
Single cell approaches

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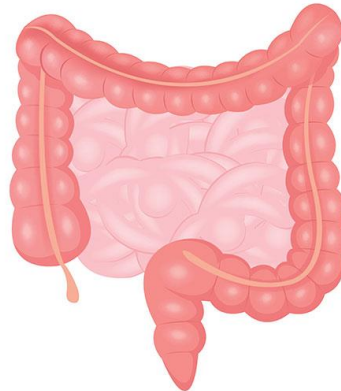
The WIMM DPhil Course, 12-11-2019

Why single cell approaches?

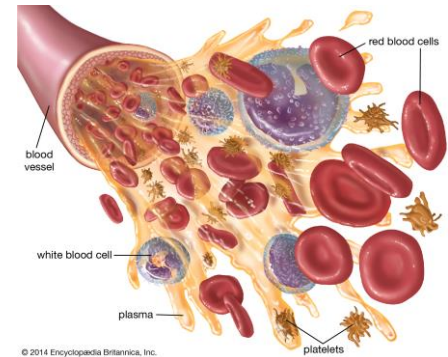
- Tissues consist of functionally different cell types
- Tissues are replenished and maintained by stem cells
- Recent technologies enable studies at single cell level
- Characterisation of single cells has emerged as key driver to unravel tissue biology



Brain



Intestine



Blood

Why single cell approaches?

Techniques

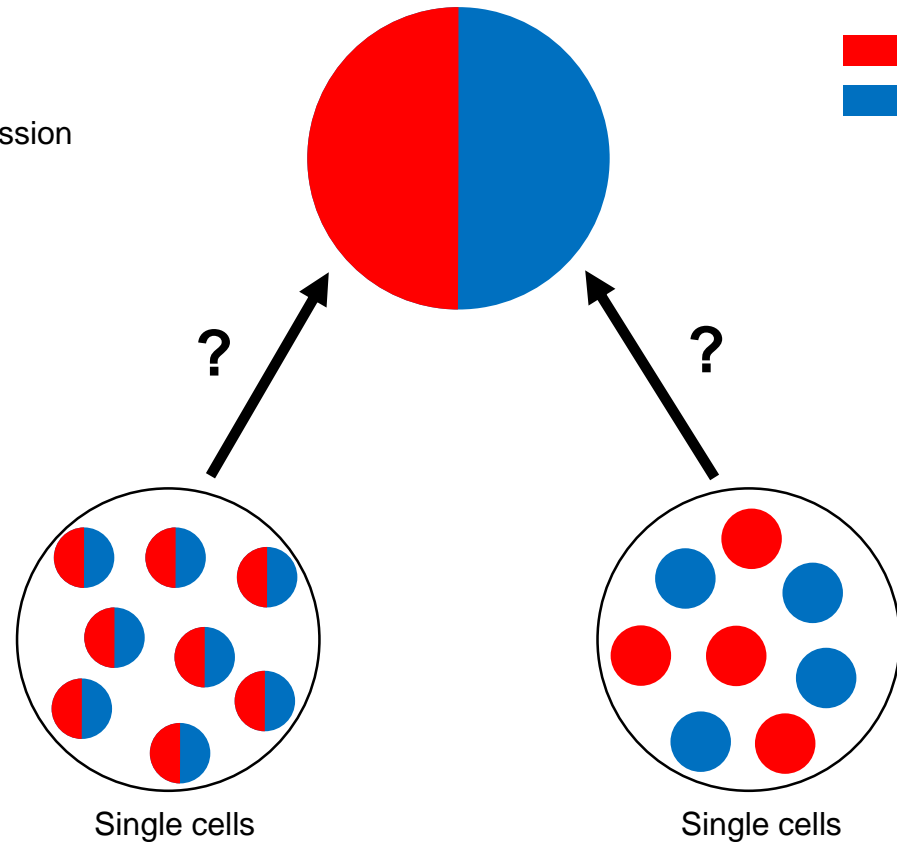
Micro array/ Northern blot
Western Blot
Chromatin ImmunoPrecipitation

Detection

Gene expression
Protein
Epigenome

Tissue/population of cells

Gene A
Gene B

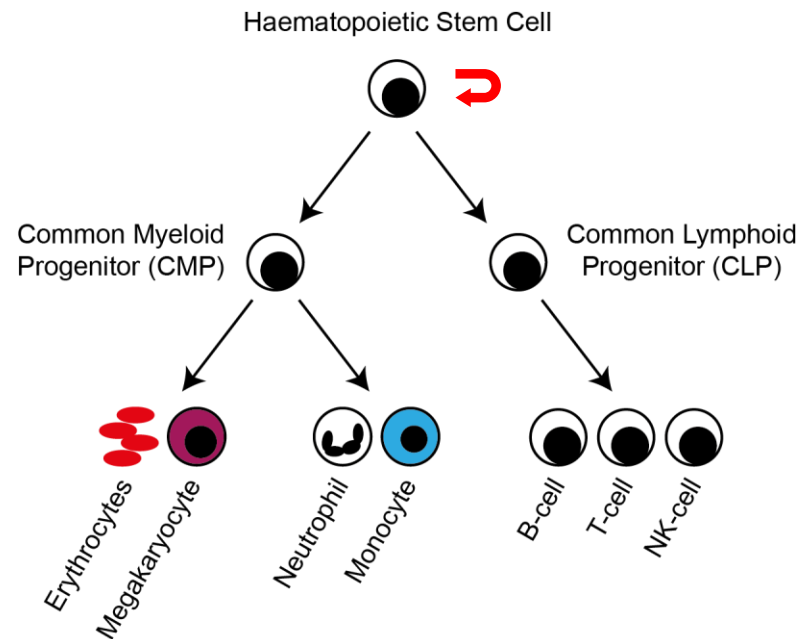


Tissues consists of multiple different cell types

Information and techniques for single cell characterisation

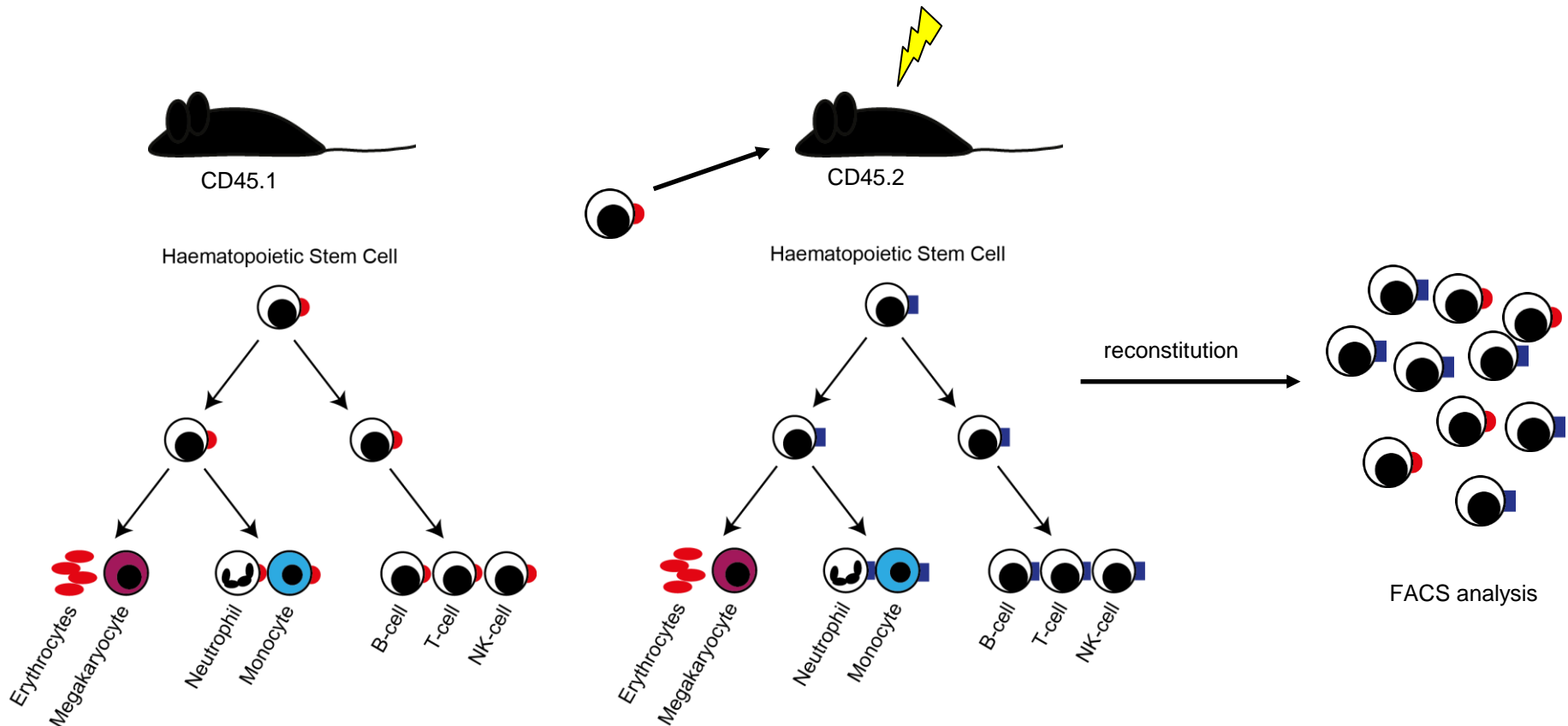
<u>Techniques</u>	<u>Detection</u>
• Transplantation studies	Cell fate/potential
• <i>In vitro</i> cultures	Cell potential
• SmartSeq/Chromium	Gene expression
• FACS	Cell surface markers
• Fluidigm	Chosen gene expression
• ATAC-Sequencing	Epigenome
• CyTOF	Protein

Haematopoiesis



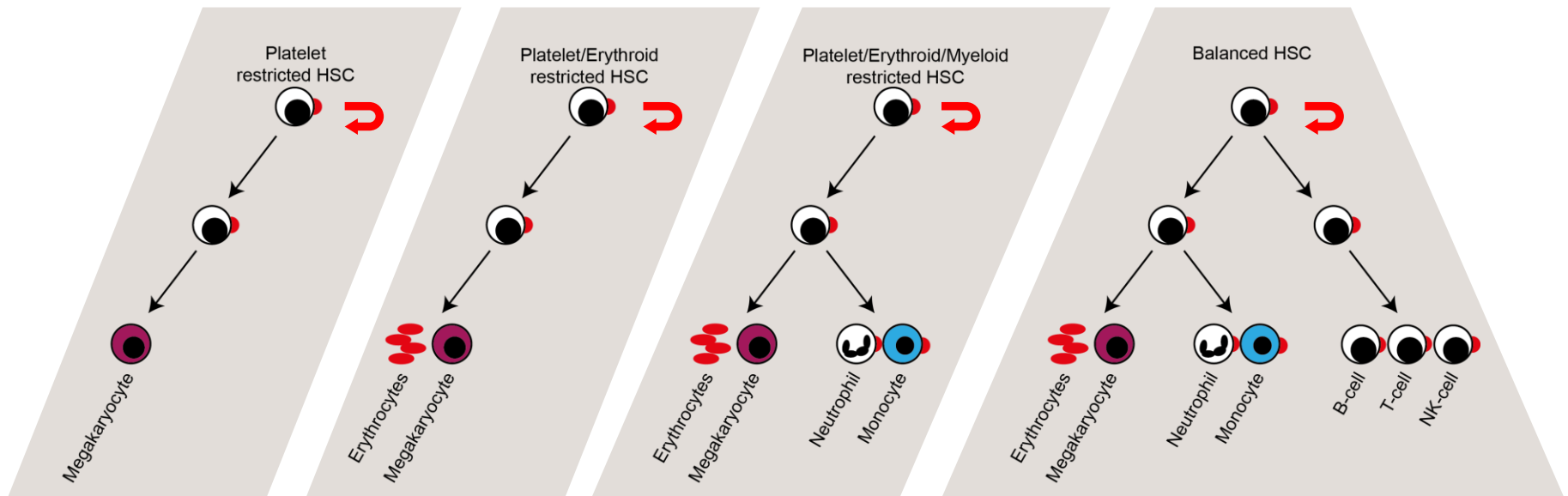
Haematopoiesis as paradigm for systems
where stem cells maintain and regenerate a tissue

Single cell approach: Single Haematopoietic Stem Cell Transplantation



Haematopoietic stem cells are defined by their ability to a life-long blood production

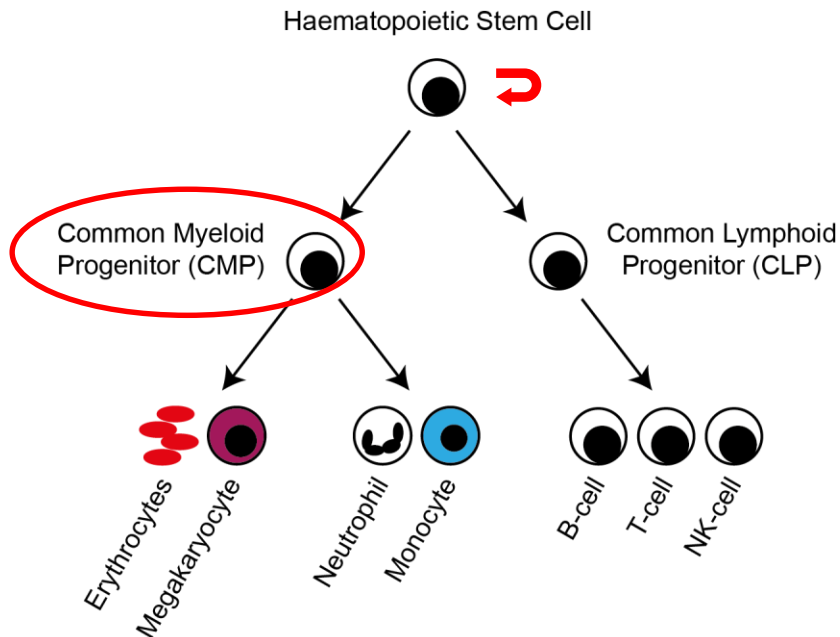
Single HSC transplantation reveals different types of HSCs



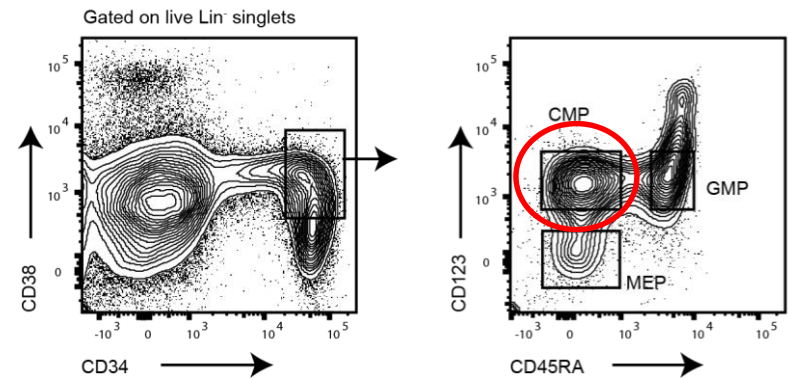
Transplantation of bulk haematopoietic stem cells show reconstitution of all blood cells

Transplantation of single haematopoietic stem cells can demonstrate limited potential of the cell

Haematopoietic Progenitors



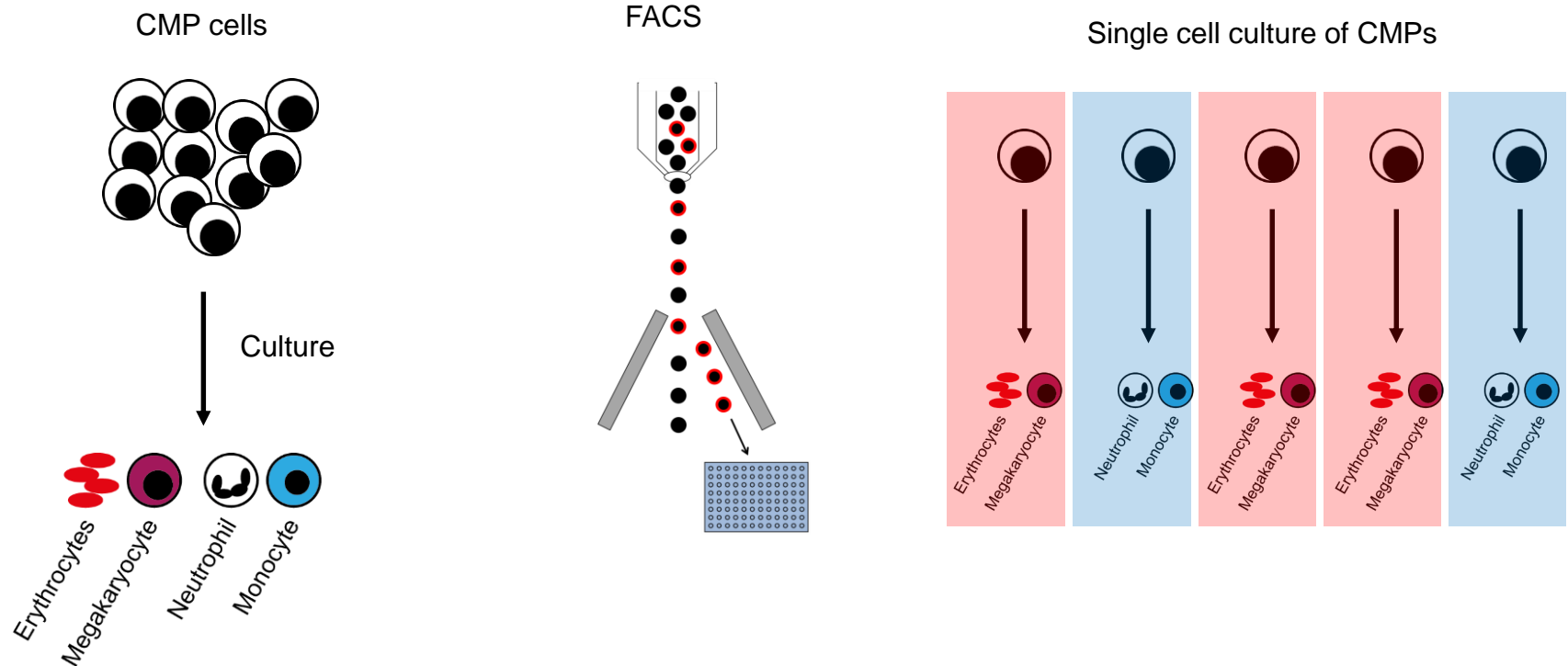
Identification and isolation of CMP cells by Flow cytometry (FACS)



Progenitors have limited lifespan and give limited progeny
Characterisation of progenitors is important for studies to

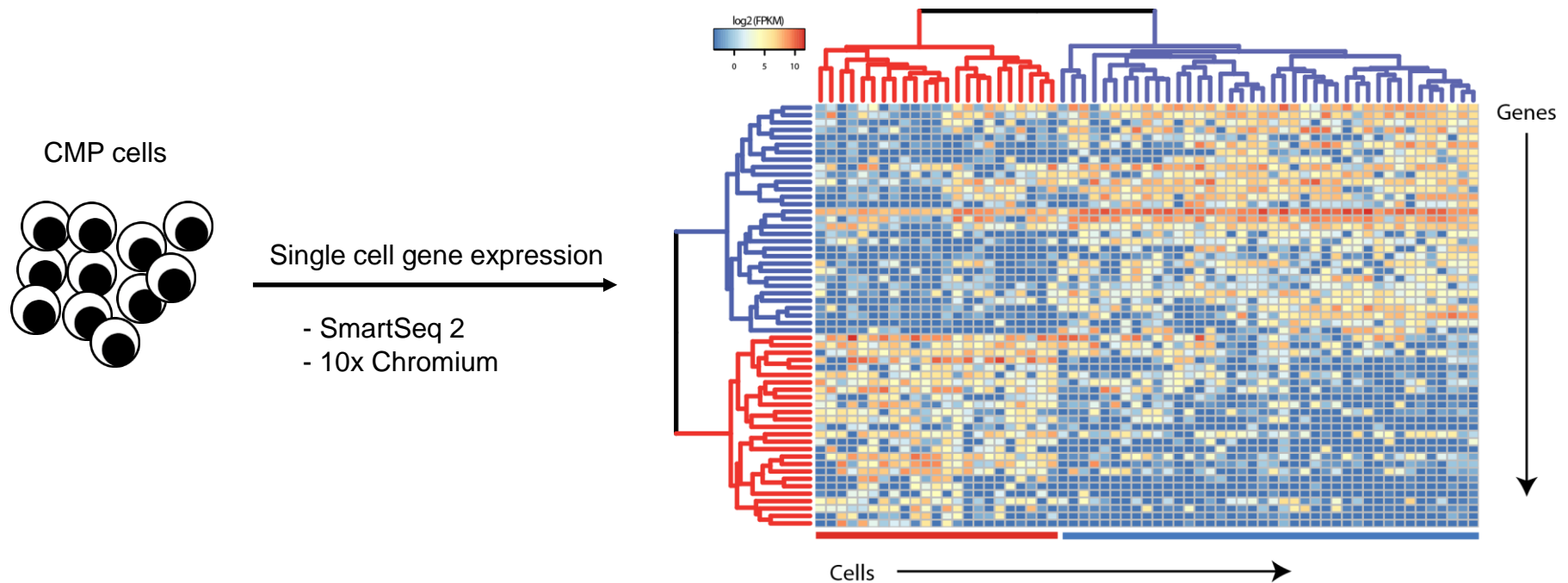
- Molecular mechanisms for lineage choice
- Characterisation of leukemic stem cells

Single cell approach: Single cell cultures



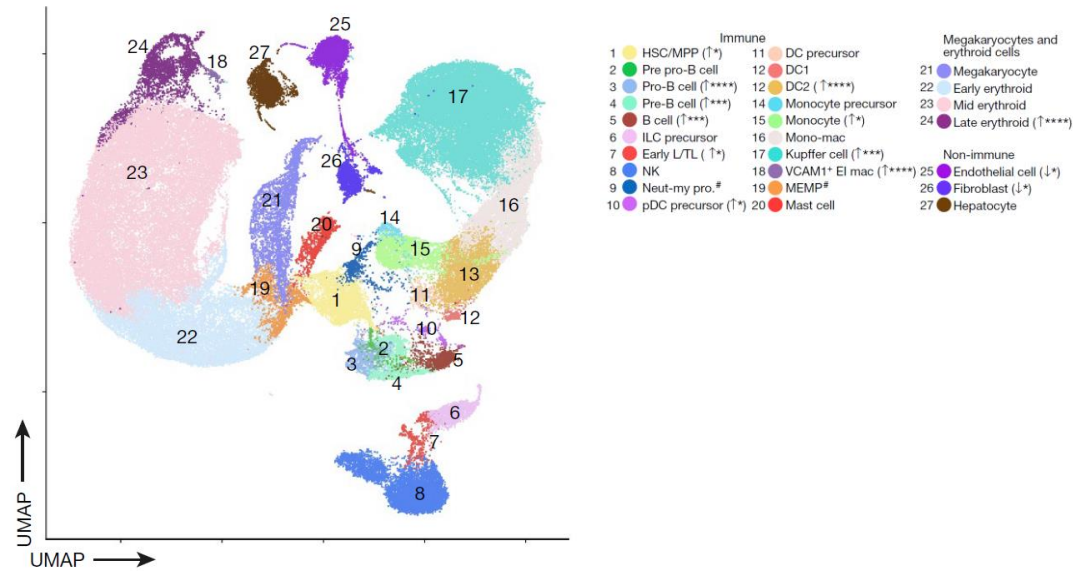
Single cell cultures of CMP show functional heterogeneity within the population

Single cell gene expression



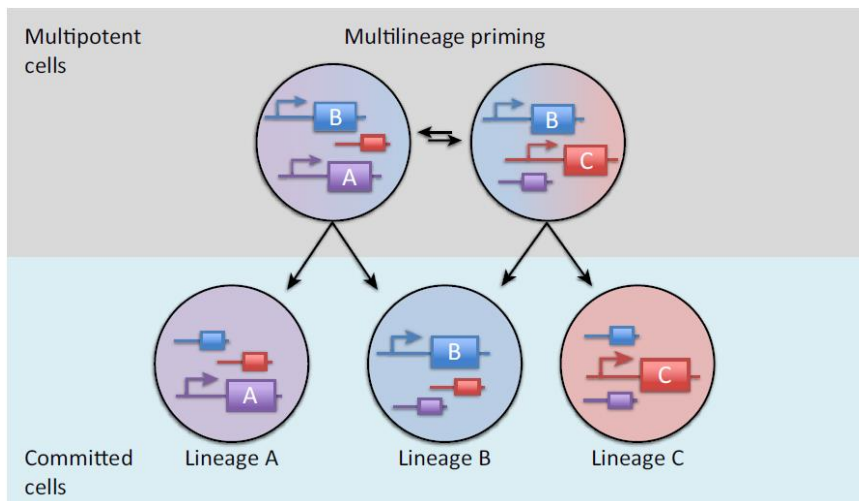
Single cell whole genome transcriptomics as an **unbiased** way of detecting heterogeneity within a population

- PCA (Principle Component Analysis)
- t-SNE (t-distributed Stochastic Neighbor Embedding)
- UMAP (Uniform Manifold Approximation and Projection)

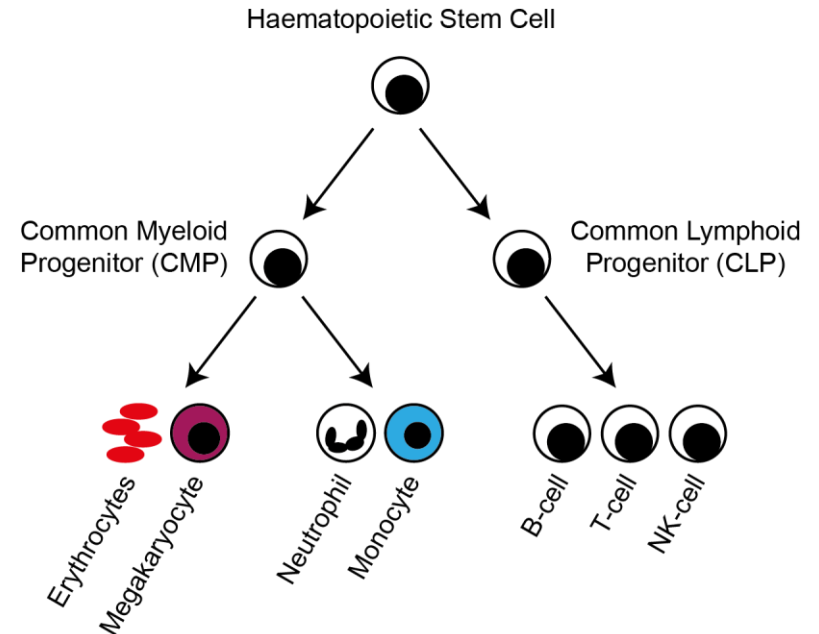


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Lineage priming; indication, but not proof for cell fate

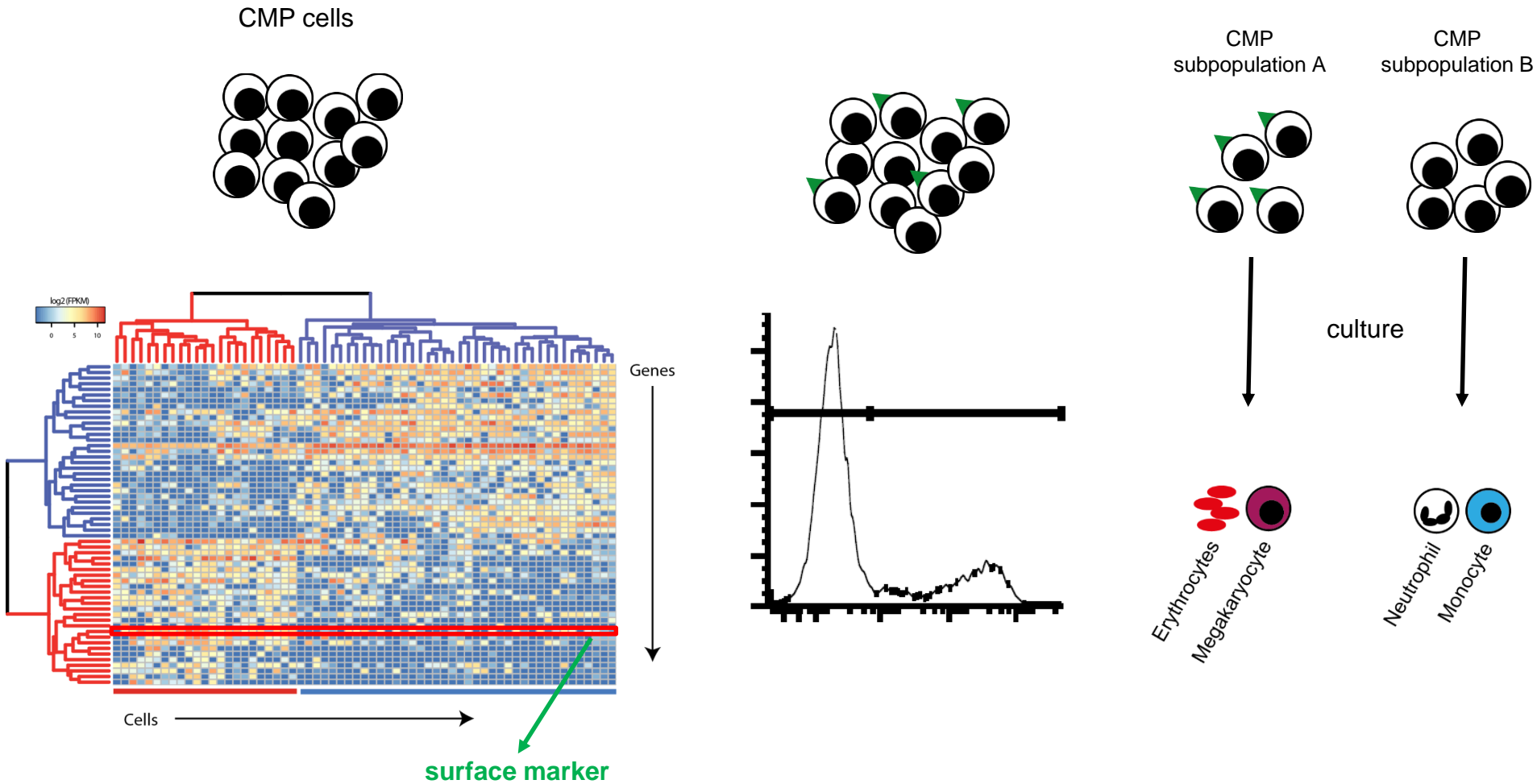


Low level of gene expression can be an indication for the potential of the progenitor

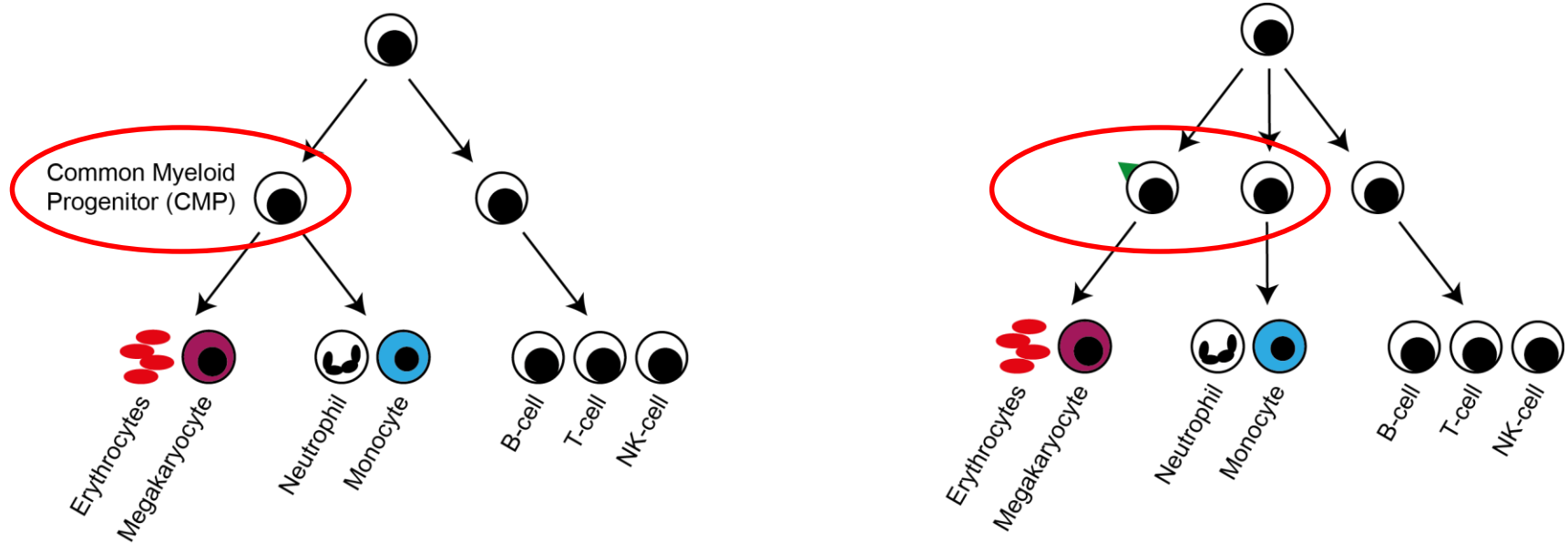


However, absence of expression does not necessarily mean absence of cell potential

Prospective isolation of cell populations

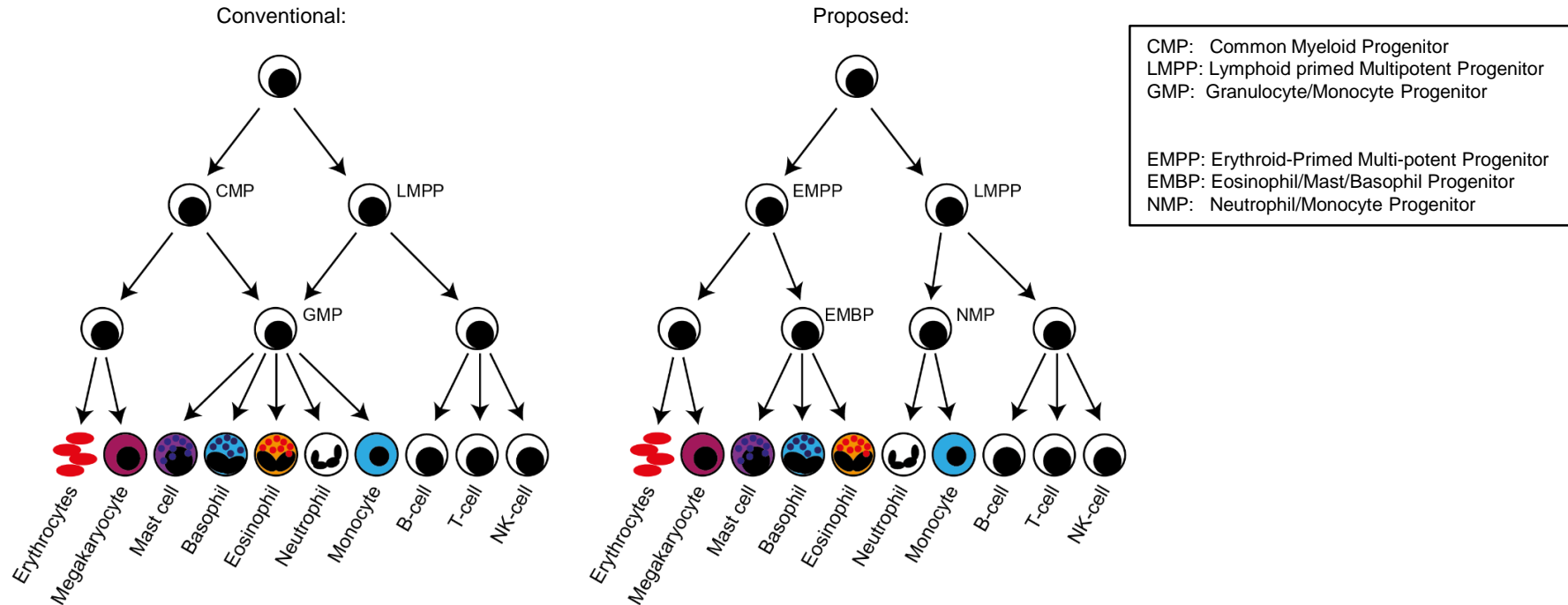


Single cell technologies reveal heterogeneity in cell population



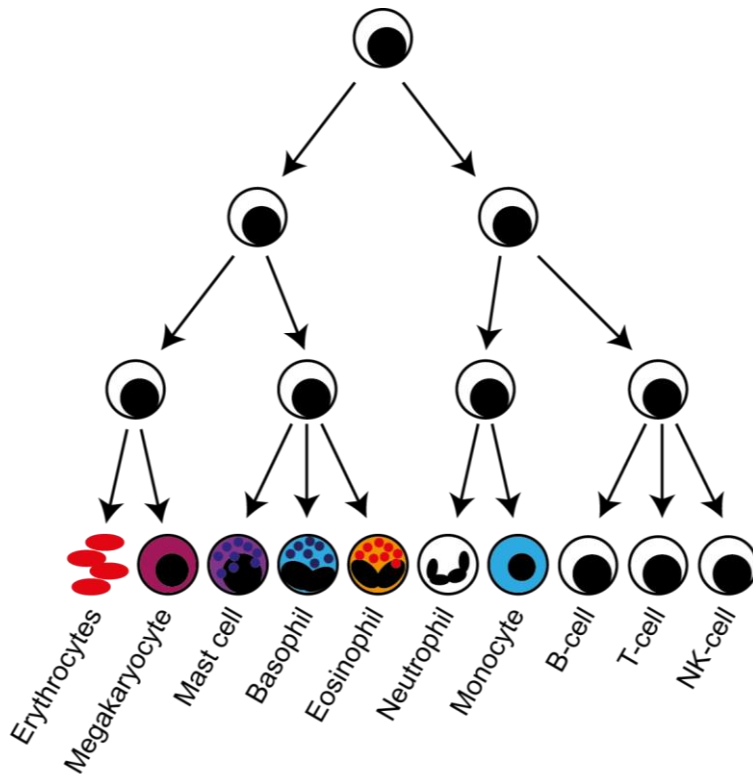
- The CMP population should not be used as a proxy for its individual cells
- Studies to molecular mechanisms for lineage choices
- Characterisation of malignant cells in leukaemia

Two distinct pathways for myelopoiesis



In both mouse and human, two pathways are identified that give rise to different sets of myeloid cell types

Summary



- Single cell techniques unravel heterogeneity of tissues
- Gene expression is an indication, but not proof, for the potential of a cell
- Prospective identification and isolation of cell types are important for studying the cellular hierarchy
- CMP populations is a heterogeneous population