Haematopoietic stem cells

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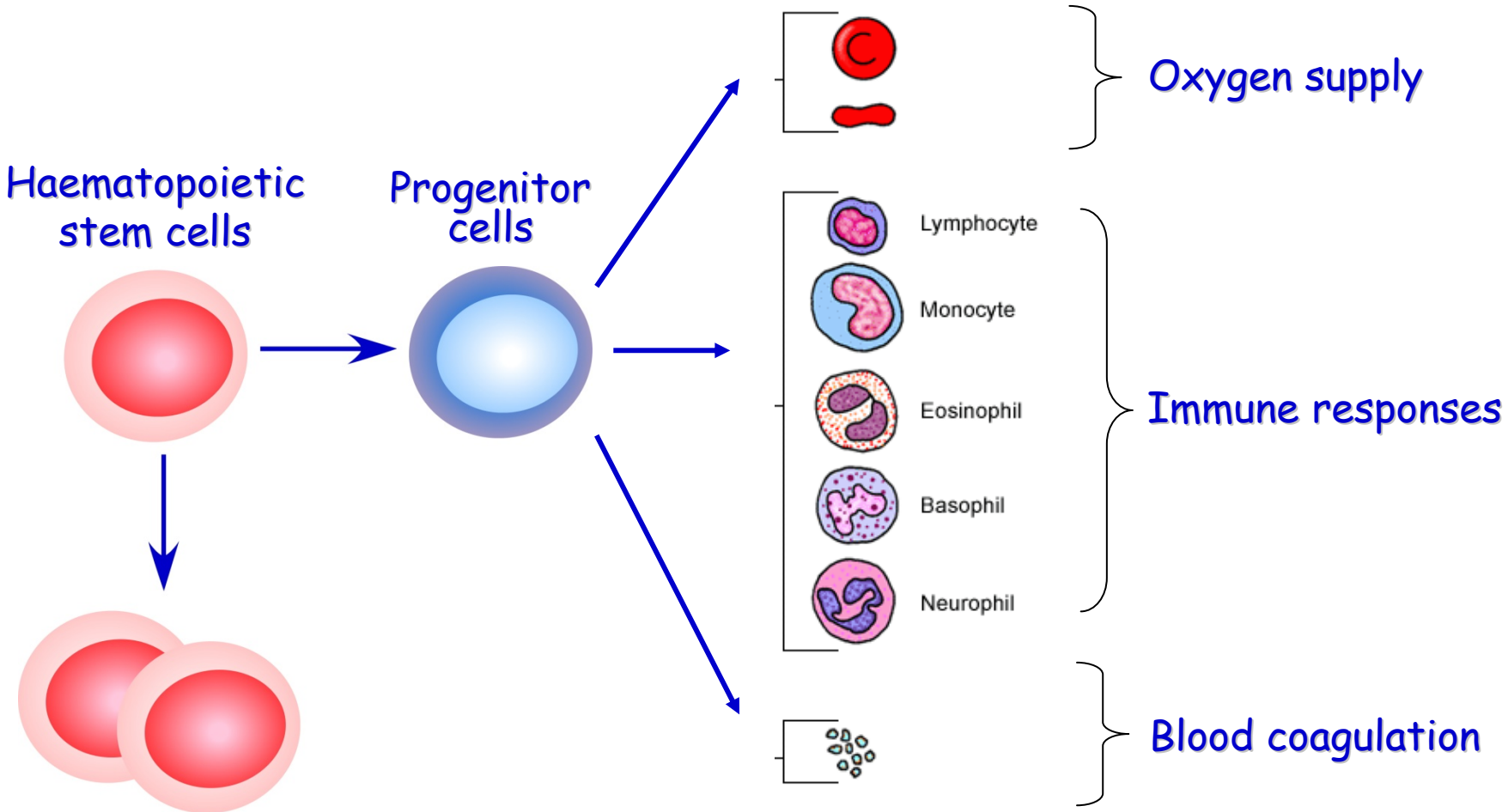
Haematopoiesis: An introduction

- **Haematopoiesis is the term used to describe the production of blood cells**
- **Blood cell production is always about balancing supply and demand**
  - **The bone marrow must be able to tightly regulate haematopoiesis to prevent over or underproduction**
- **Haematopoiesis controlled by**
  - **Stem and progenitor cells**
  - **Internal cues such as transcription factors**
  - **External cues such as growth factors: (cytokines)**
  - **Environmental factors such hematopoietic stem cell niche**

Hierarchical production of blood cells are generated from haematopoietic stem cells (HSCs)



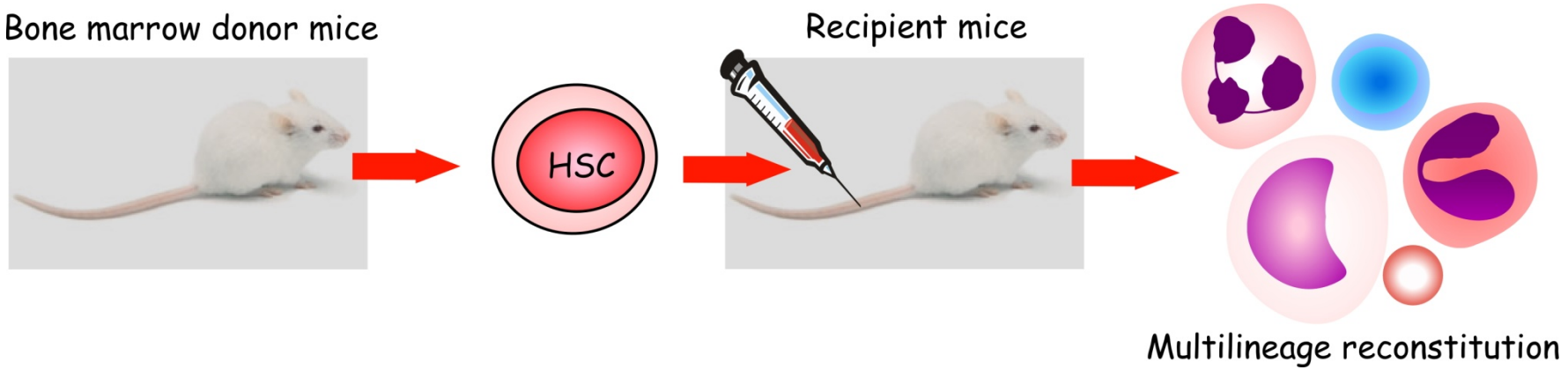
Haematopoietic stem cells (HSCs): how can they be isolated and studied?

Flow cytometry:

Allows examination and/or isolation of cells of interest based on physical or biological characteristics of individual cells passing through an optical and electronic detection system

Cell surface markers (glycoproteins expressed on surface of cells) identify HSCs

Haematopoietic stem cells (HSCs) - *in vivo* study



- Ability to stably reconstitute mouse haematopoiesis after lethal irradiation (Purton & Scadden Cell Stem Cell 2007)

Lineage negative Sca-1+c-kit+ marks mouse HSC compartment:

LT-HSC (CD34-Flt-3-)

ST-HSC (CD34+Flt-3-)

MPP/LMPP (CD34+Flt-3+)

Haematopoietic stem cells (HSCs) - different classes of HSCs

LT-HSC:

- Rare
- Relatively quiescent (dormant) compared to progenitor intermediates
- Able to reconstitute later (> 16 weeks after transplantation)
- A single cell is able to reconstitute all blood lineages for a lifetime after transplantation

ST-HSC:

- More abundant
- Still relatively quiescent, but more actively cycling than LT-HSC
- Able to reconstitute early (2 weeks) and up to 16 weeks after transplantation: particularly important in clinical transplantation when patient may be immuno-compromised after chemotherapy/conditioning: LT-HSCs CANNOT do this job

Study of human haematopoiesis in NOD/SCID mice

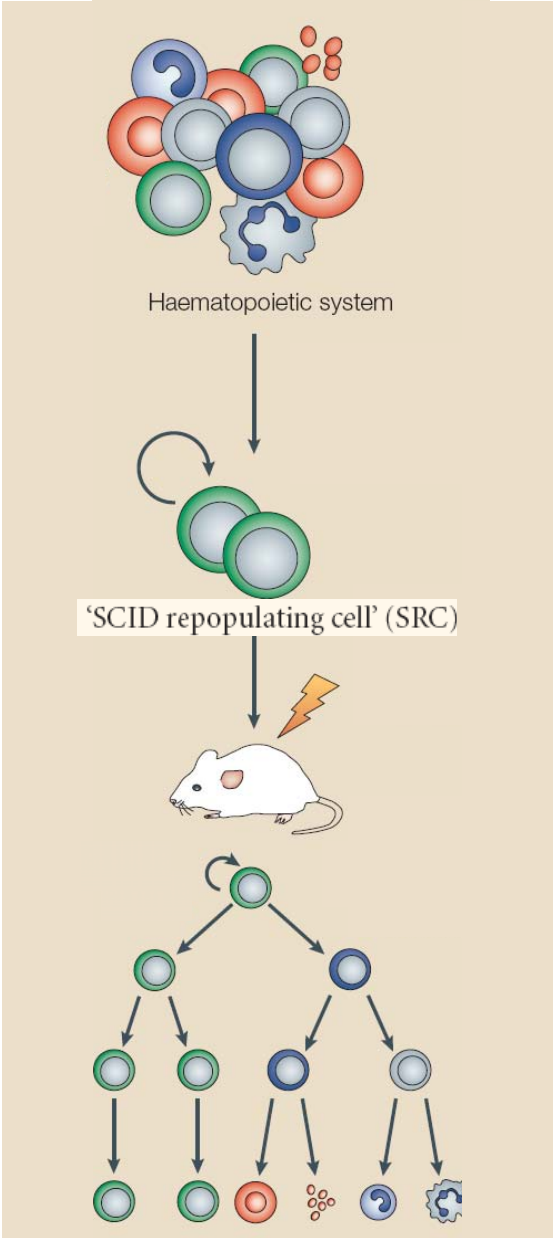
NOD SCID mouse
Non-obese diabetic
Severe combined
immunodeficiency

The Nod strain (non obese diabetic) is characterized by a functional deficit in NK cells, an absence of circulating complement and defects in the differentiation and function of APCs (antigen-presenting cells).

Animals homozygous for the SCID mutation have impaired T and B cell lymphocyte development.

Due to the immunodeficiency the NOD/SCID mice are suitable as recipients of a human cells

Lineage neg CD34+CD38- marks human HSC compartment



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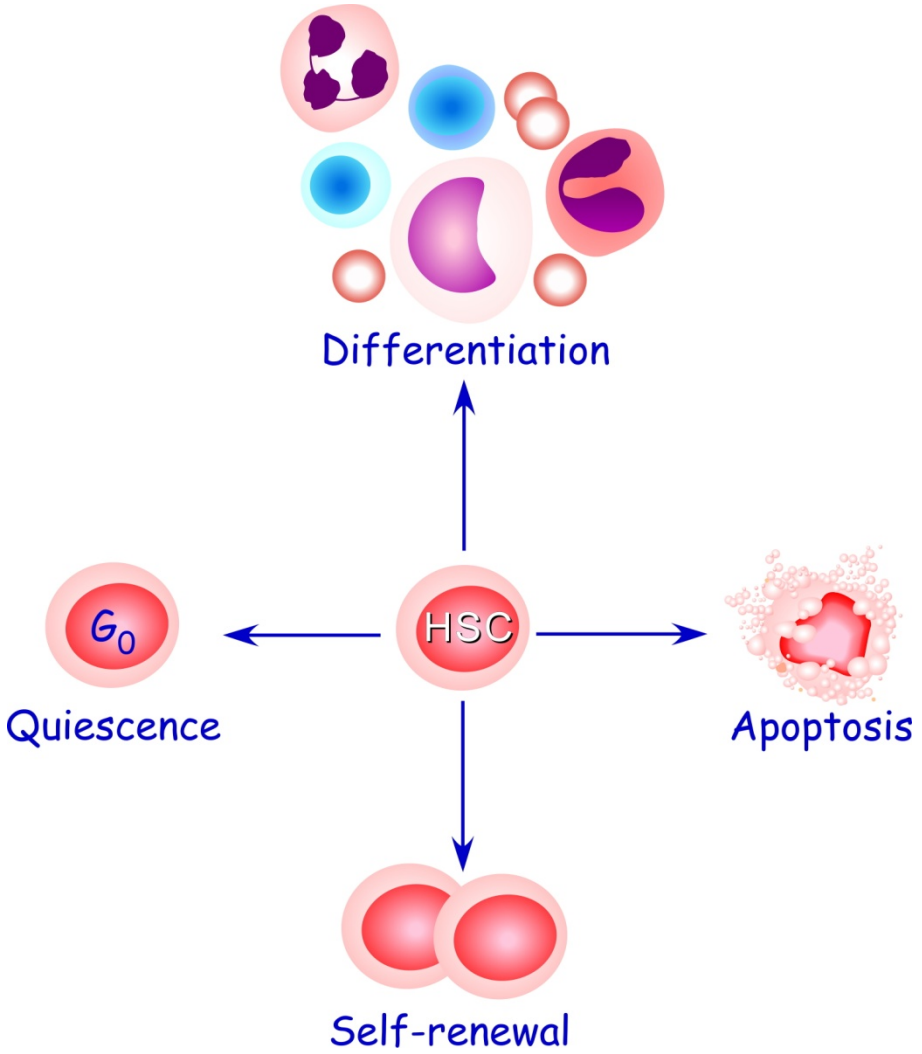
Strict regulation of haematopoietic stem cell (HSC) fates is critical for normal haematopoiesis

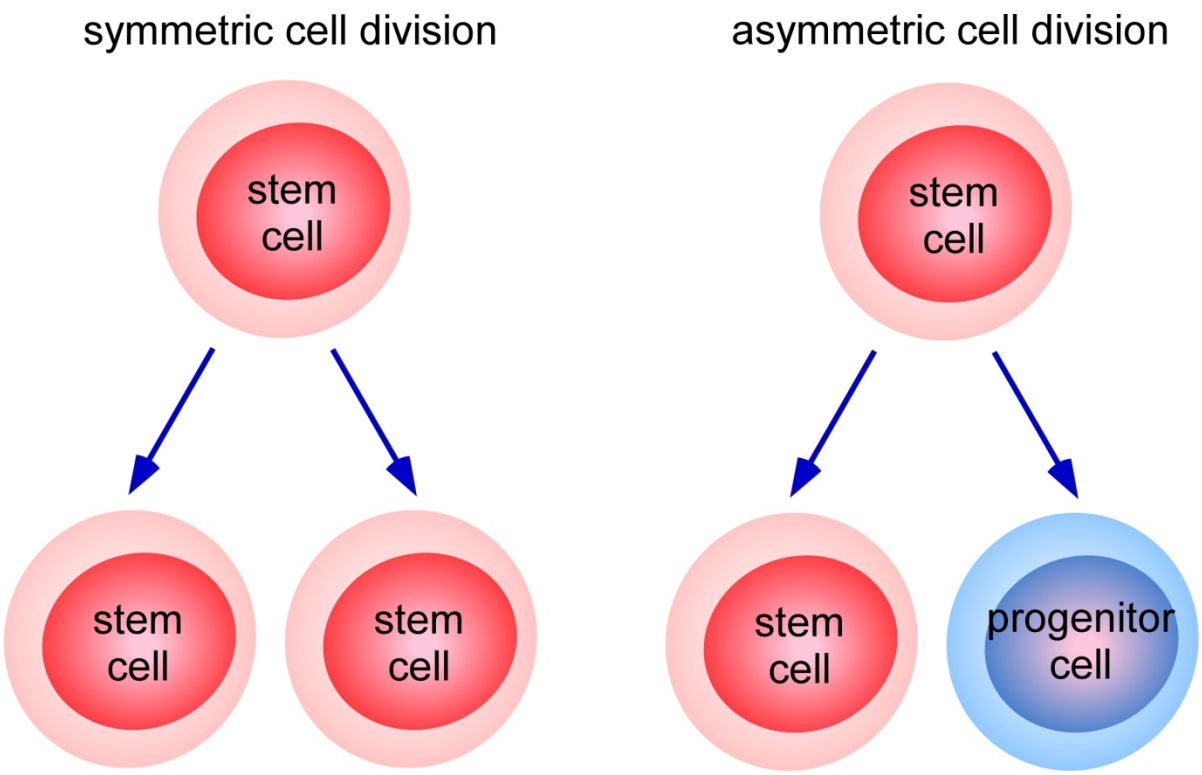


- Self-renewal: allows preservation of the stem cell pool for lifetime of organism
- Quiescence (dormancy): tight regulation to stop superfluous proliferation or to prevent accumulation of mutations (that could cause cancer)
- Apoptosis: cell death mechanism to eliminate excess cells or damaged cells
- Differentiation or lineage specification: to produce intermediates that ultimately yield blood and immune cells!

Disruption of the balance between these processes is a hallmark of leukaemogenesis

What is haematopoietic stem cell (HSC) self-renewal ?

Self-renewal: Cycles of division that repeatedly generate at least one daughter equivalent to the parental cell with equal capacity for differentiation. This is the defining property of stem cells.



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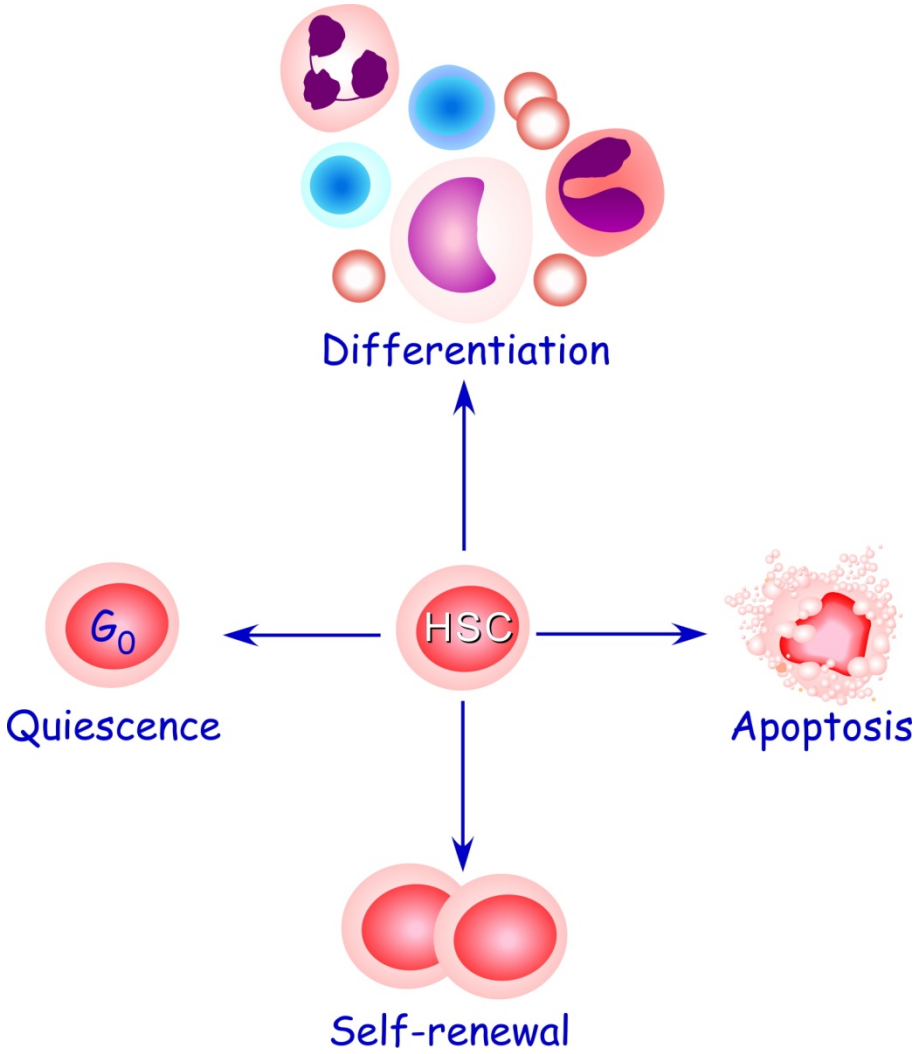

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Regulators of stem cell functions and haematopoiesis: growth factors

- **Critical aspect of blood cell production**
- **Cytokines promote**
  - **Stem cell activity**
    - **Stem cell factor (SCF)**
    - **Flt3 Ligand**
    - **Thrombopoietin (TPO)**
  - **Progenitor maturation**
    - **Erythropoietin : Red cells**
    - **Granulocyte colony stimulating factor: Granulocytes**
    - **Monocyte colony stimulating factor: Monocytes**

Regulators of stem cell functions and haematopoiesis: transcription factors

- **Cell behaviour is also controlled by switching on (expression) or switching off (repression) of genes**

- **In hematopoietic stem and progenitor cells this is controlled by nuclear transcription factors (10% of genome) that co-ordinately regulate gene expression**

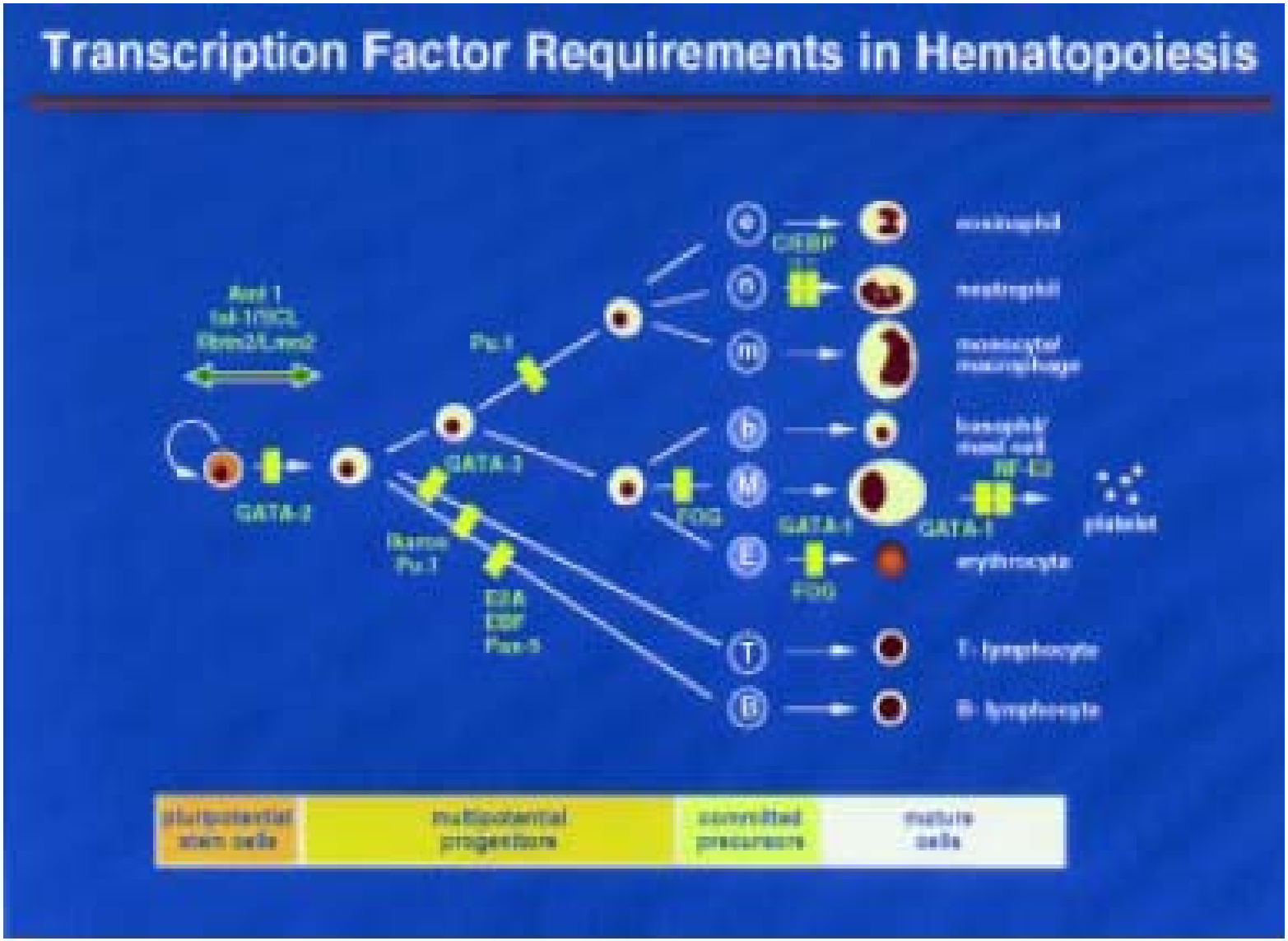
- **How do we know this?**

- **Examine the requirement of specific transcription factors in hematopoietic stem and progenitor cells**

- **Engineered mice that are missing (knockout) or over-express a particular gene and study the biology of this transcription factor**

- **Abnormalities in blood cell production often occur**

Regulators of stem cell functions and haematopoiesis: transcription factors



Regulators of lineage commitment from HSCs: transcription factors

- **Lineage commitment essential for formation of blood**
- **How is it determined?**
- **At the molecular level by transcription factors**
- **Transcription factors: lineage priming**
- **Lineage priming: in HSCs there is low level expression of transcription factors (or priming) that are normally expressed at high levels in specific blood cells lineages**
- **READY STATE for blood formation: all options are open!**
- **On differentiation, there is a shutdown of extraneous transcriptional programmes: transcription factor antagonism (e.g. GATA-2 and PU.1 in myeloid choice)**

Leukaemias: The involvement of transcription factors

- **Transcription factors are very important for normal haematopoiesis**

- **Their importance in normal haematopoiesis is exemplified by their involvement in leukaemia**

- **Leukaemia is often associated with either (i) dysregulated transcription factor expression or (ii) chromosomal translocation can give rise leukaemia**

- **Chromosomal translocations: Caused by rearrangement of genes between nonhomologous chromosomes. A fusion genes may be created when the translocation joins two otherwise separated genes, an event which is common in leukaemia**

- **Chromosomal translocations: SCL/tal-1, AML-1 and Tel**

- **Mis-expressed transcription factors that give rise to leukaemia: PU.1 and Ikaros**

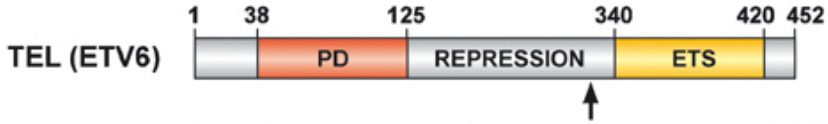
- **These epigenetic or genetic changes are normally termed the "first" hits**

- **How do these "first hits" contribute to leukaemia?**

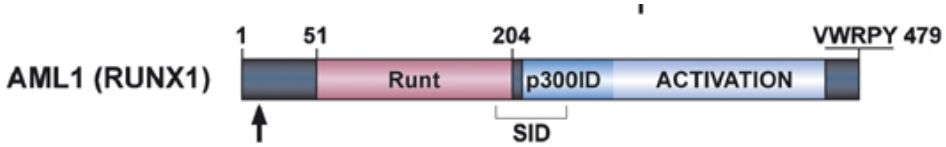
- **They can expand an abnormal clone of cells: transcriptional program and gene expression is dysregulated.**

- **Environmental, extrinsic factors or further genetic changes in these clones are normally termed the "second" hits and can give rise to leukaemia**

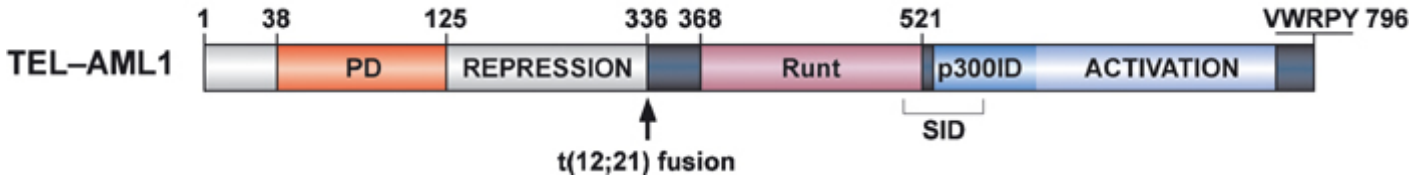
1st hit – Tel-AML1



The TEL gene is required specifically for haematopoiesis in the bone marrow (Genes & Dev. 1998. 12: 2392-2402)



AML1 is required for the establishment of definitive haematopoiesis during development and for megakaryocytic maturation and lymphocytic differentiation

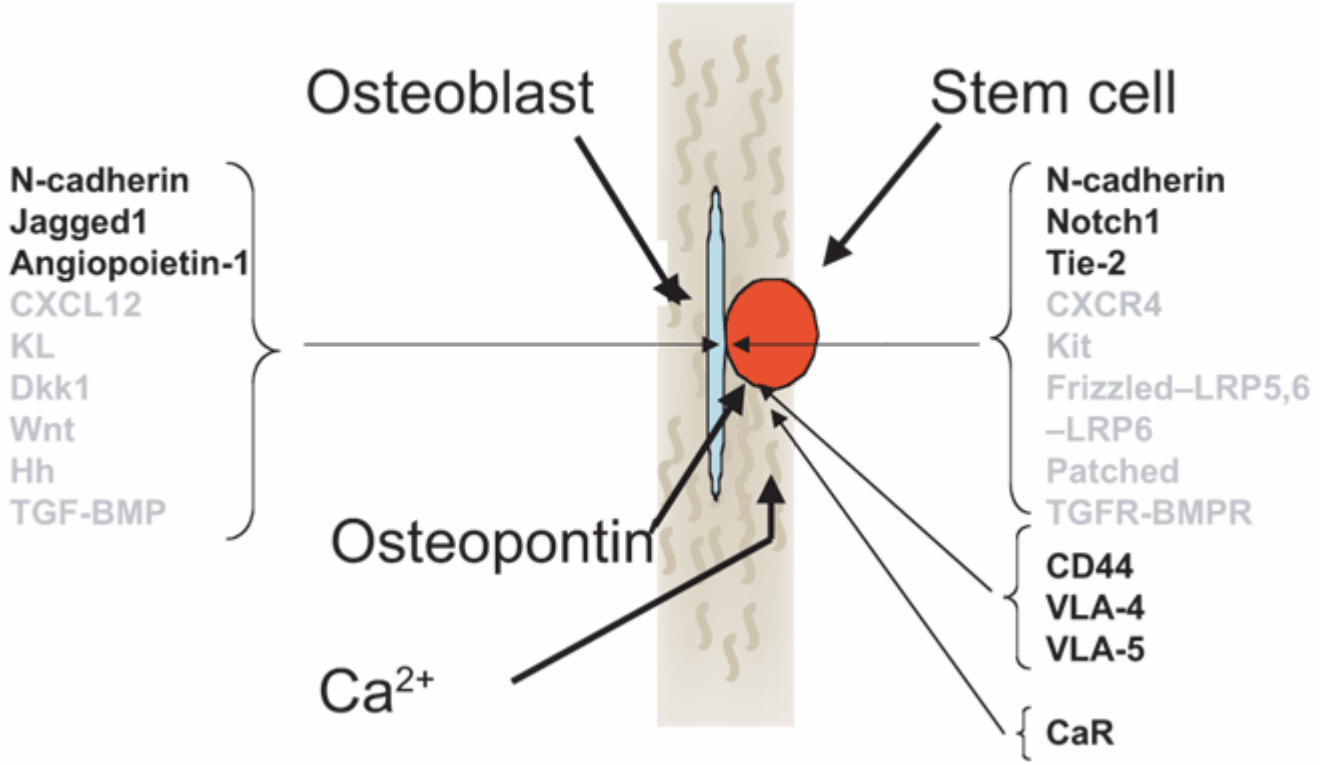


The t(12;21) chromosomal translocation is the most frequent gene recombination in paediatric ALL **(first hit in leukemogenesis).**

Oncogene (2004) **23, 4275–4283**

Hematopoietic stem cell niche as a determinant of stem cell fate decisions

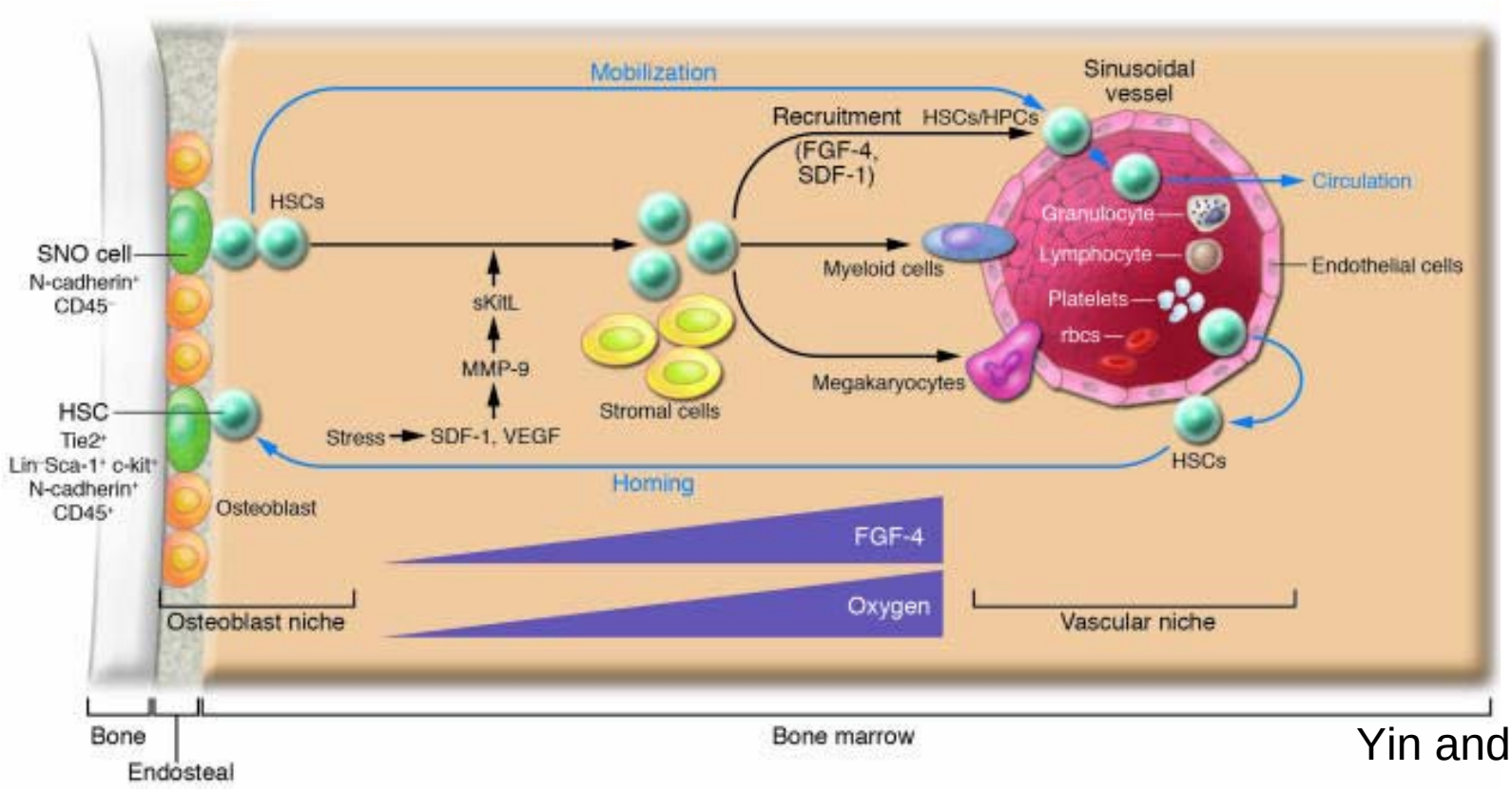
The niche in the endosteum: endosteal surface is a thin layer of connective tissue that lines the walls of the bone marrow cavities; contains osteogenic (bone) and hematopoietic stem cells



Adams and Scadden, Nature Immunology, 2006

Hematopoietic stem cell niche as a determinant of stem cell fate decisions

The vascular niche: located away from the endosteum in sinusoidal vessels (type of blood vessel in marrow cavity with discontinuous endothelium allowing secretion of cells or proteins); interacts with endosteal (osteoblastic) niche to regulate stem cell fate as depicted below



Yin and Li, JCI, 2006

Perspective

- **Bone marrow or stem cell transplantation to treat cancers:** Stem cells are also important in bone marrow transplantation to treat blood cancers: the first and only established stem cell therapy

- **Working in multiple settings but not perfect.**

- **Cord blood stem cells to treat adults limited: HSC expansion would be a solution but these cells are difficult to grow. Studying and gaining new insights into biology is important as it may hint at pathways that can be manipulated for this purpose.**

- **Ex vivo manipulation of HSCs through drugs (small molecule) screening**

- **Activation of endogenous HSCs through small molecules may also hold promise (e.g. directly after transplantation when patient is immunocompromised)**

- **Modulation of niche components through drugs: targeted therapy for leukaemia and expand HSCs endogenously**