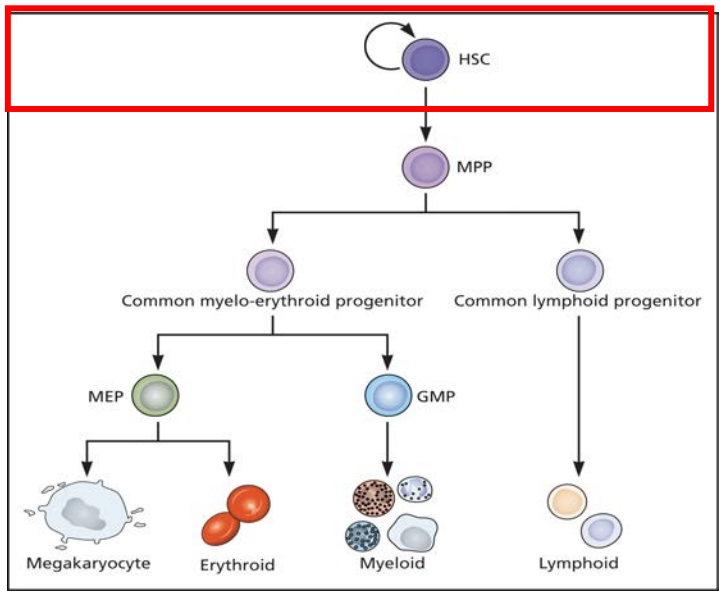
MRC WIMM DPhil Course November 2020

Learning Objectives

- What are the defining properties of a **stem cell?**
- **Key concepts** in HSC biology during ontogeny and adult life
  - Properties of HSCs and progenitors
  - HSC niches
  - Intrinsic / extrinsic regulators
- **Experimental approaches** to study HSC biology
  - *In vitro*
  - *In vivo*
- **Cancer Stem cells**

Defining properties of stem cells. Toti-potent, multi-potent, oligopotent,..





- Perpetual self renewal
- Able to differentiate into a mature adult cell type (mostly multi-lineage)
- Life-long reconstitution following transplantation

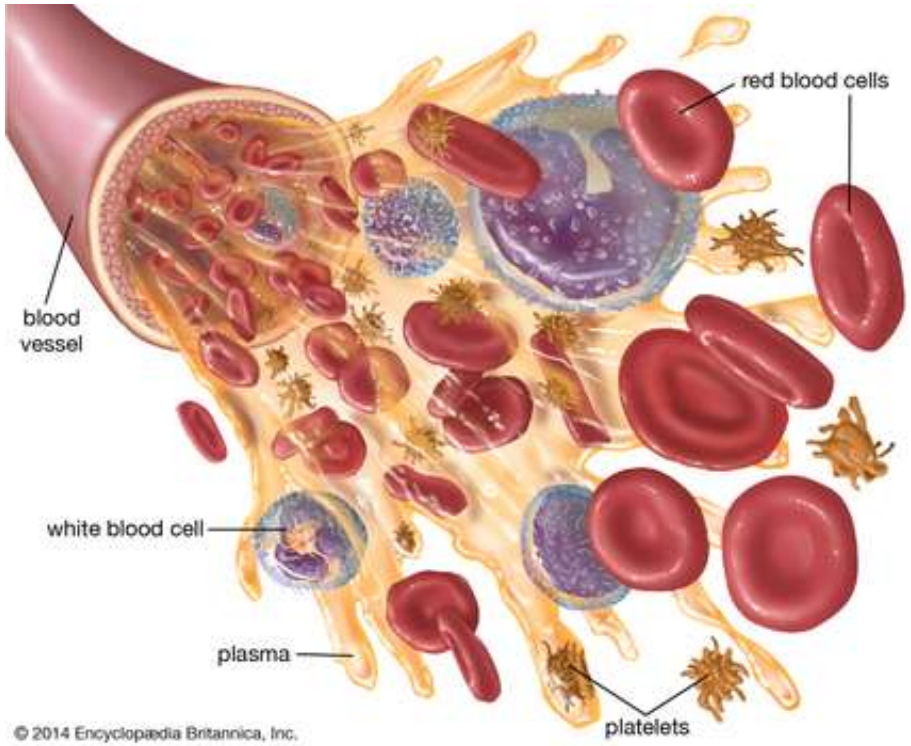
HSCs are arguably the best studied stem cell in mammals

- Ease of access
- Blood lineages are morphologically and immunophenotypically distinct
- Experimental assays now well developed
- Only stem cell therapy in routine clinical use
- While stem cell transplant is widely used, there are many limitations
  - Mortality <25%
  - Graft-versus-host-disease
  - Relapse

Why study HSCs?

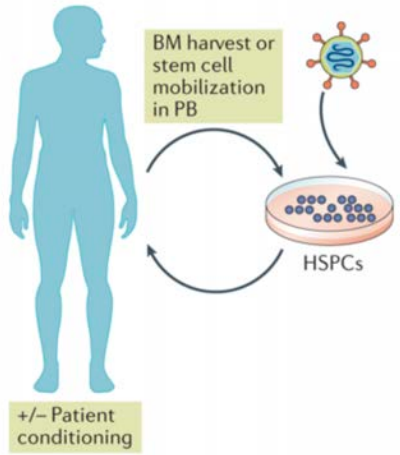
Abnormalities of blood cell production

- **Too few cells produced**
  - Bone marrow failure e.g. aplastic anaemia
  - Anaemia, neutropenia, thrombocytopenia
- Too **many** cells produced
  - Essential thrombocythaemia
  - Polycythaemia vera
  - Chronic myeloid leukaemia
- **Abnormal** cell production
  - Malignant: leukaemia, myelodysplasia
  - Non-malignant: sickle-cell anaemia



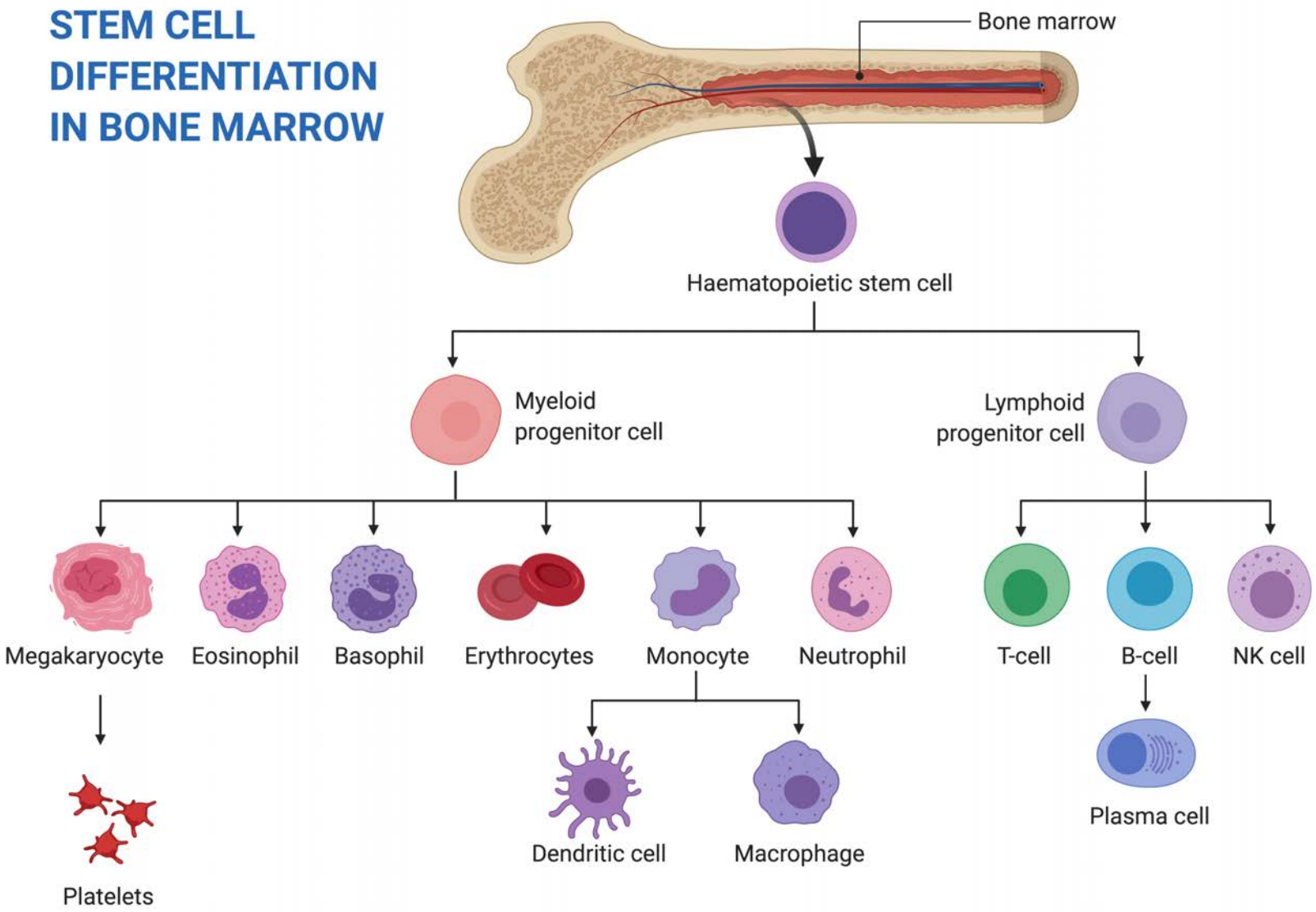
What are the potential clinical uses of HSCs?

- Transplantation for bone marrow disorder e.g. leukaemia
- In vitro production of red cells, platelets
- Repair of damaged tissue e.g. heart, liver
- Gene therapy e.g. SCID, thalassaemia

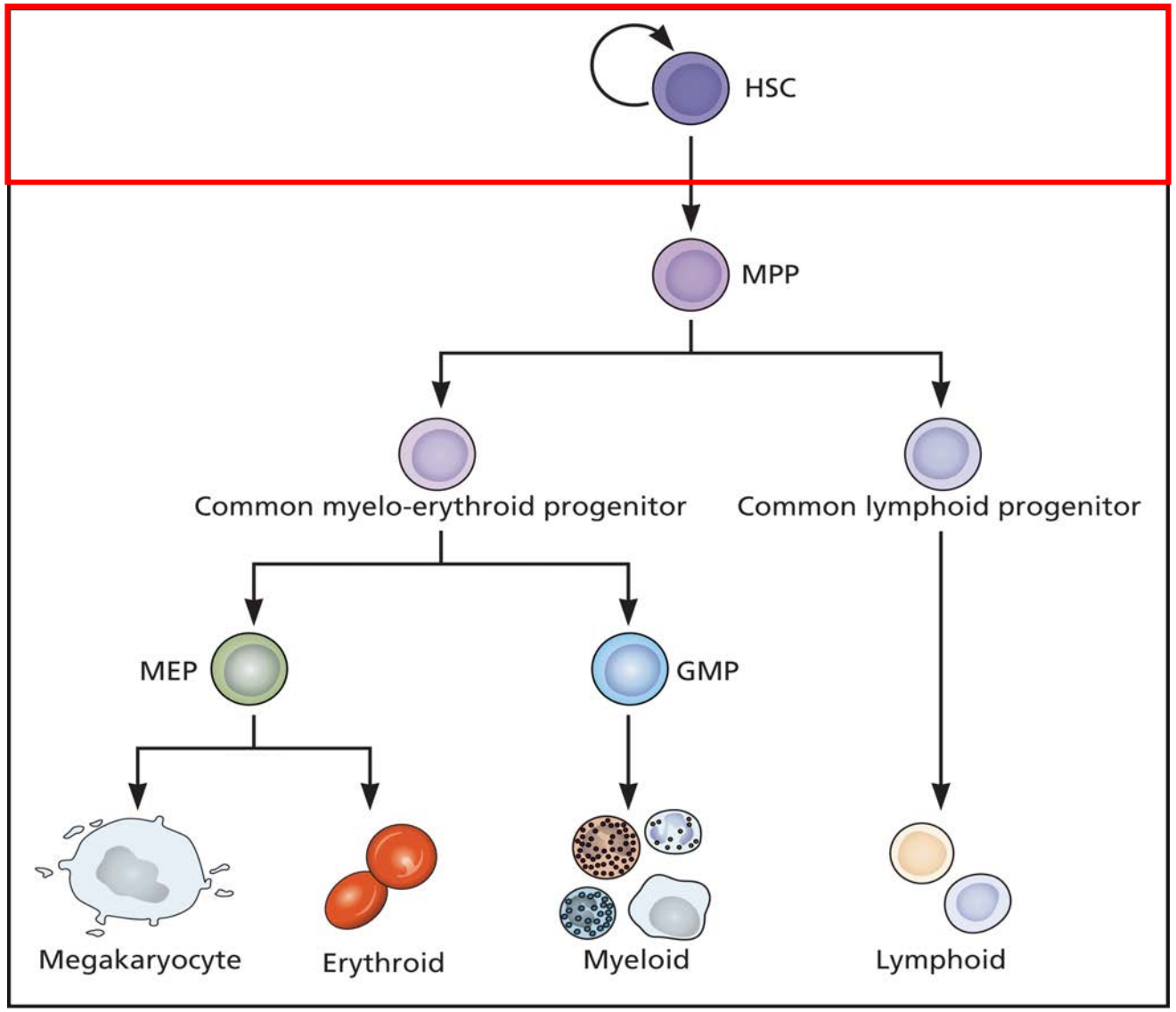


See review by Cavazzana et al, Nature Reviews 2019

Haematopoiesis: classical hierarchical model



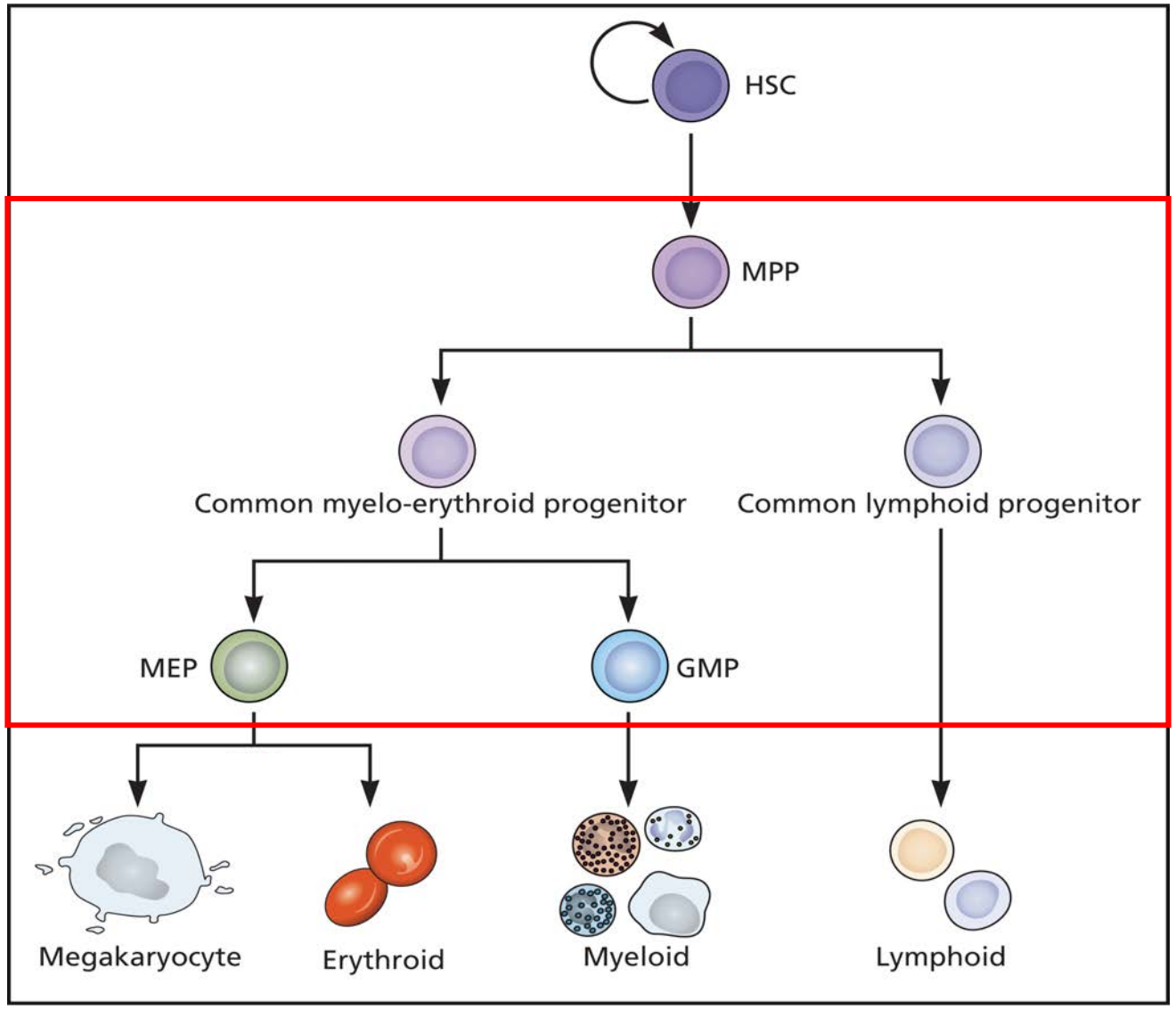
Haematopoietic stem *vs*. progenitor cells



What are the key defining properties of an HSC?

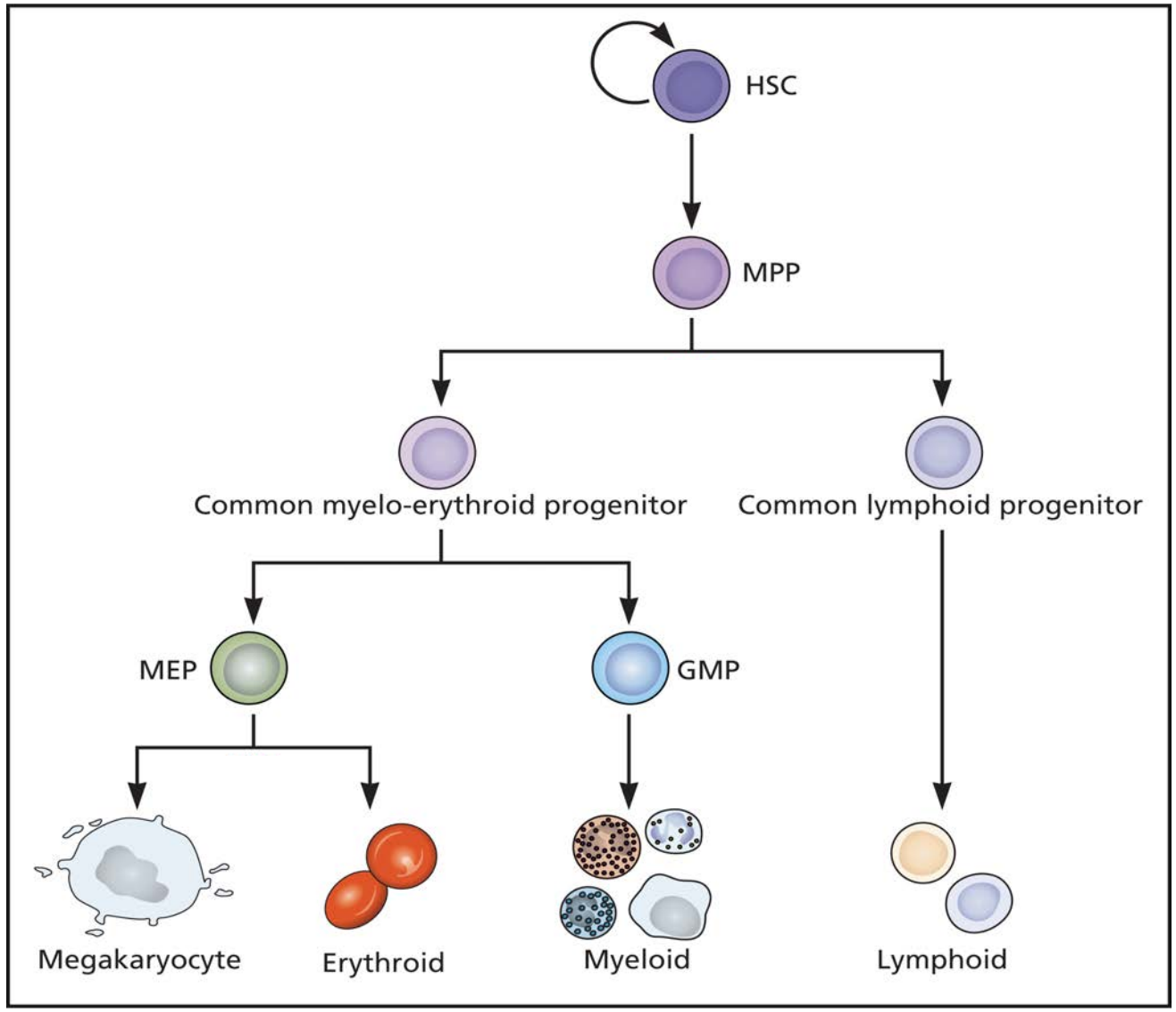
- Self renewal
- Life-long reconstitution following transplantation
- Multi-lineage differentiation

Haematopoietic **progenitor** cells



- Less self-renewal capacity (short term engraftment)
- Already made some lineage commitment decisions (oligopotent)

Haematopoietic **progenitor** cells



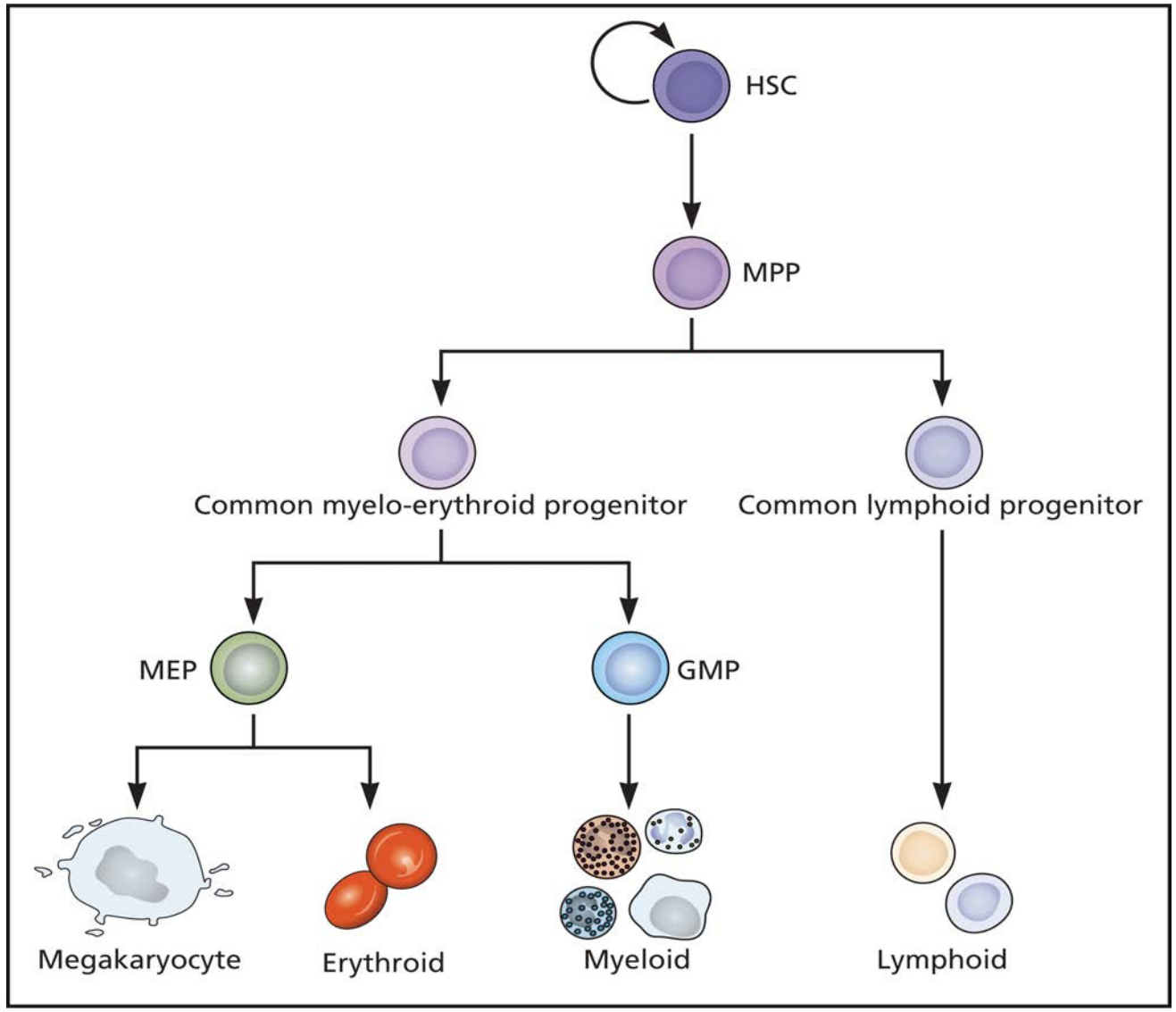
- Common **lymphoid** progenitor

(B, T, NK cells)

- Common **myeloid** progenitor

(granulocytes, erythroid, megakaryocyte and monocyte/macrophage)

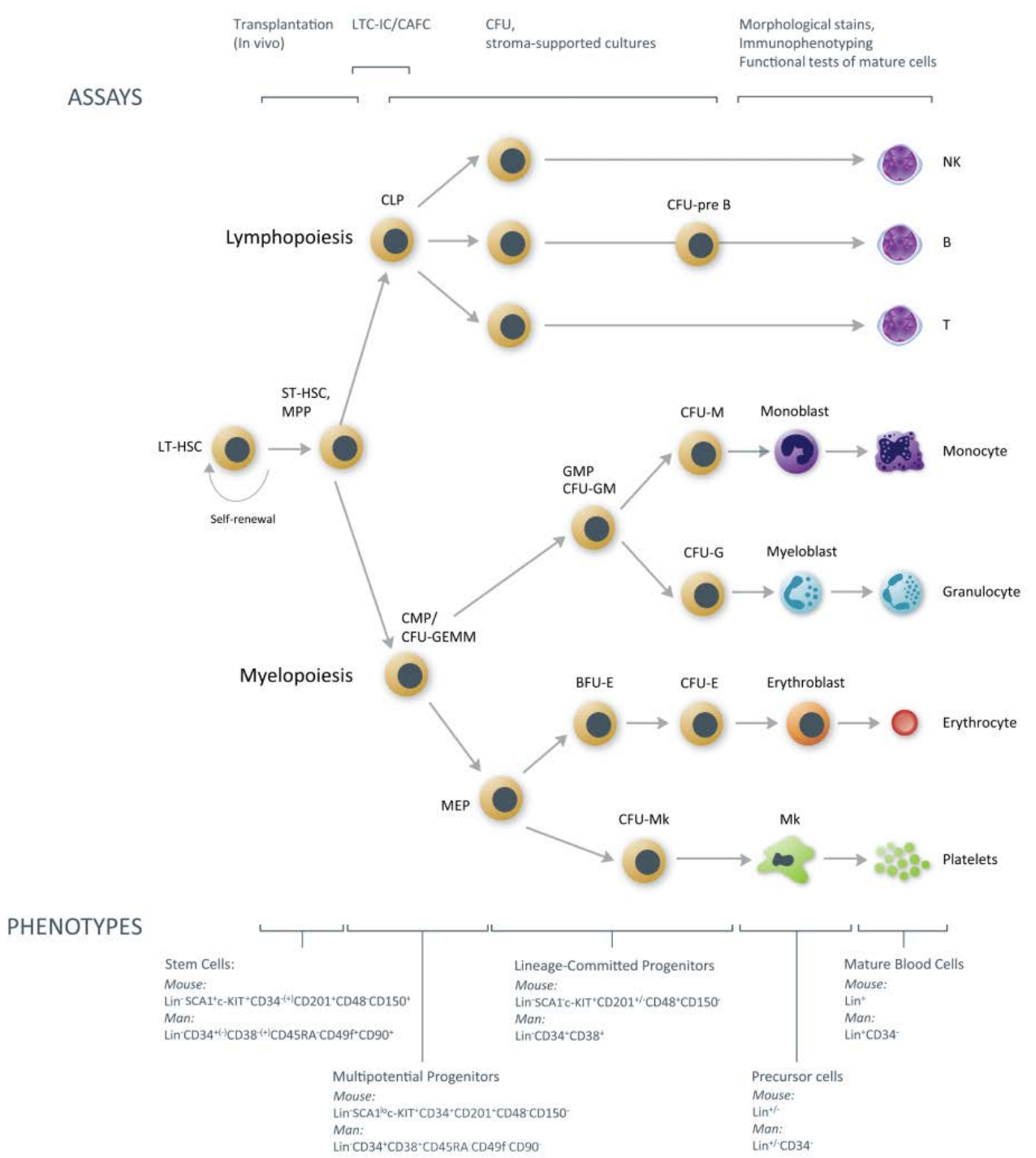
Haematopoietic stem/progenitor cells



Identified by their expression of cell surface markers

e.g. Human HSC = **CD34+**lineage-CD38-CD45RA-CD90+CD49f+

Identification of HSC/HSPCs: Human/mouse



B

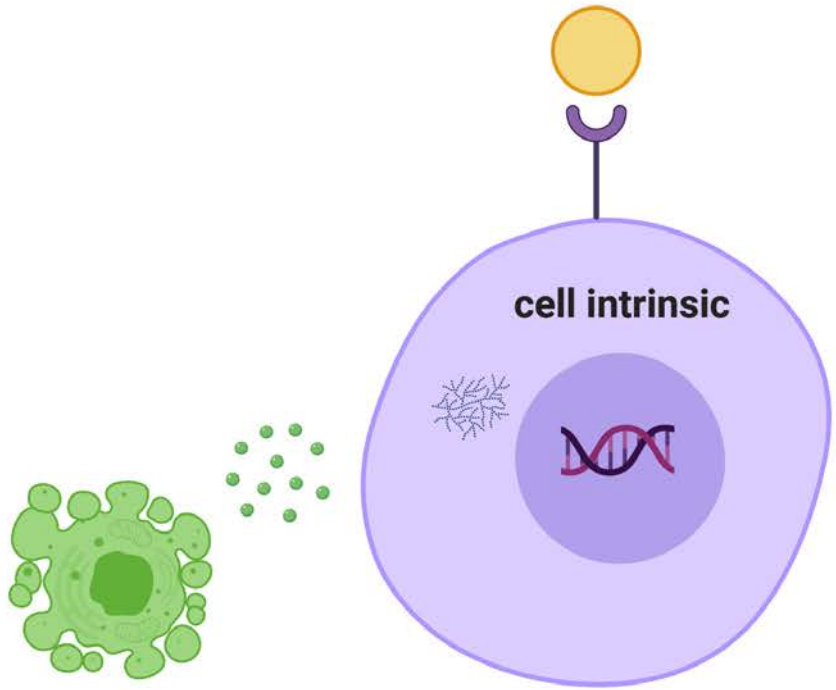
Cell type	Mouse marker		Human marker
HSCs	LT-HSC	Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$ $CD34^-$ $Flk2^-$	$CD34^+$ $CD38^-$ $CD90^+$ $CD45RA^-$ $CD49F^+$
	ST-HSC	$Line^-$ $Sca\text{-}1^+$ $c\text{-}Kit^+$ $CD34^+$ $Flt2^-$	
	MPPs	Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$ $CD34^+$ $Flt2^+$	$CD34^+$ $CD38^-$ $CD90^-$ $CD45RA^-$ $CD49F^-$
SLAM-HSCs	SLAM-HSC	Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$ $CD150^+$ $CD48^-$ $CD229^-$ $CD244^-$	
	SLAM-MPP	Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$ $CD150^-$ $CD48^-$ $CD229^-$ $CD244^-$	
Progenitor cells	CLP	Lin^- $Flt2^+$ $IL7Ra^+$ $CD27^+$	$CD34^+$ $CD10^+$ $CD7^+$
	CMP	$Line^-$ $Sca\text{-}1^-$ $c\text{-}Kit^+$ $CD34^+$ $Fc\gamma RII/III^-$	$CD34^+$ $CD38^+$ $CD123^{med}$ $CD135^+$ $CD45RA^-$
	GMP	Lin^- $Sca\text{-}1^-$ $c\text{-}Kit^+$ $CD34^+$ $Fc\gamma RII/III^+$	$CD34^+$ $CD38^+$ $CD123^{med}$ $CD135^+$ $CD45RA^+$
	MEP	Lin^- $Sca\text{-}1^-$ $c\text{-}Kit^+$ $CD34^-$ $Fc\gamma RII/III^-$	$CD34^+$ $CD38^+$ $CD123^-$ $CD135^-$ $CD45RA^-$ $CD110^+$
Niche supporting cells	Mφ	$CD11b^+$ $Gr1^{low}$ $F4/80^+$ SSC^{low}	
	T cell	$CD3^+$	$CD4^+$ $CD25^+$ $CD127^{low}$ $CD45RA^{+/-}$
	B cell	$CD45R/B220^+$	
	Erythrocyte	$CD45^-Ter119^+$	
	EC	$CD45^-Ter119^-CD31^+$	
	BMSC	$CD45^-Ter119^-CD31^-$ $LepR^+$	$CD45^-$ $CD34^-$ $CD73^+$ $CD105^+$ $CD90^+$
	OB	$CD45^-Ter119^-CD31^-$ $Sca1^-$ $CD51^+$	

Key markers
HUMAN – CD34, CD38

MOUSE – LSK, CD34-

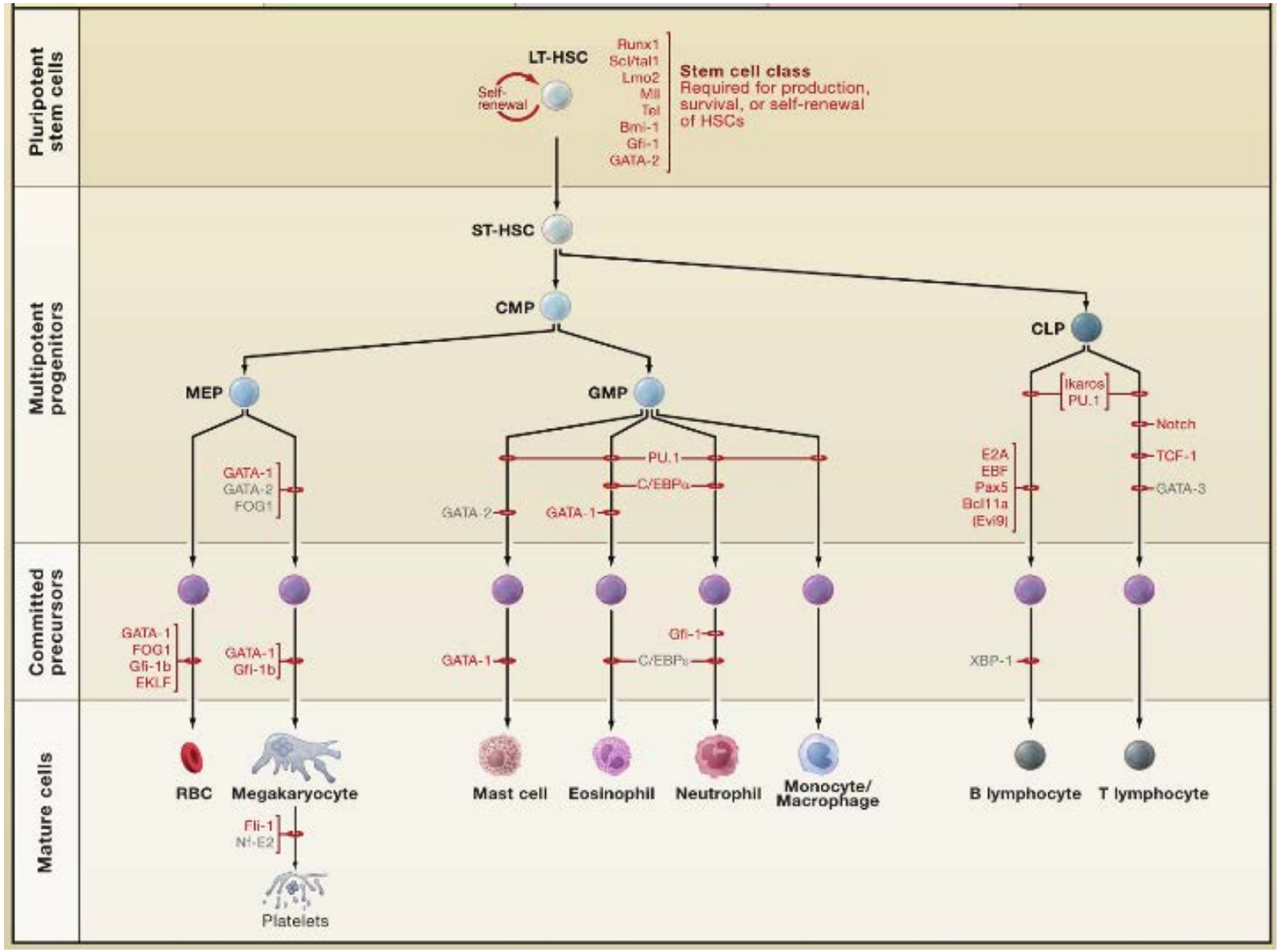
Lim & Son, 2017

Regulation of haematopoiesis



cell extrinsic
- cell-cell interactions
- secreted factors
- metabolic components
- non-cellular matrix factors
- mechanical forces

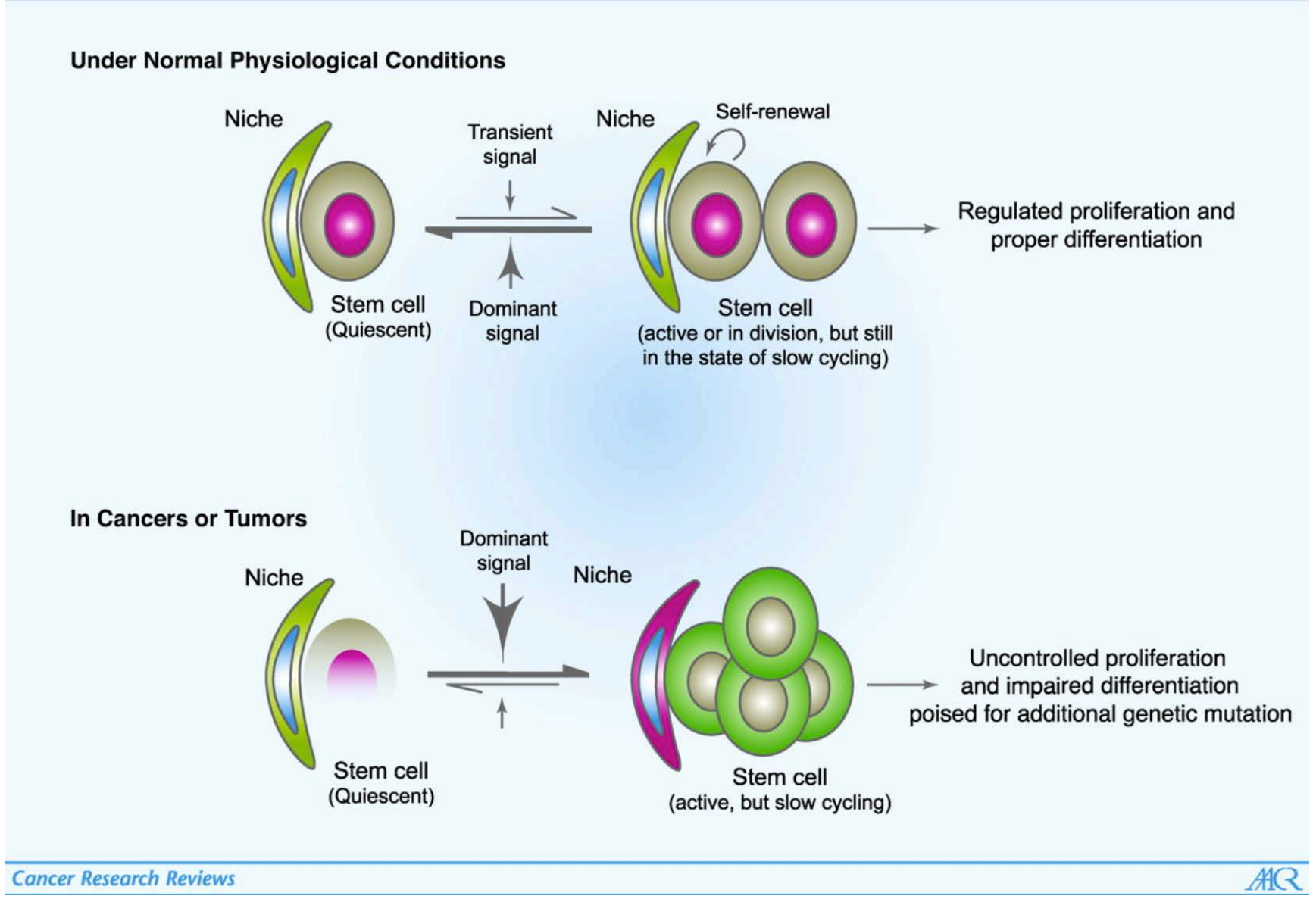
Cell intrinsic controls on haematopoiesis: finely tuned expression of transcription factors



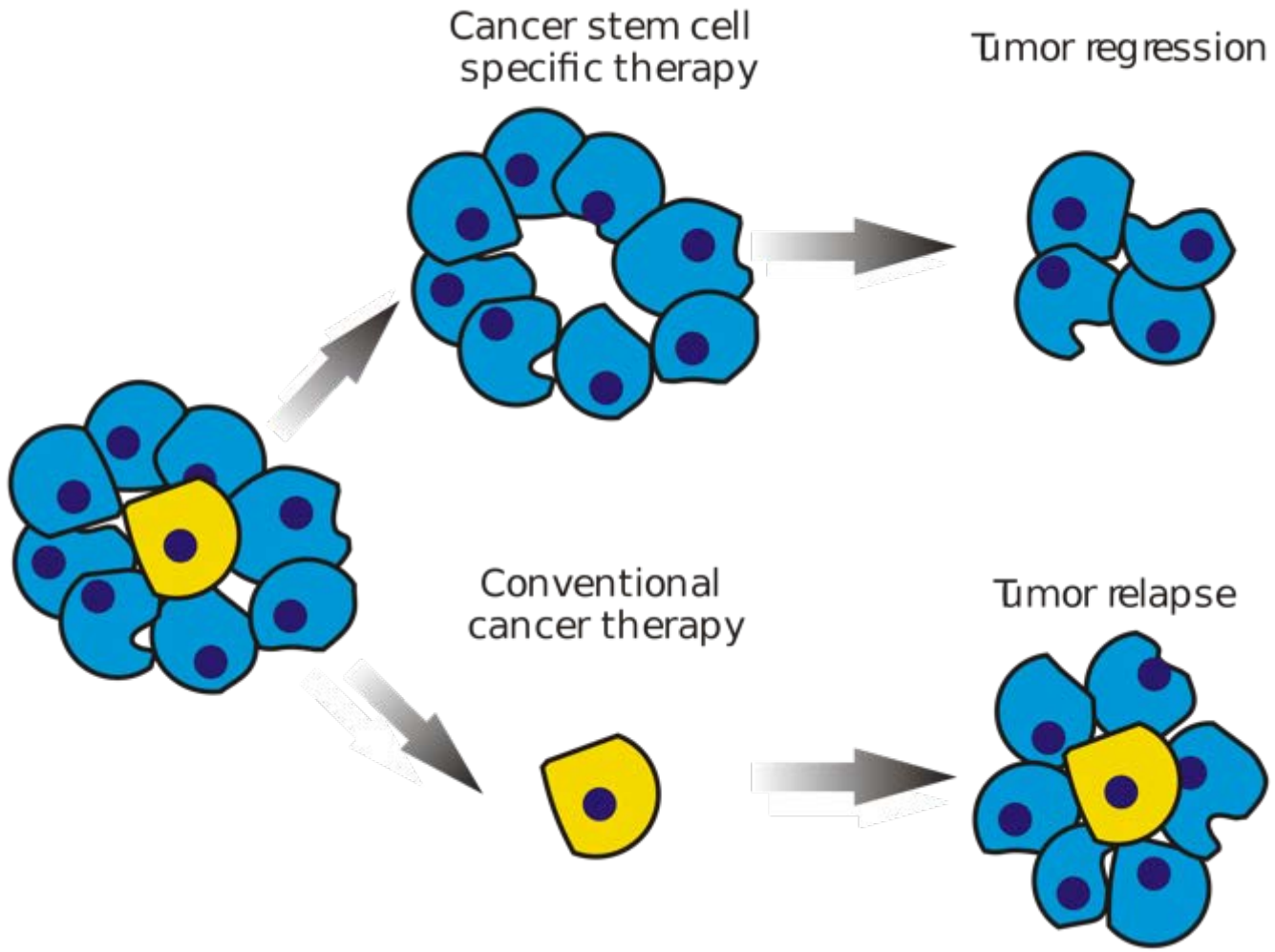
External control: role of microenvironment in haematopoiesis

- Produce growth factors
- Support and regulate balanced self-renewal and differentiation
- Physical support
- Homing of stem and progenitor cells

Niche-HSC interactions



Importance of cancer stem cells



HSC terminology – can be confusing!

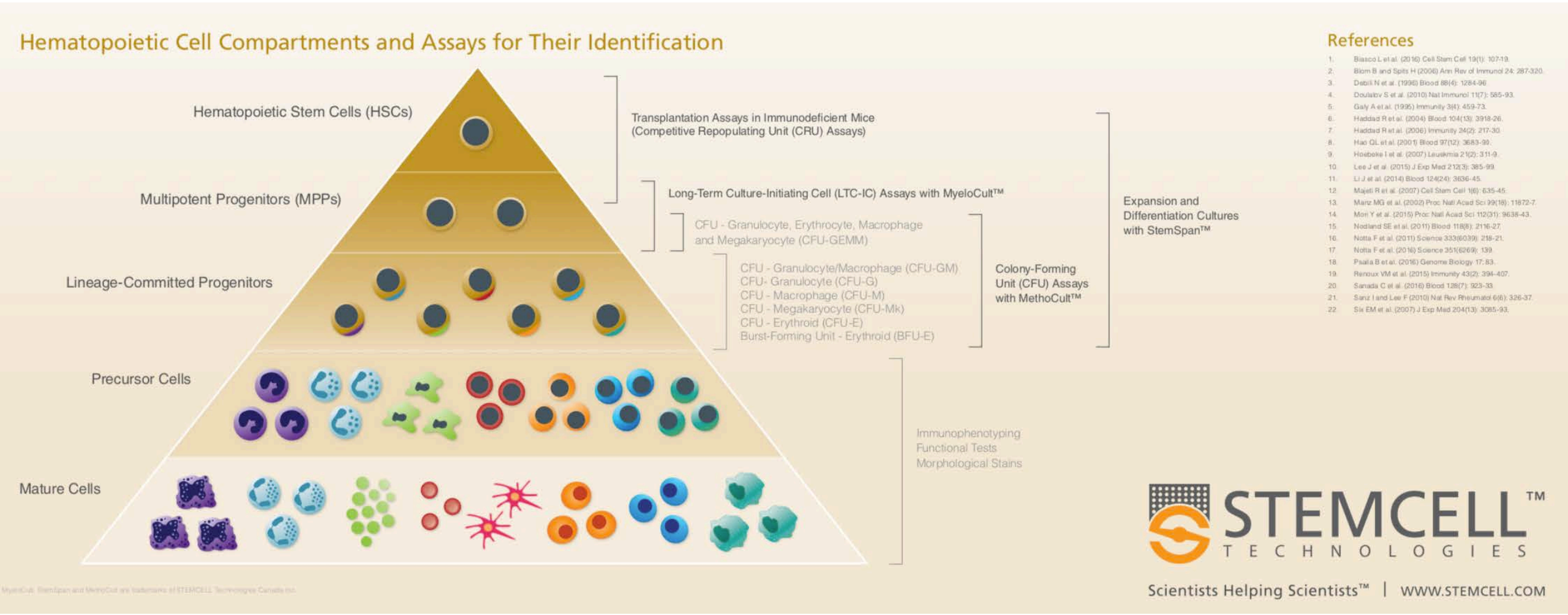
Table 1.

Unique and overlapping commonly used stem cell terms and definitions

Term	Widely accepted definition
Long-term HSC	Cells able to reconstitute all 5 types of mature blood cells in recipient mice over the long term (≥16-wk after transplantation) and in secondary and tertiary transplants[28]
Intermediate-term repopulating HSC	Cells that reconstitute multilineage blood cells over the medium term (6-8 mo), but show some loss of self-renewal in secondary transplant recipients[28]
Short-term repopulating HSC	Cells that transiently reconstitute multiple blood cell types for up to 8 wk in transplant recipients, or in which 1 or more donor-derived lineages disappear before 24 wk after primary transplant
Lineage priming	Expression of a transcriptional program associated with potential for differentiation to mature cells of a specific lineage in a multi/oligopotent cell
Lineage bias	Multi-/oligopotent cells that preferentially give rise to a single lineage but retain potential for alternative differentiation
Lineage restricted/committed	Oligo-/unipotent cells not able to give rise to cells of certain lineages
Lineage potential	The mature lineages that a cell has the potential to give rise to, depending on external stimuli
Lineage fate	The lineage that a stem/progenitor will give rise to in vivo

Psaila & Mead, Blood 2019

Experimental methods to study HSCs



Experimental methods to study HSCs

1. Stem cell assays to confirm ***self renewal and multi-potency***
   - Single cell transplantation of human cells into immunodeficient mice (serial transplantation)
   - Long term culture-initiating cell (LTC-IC) assays
   - Lineage tracing and barcoding approaches

2. Methods to **determine lineage potential** of stem and progenitor cells
   - What lineages can this cell produce?
     - Colony forming assays (e.g. Methocult)
     - Liquid culture assays (supportive media with expansion/differentiation cytokines)
     - Lineage tracing / barcoding

3. 'Phenotypic' assays
   - Immunophenotyping / proteomics
   - Genome/transcriptome/epigenomic analysis
   - Morphology

Need for a single cell approach

Bulk analysis

Information is ‘averaged’

How do you determine if a population is homogeneous?

Is any population truly homogeneous??

Unable to understand functional read-outs and to properly compare two populations using ‘bulk’ assays

Need for a single cell approach

Bulk analysis

Information is ‘averaged’

Single cell analysis

Unveil rare cell populations
Cellular hierarchies
Combinatorial patterns of gene expression

Need for a single cell approach

Bulk analysis

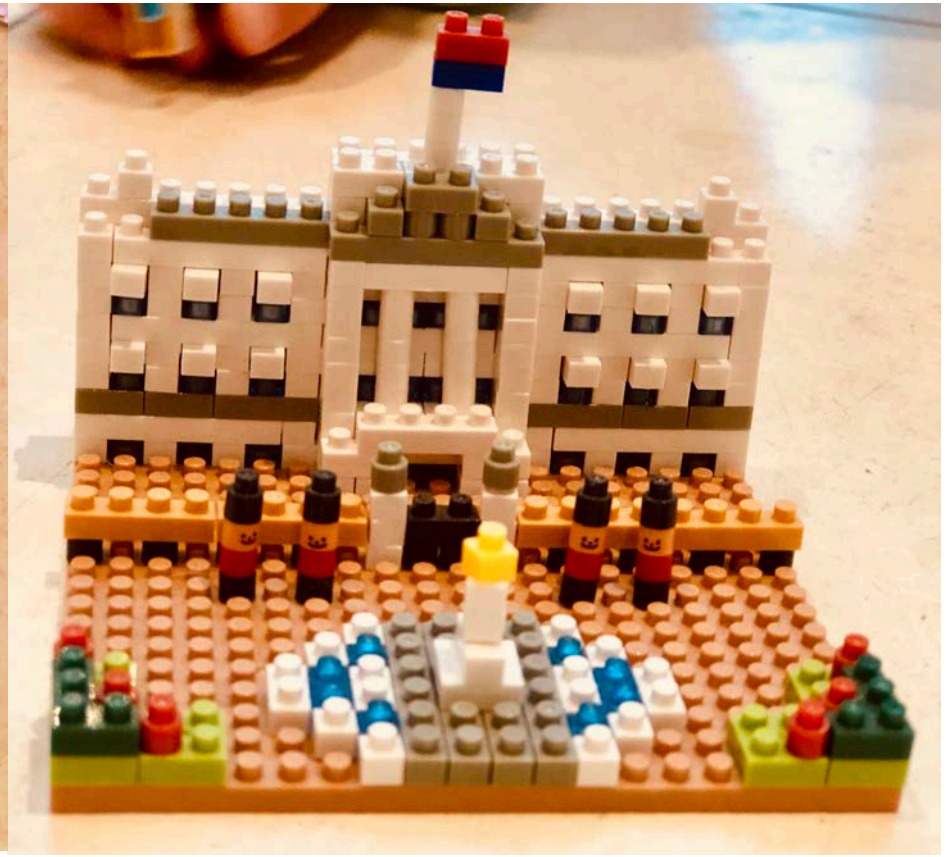
Information is ‘averaged’

Jnr Scientists a, b and c

Single cell analysis

Unveil rare cell populations
Cellular hierarchies
Combinatorial patterns of gene expression

3D context/spatial positioning



Organization in native context is crucial to understand cell-cell interactions

Single cell transplantation to identify lineage priming

- 1st in vivo transplantation assay was developed in 1950s by Ford et al (1956) and McCulloch & Till 1960
- CFU-S: colony forming unit-spleen cells
  - Cells injected into an irradiated recipient formed macroscopic splenic colonies 1-3 weeks later

- **Long term repopulating assays**
  - Usually done as competitive repopulation assay, to measure a population with unknown repopulating ability against competitor whole BM containing a known number of HSC → calculate the repopulating units
  - Frequency of HSC can be measured using a limiting dilution assay (dilutions of the test cells against constant number of competitors, measure the number of mice that are reconstituted)

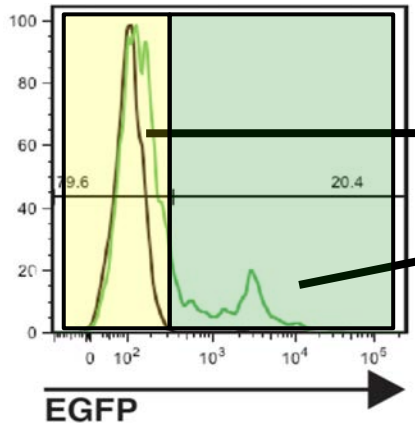
Single cell transplantation to identify lineage priming

LETTER

doi:10.1038/nature12495

Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy

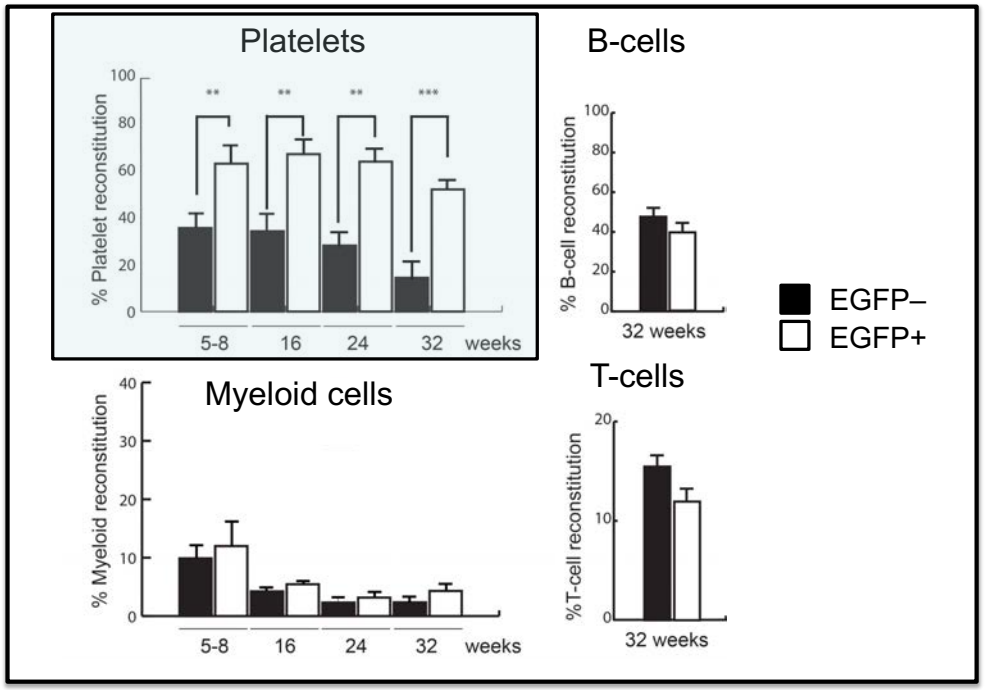
Alejandra Sanjuan-Pla[1,2], Iain C. Macaulay[3,4], Christina T. Jensen[3,4], Petter S. Woll[3,4], Tiago C. Luis[3,4], Adam Mead[3,4], Susan Moore[1,2], Cintia Carella[2], Sahoko Matsuoka[3,4], Tiphaine Bouriez Jones[3,4], Onima Chowdhury[3,4], Laura Stenson[3,4], Michael Lutteropp[3,4], Joanna C. A. Green[3,4], Raffaella Facchini[3,4], Hanane Boukarabila[3,4], Amit Grover[3], Adriana Gambardella[3], Supat Thongjuea[3], Joana Carrelha[3,4], Paul Tarrant[3,4], Deborah Atkinson[3,4], Sally-Ann Clark[3,4], Claus Nerlov[1,2,3]* & Sten Eirik W. Jacobsen[3,4]*



CD45.1 wild type
competitor bone marrow

VWF-EGFP– or +
LT-HSCs (CD45.2)

CD45.1 lethally
irradiated recipient

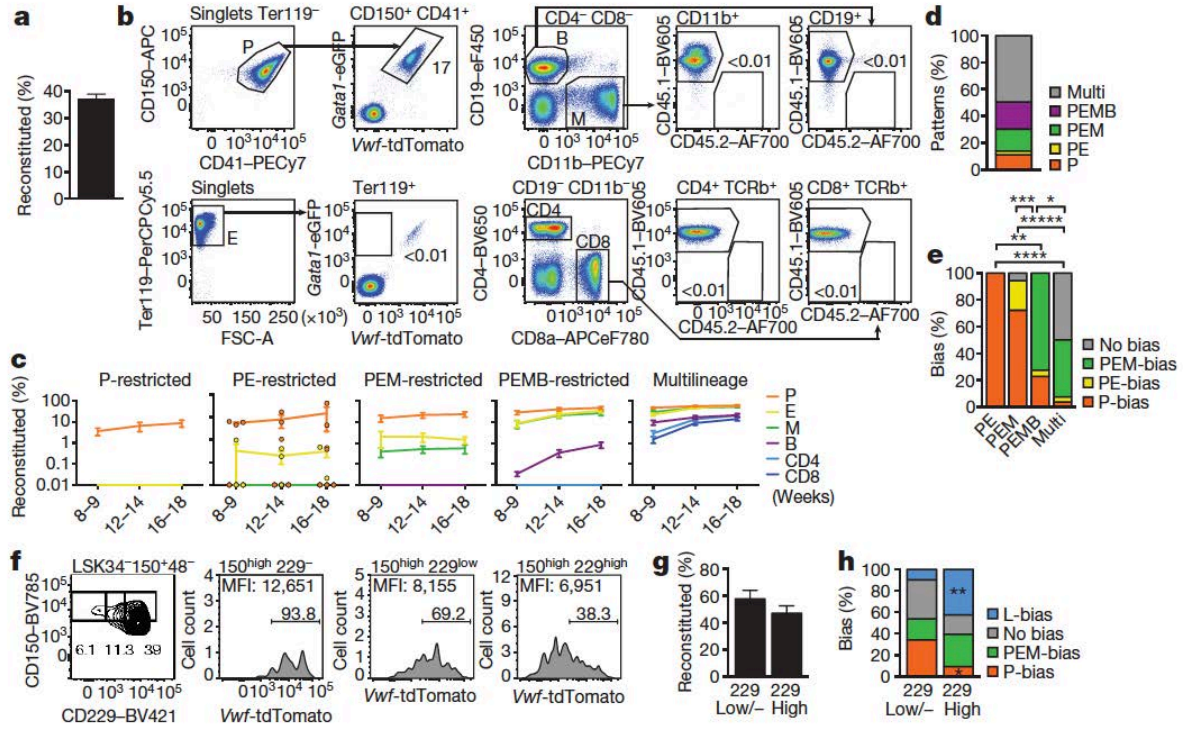


LETTER

doi:10.1038/nature25455

Hierarchically related lineage-restricted fates of multipotent haematopoietic stem cells

Joana Carrelha[1,2], Yiran Meng[1,2], Laura M. Kettyle[3,4], Tiago C. Luis[1,2], Ruggiero Norfo[1,2], Verónica Alcolea[1,2], Hanane Boukarabila[1,2]†, Francesca Grasso[4,5], Adriana Gambardella[2], Amit Grover[2], Kari Högstrand[3,4], Allegra M. Lord[3,4], Alejandra Sanjuan-Pla[2]†, Petter S. Woll[4,5], Claus Nerlov[2]* & Sten Eirik W. Jacobsen[1,2,3,4,5]*



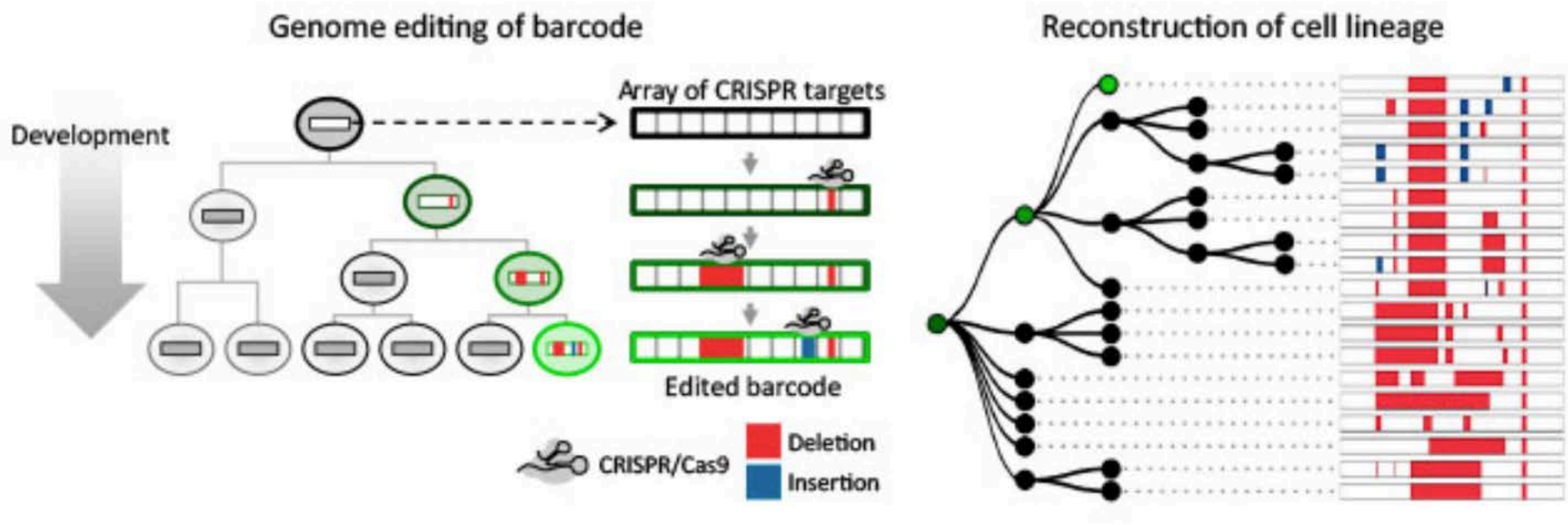
- Reveals a highly organized and stable framework for lineage restricted fates of LT HSCs
- A distinct class of HSC adopts a MK fate but none other (the only single lineage HSC)
- Single multipotent HSCs may restrict their fate to Mk, Mk-E, Mk-E-Mye without executing their lymphoid potential

Considerations in single cell transplant experiments

- Extremely technically challenging, expensive and use a lot of mice!
- Cut off for engraftment is arbitrary – 1% for all lineages?
- Time point for analysis?
- Differences in genetic background of mice
- What method has been used to identify the donor *vs*. recipient cells and how robust is this
- Assay also dependent on homing and engraftment potential of HSCs
- Has BM microenvironment been perturbed by irradiation?
- Not all lineages can be easily assessed e.g. Mk-E

Lineage tracing / barcoding assays

- CRISPR-Cas9: ‘dropping genetic breadcrumbs’ into developing cells



GESTALT – Science 2016. CRISPR-Cas9 used to introduce a 300 bp ‘barcode’ into dividing cells and cell progeny then tracked as they accumulate mutations. Progeny cells then collected and DNA amplified to ‘read’ the barcode and reconstruct the lineage tree

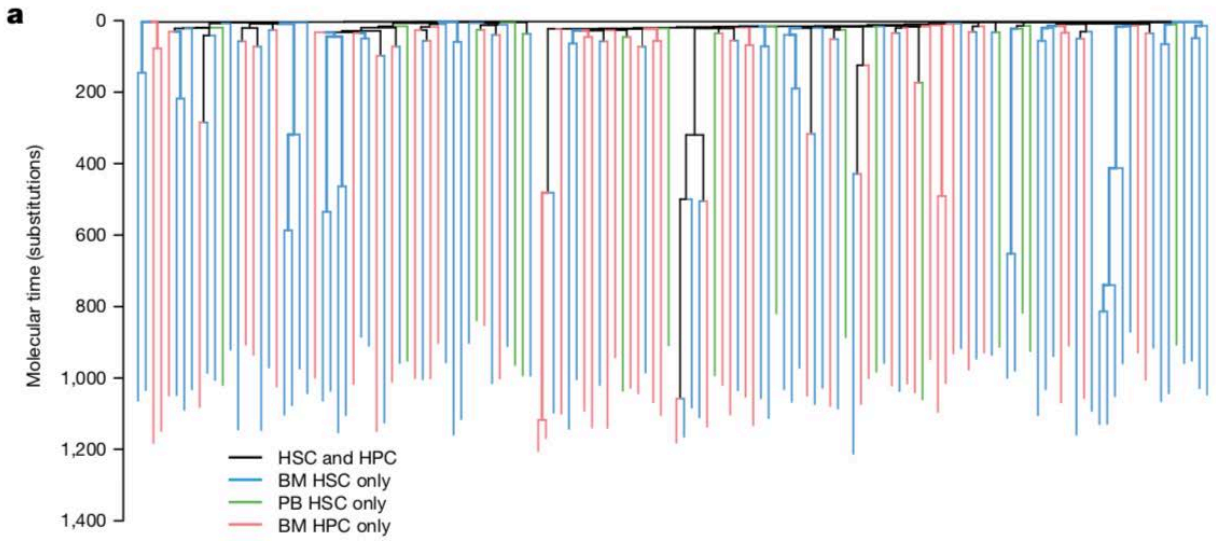
Acquisition of somatic barcodes confers an endogenous ‘barcode’

ARTICLE

https://doi.org/10.1038/s41586-018-0497-0

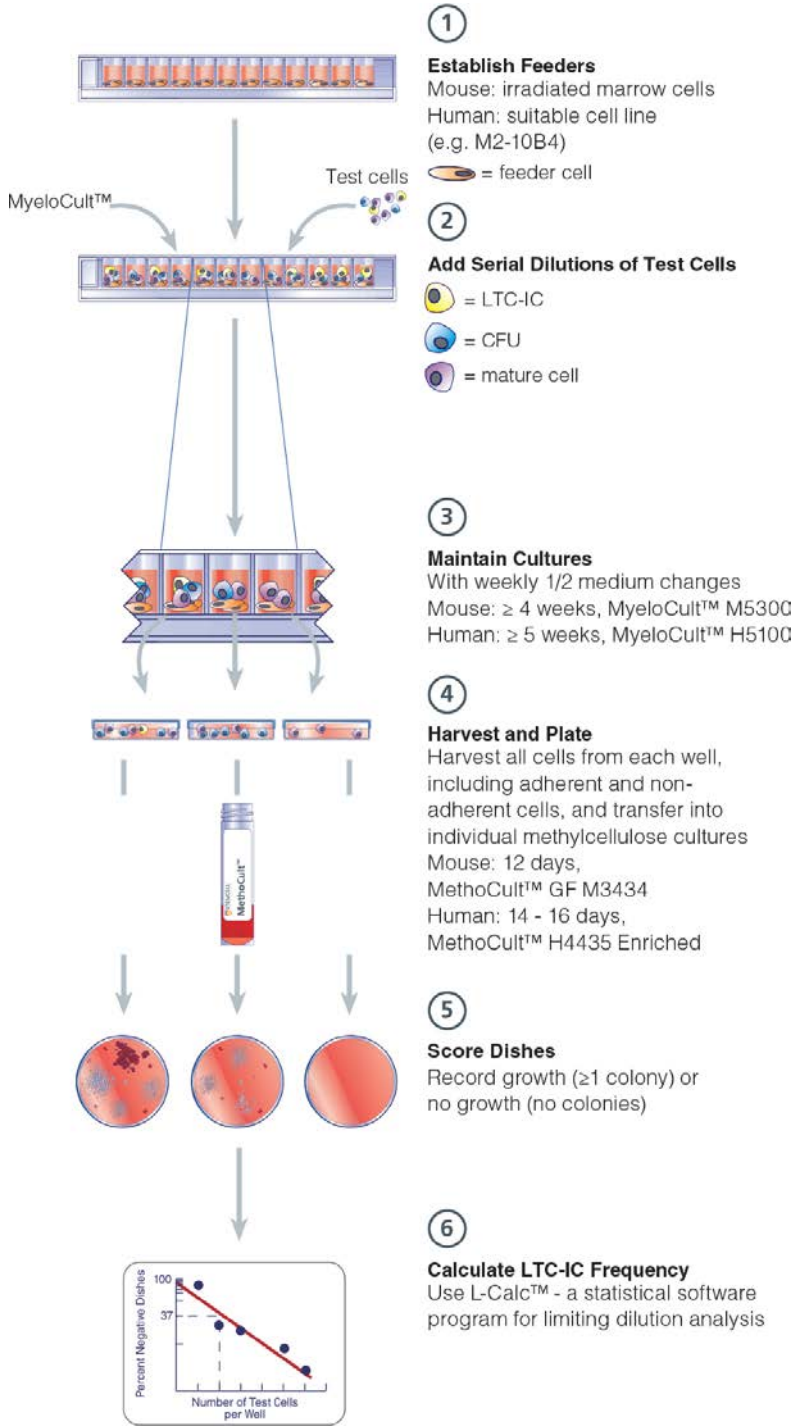
Population dynamics of normal human blood inferred from somatic mutations

Henry Lee-Six[1], Nina Friesgaard Øbro[2], Mairi S. Shepherd[2], Sebastian Grossmann[1], Kevin Dawson[1], Miriam Belmonte[2], Robert J. Osborne[1], Brian J. P. Huntly[2], Inigo Martincorena[1], Elizabeth Anderson[1], Laura O’Neill[1], Michael R. Stratton[1], Elisa Laurenti[2], Anthony R. Green[2,3]*, David G. Kent[2,3]* & Peter J. Campbell[1,3]*



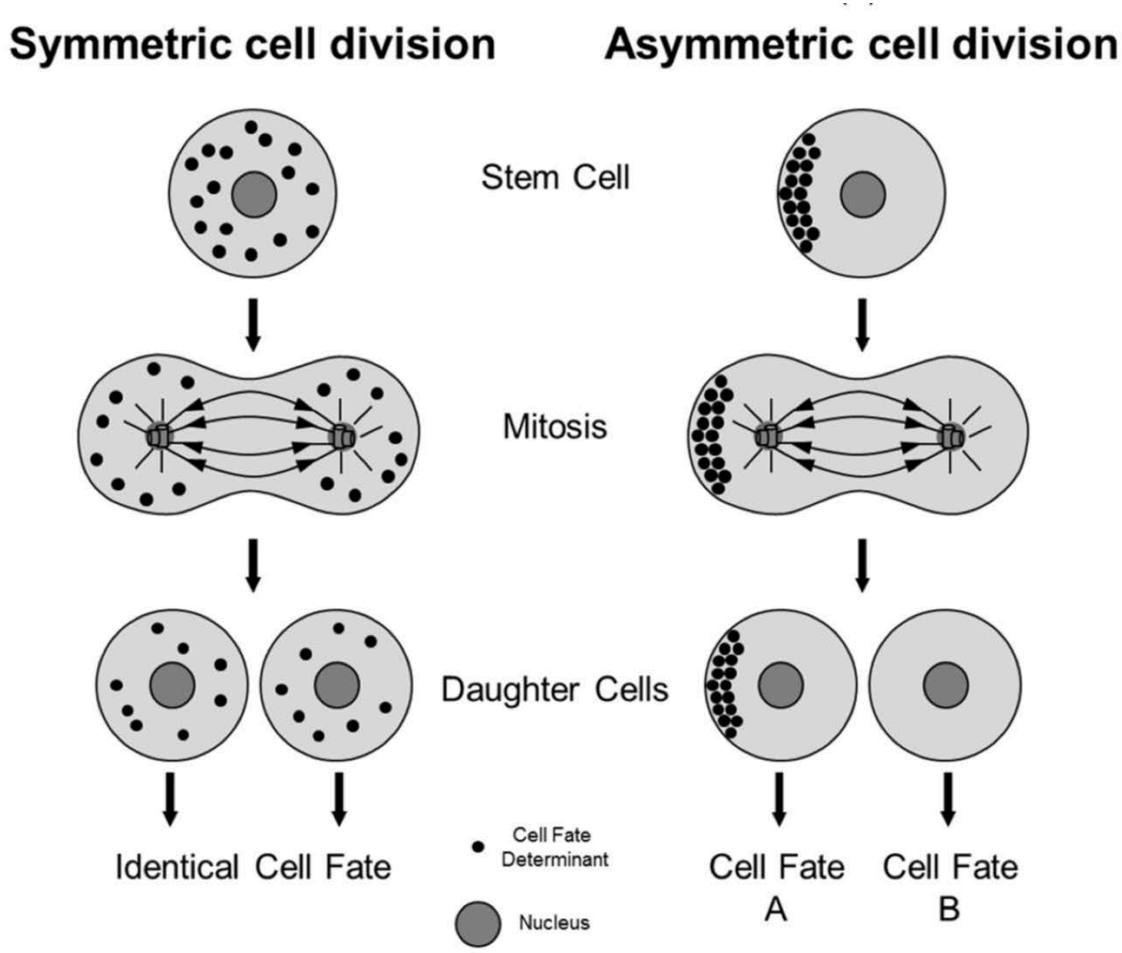
In vitro stem cell assay: Long term Colony-initating cell assay (LTC-IC)

Plate cells on stromal support cells for 4-6 weeks
Then transfer to methylcellulose to see if can initiate colonies



Paired daughter cell assays

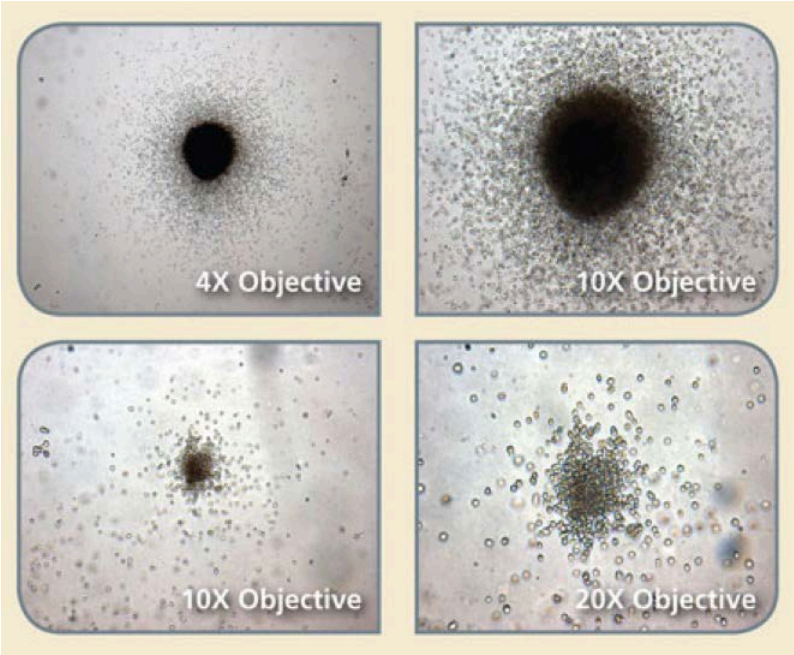
Micromanipulation of progeny following 1st cell division to test symmetric vs asymmetric division



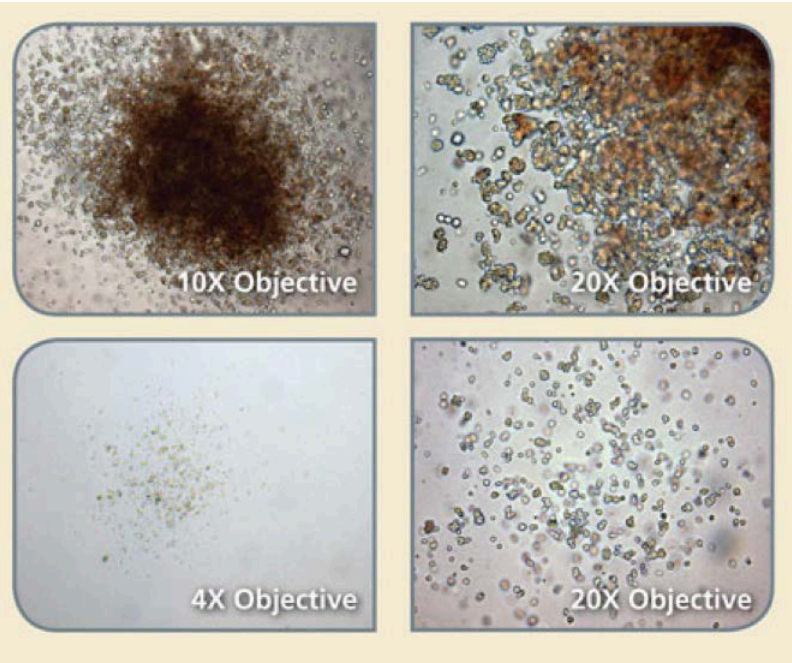
In vitro differentiation assays

Semi-solid ‘clonogenic’ assays: mix cells with methylcellulose (+ cytokines)

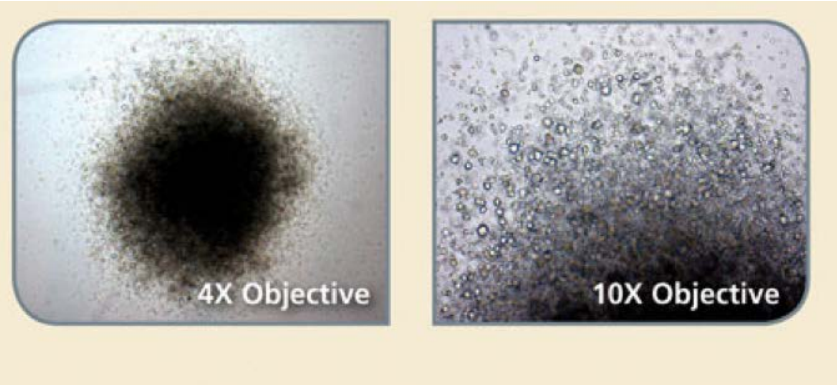
CFU-GM
Ery



BFU-E
Ery

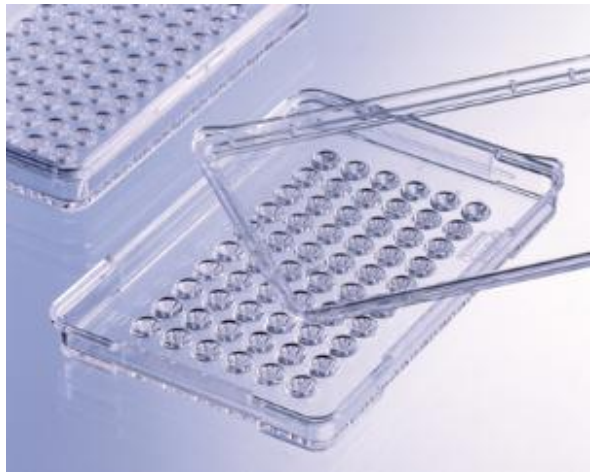


CFU-GEMM
Gran, ery, macrophage, mega



In vitro differentiation assays

- Liquid culture (single cell 96-well plate or Terasaki plates)



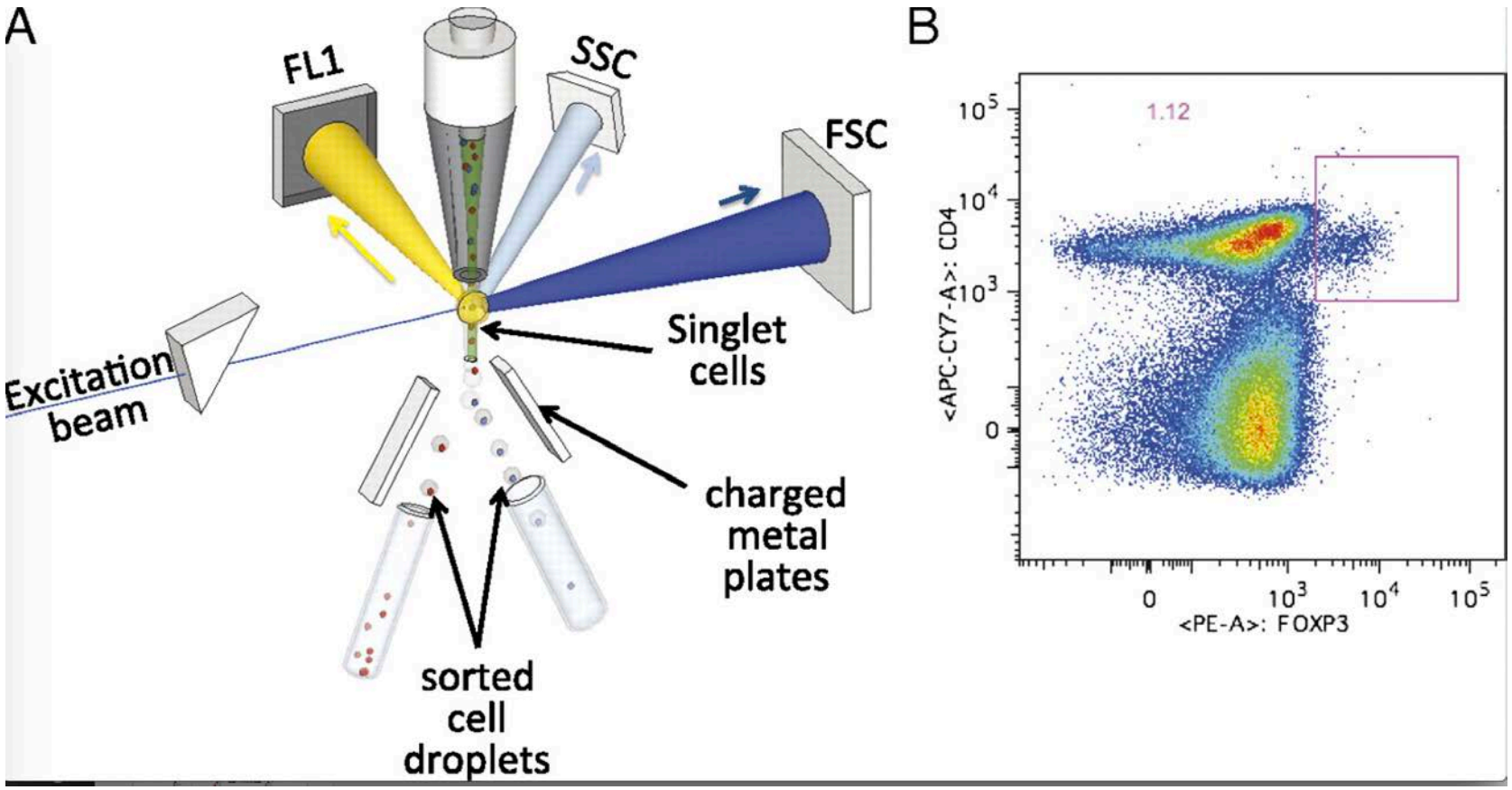
Stemspan (serum free media)
+ Stem cell Factor
+ erythropoietin
+ thrombopoietin
+ G-CSF, GM-CSF
+ Interleukins IL3, 6
+ FLT3-Ligand

May need stromal support cells
- OP9 for B –ells
- OP9DL1 for T cells

Semi-solid / liquid culture pitfalls

- Measure progenitor frequency rather than HSCs
- Assessing multipotency of single cells is really challenging
- Readout is very dependent on skills and experience of observer!
- Biased by culture conditions / cytokines added

Phenotypic assays: immunophenotyping



Cells are excited by laser beams, to delineate forward scatter (FSC) and side scatter (SSC) and labelled with fluorescently-labelled antibodies detected by fluorescent light (FL1)

Jaye et al, J of Immunol 2012

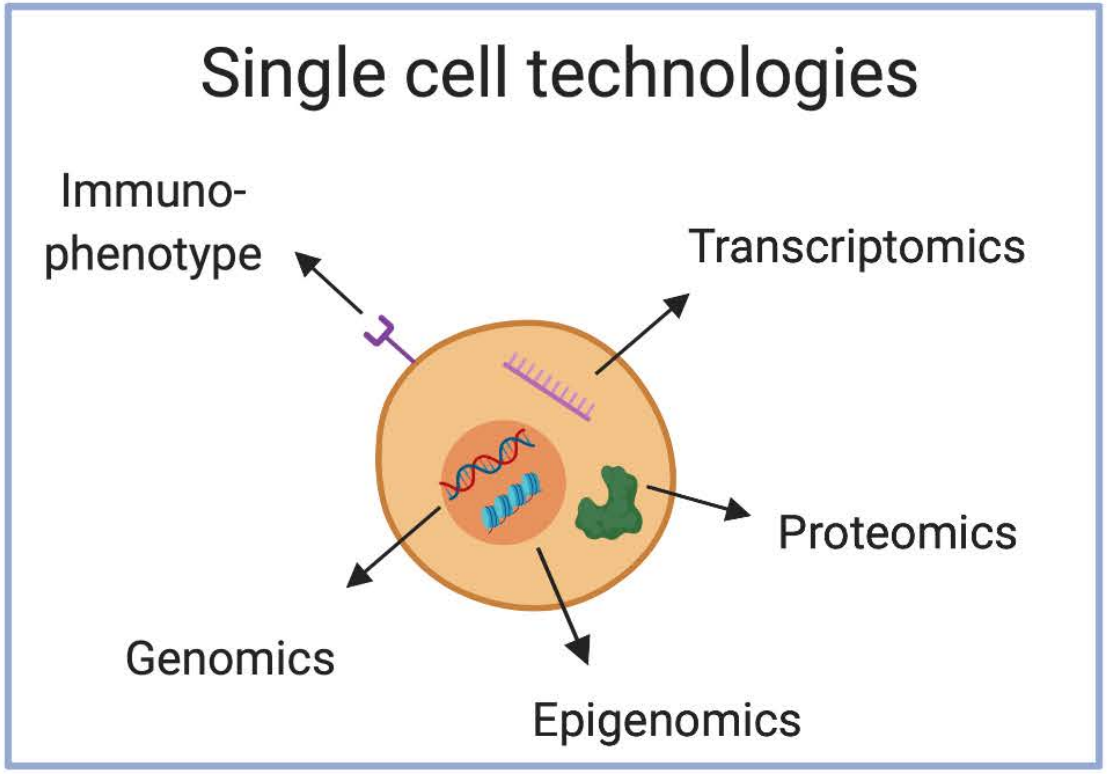
Phenotypic assays: immunophenotyping

- Various different immunophenotyping strategies for mouse and human

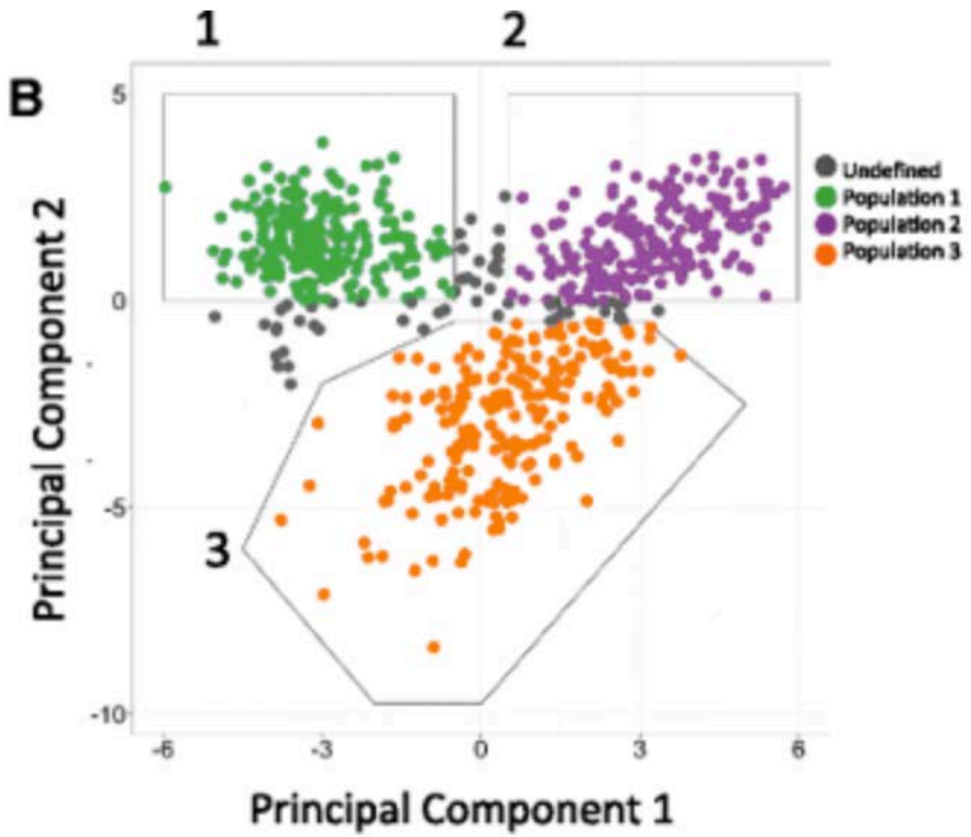
- Purity of HSCs identified using the most state-of-the-art FACS methods is thought to be around 50% (assessed by the transplantation assay)
  - Variability in HSC antigens between different mouse strains
  - Different stages of ontogeny
  - Cell cycle
  - Non-steady state e.g. post transplant/5-FU treatment

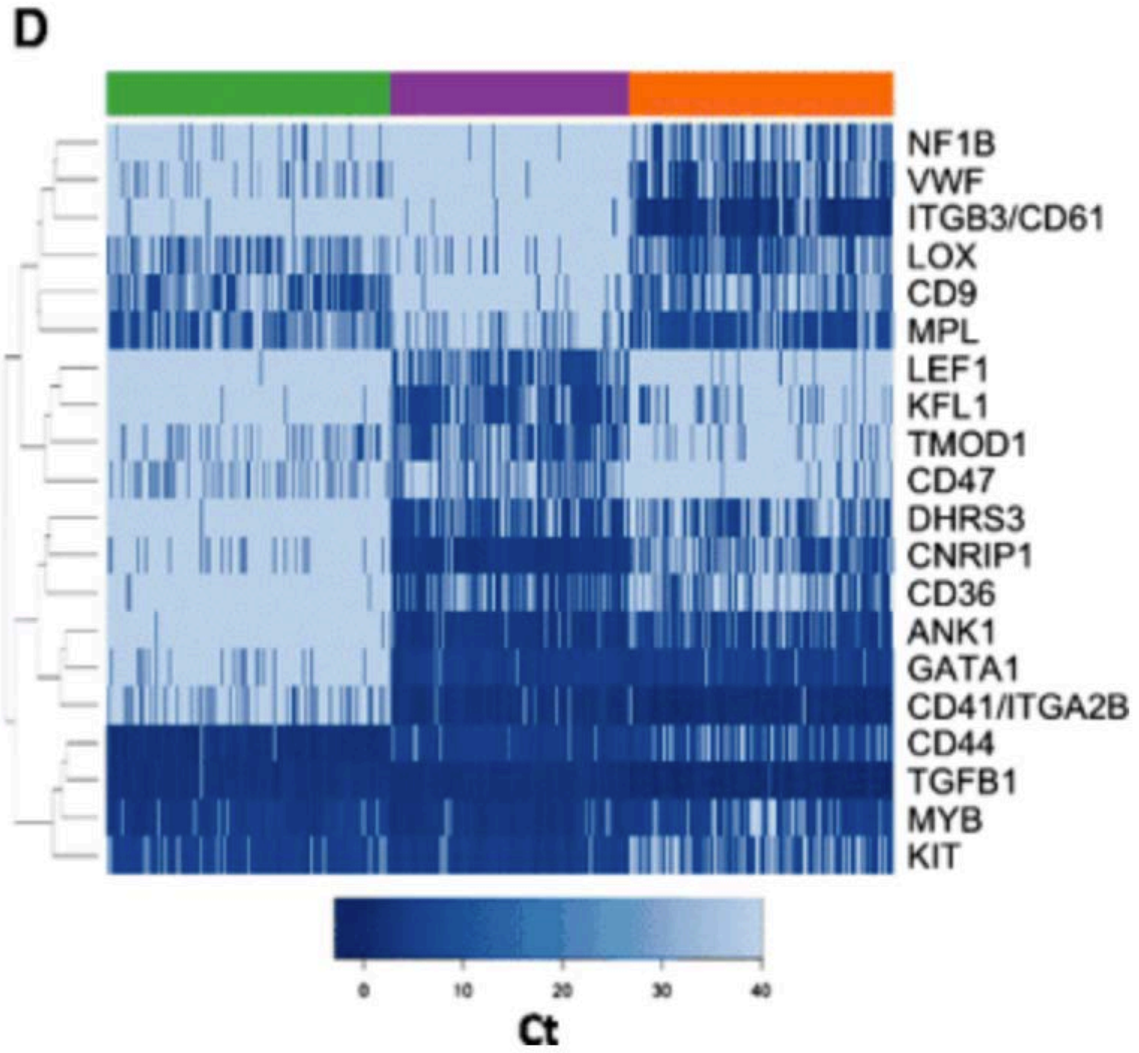
→ 'true' HSC need to be defined by functional analyses

Molecular profiling to identify stem/progenitor priming



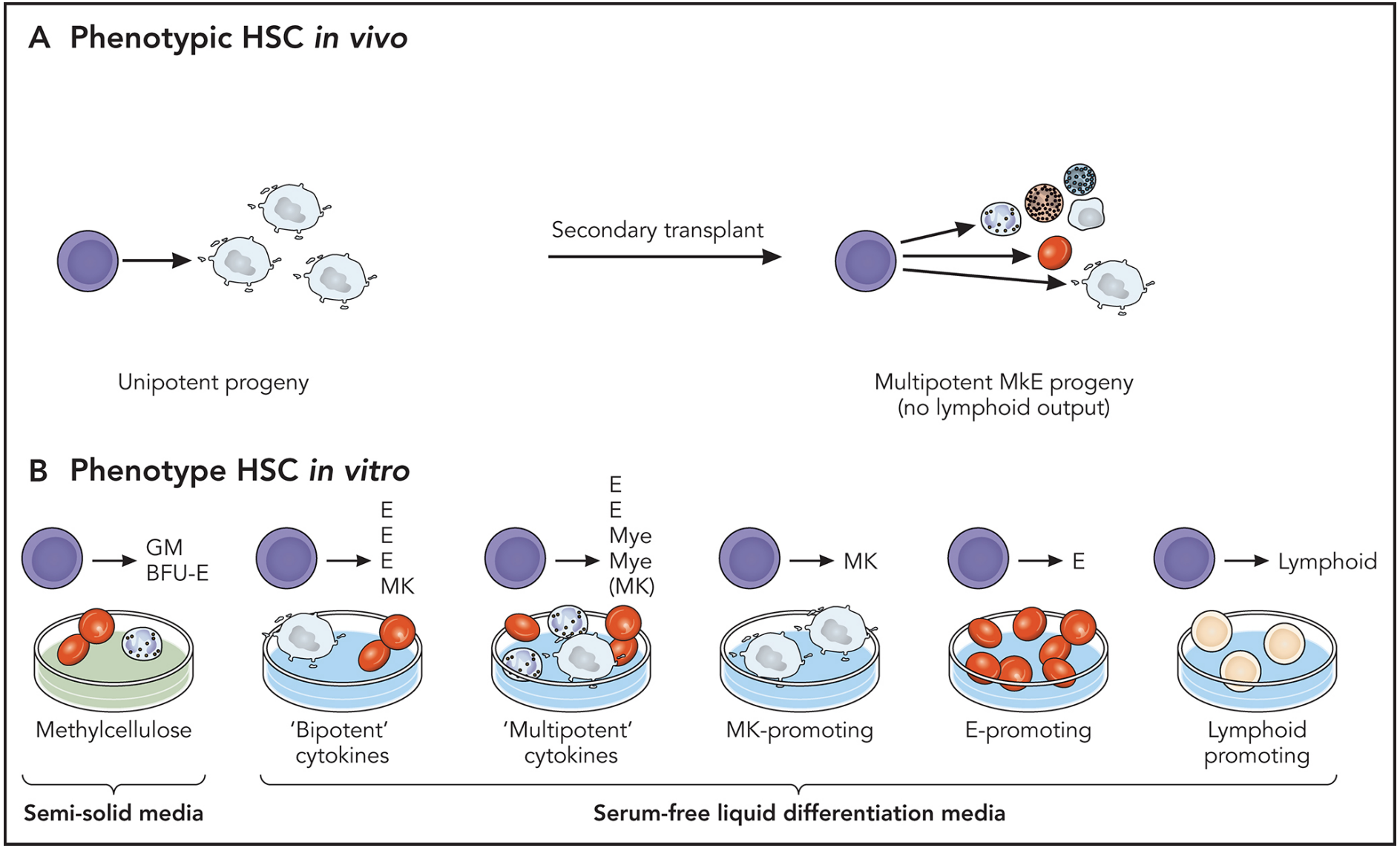
Molecular profiling to identify stem/progenitor priming





Psaila, Genome Biology 2016

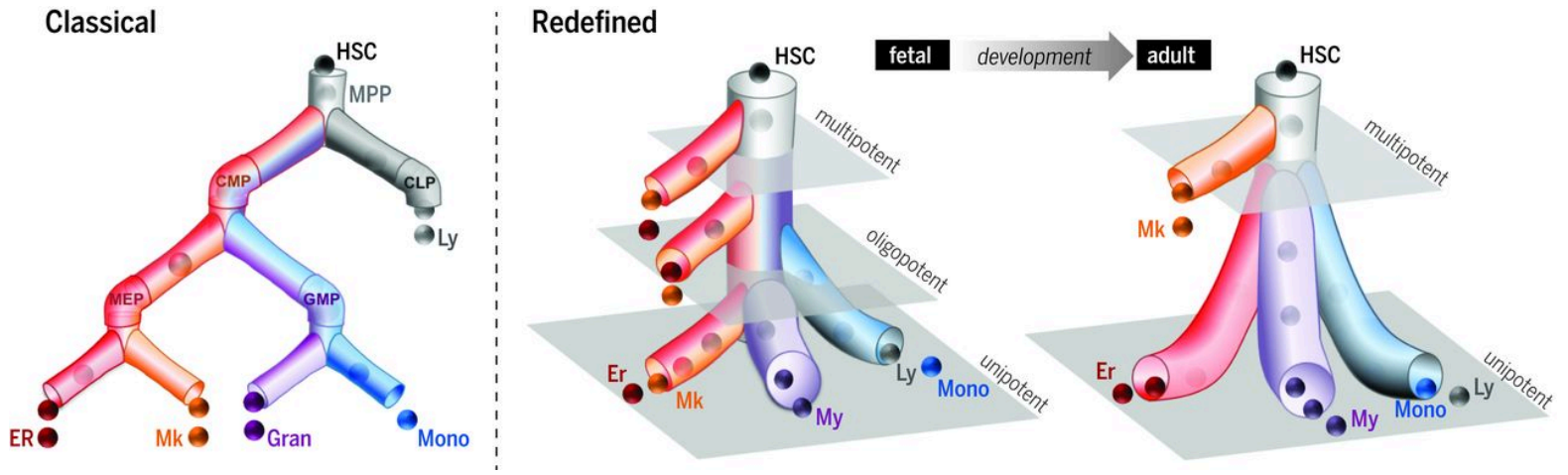
Limitations in stem cells assay even at single cell level



Questions/controversies

- Does haematopoiesis occur via step-wise transitions or over a gradual continuum of differentiation?

- What % of HSCs are truly ‘multipotent’? Recent data suggests that majority of HSCs in adult life are ‘biased’ towards certain differentiation fates



Science
AAAS

Faiyaz Notta et al. Science 2016

Questions/controversies

- Do HSCs contribute to steady-state haematopoiesis or are they a ‘reserve’ (with most contributions coming from progenitors)?

- How much similarity is there between humans and animal models?

Summary of stem cell assays

1. Stem cell assays to confirm ***self renewal and multi-potency***
   - Single cell transplantation of human cells into immunodeficient mice (serial transplantation)
   - Long term culture-initiating cell (LTC-IC) assays
   - Lineage tracing and barcoding approaches

2. Methods to **determine lineage potential** of stem and progenitor cells
   - What lineages can this cell produce?
     - Colony forming assays (e.g. Methocult)
     - Liquid culture assays (supportive media with expansion/differentiation cytokines)
     - Lineage tracing / barcoding

3. 'Phenotypic' assays
   - Immunophenotyping / proteomics
   - Genome/transcriptome/epigenomic analysis
   - Morphology

Many complex experimental systems exist to test stem cell function

→ need to carefully evaluate what has been done and what the assay is ***actually*** testing (e.g. cell fate *vs*. potential) to interpret the literature

Questions?