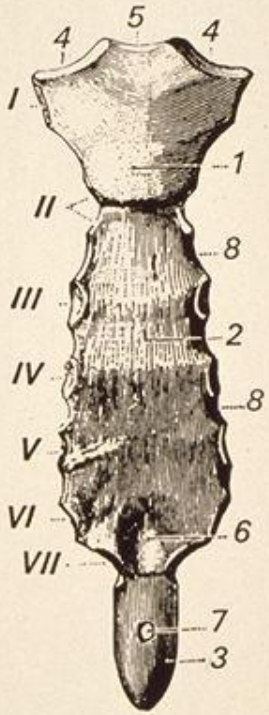
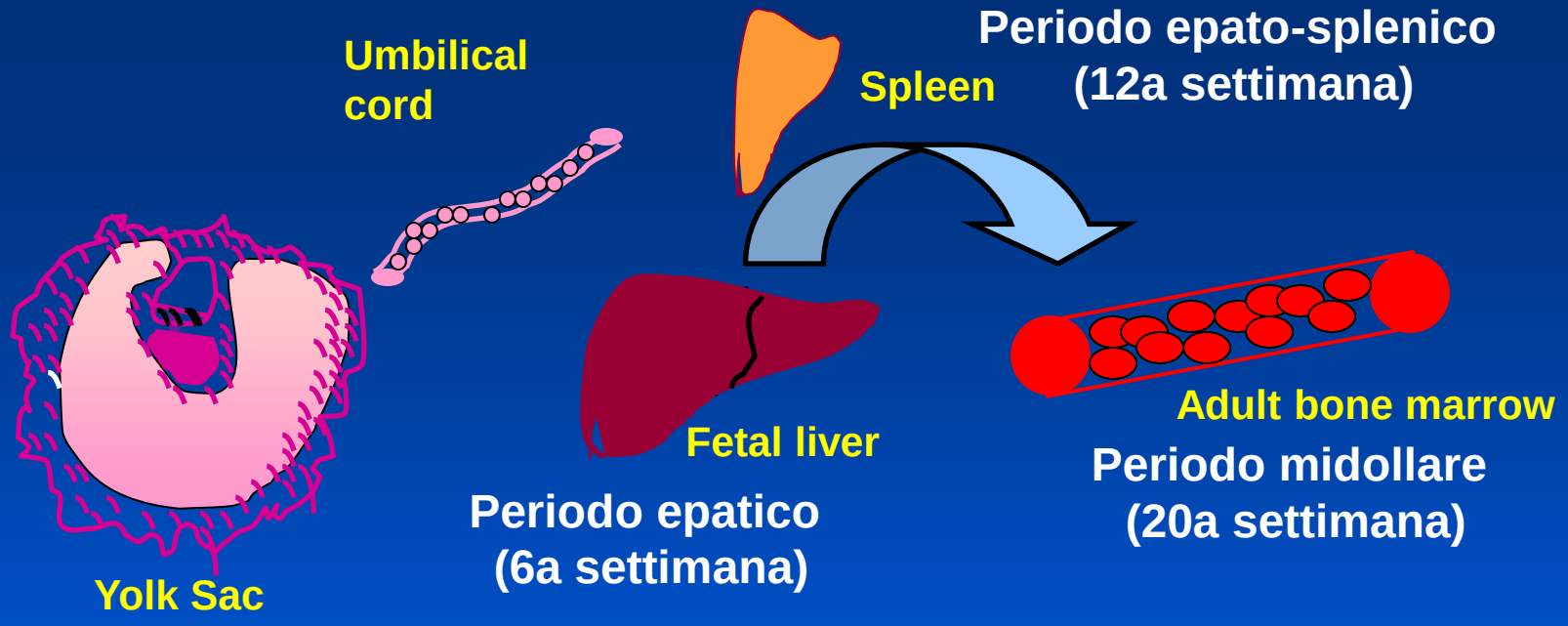
BIOPSIA MIDOLLARE DELLO STERNO

SEDI DI PRELIEVO

- MANUBRIO : 1 cm AL DI SOPRA DELL'ANGOLO STERNALE
- CORPO : II o III SPAZIO INTERCOSTALE

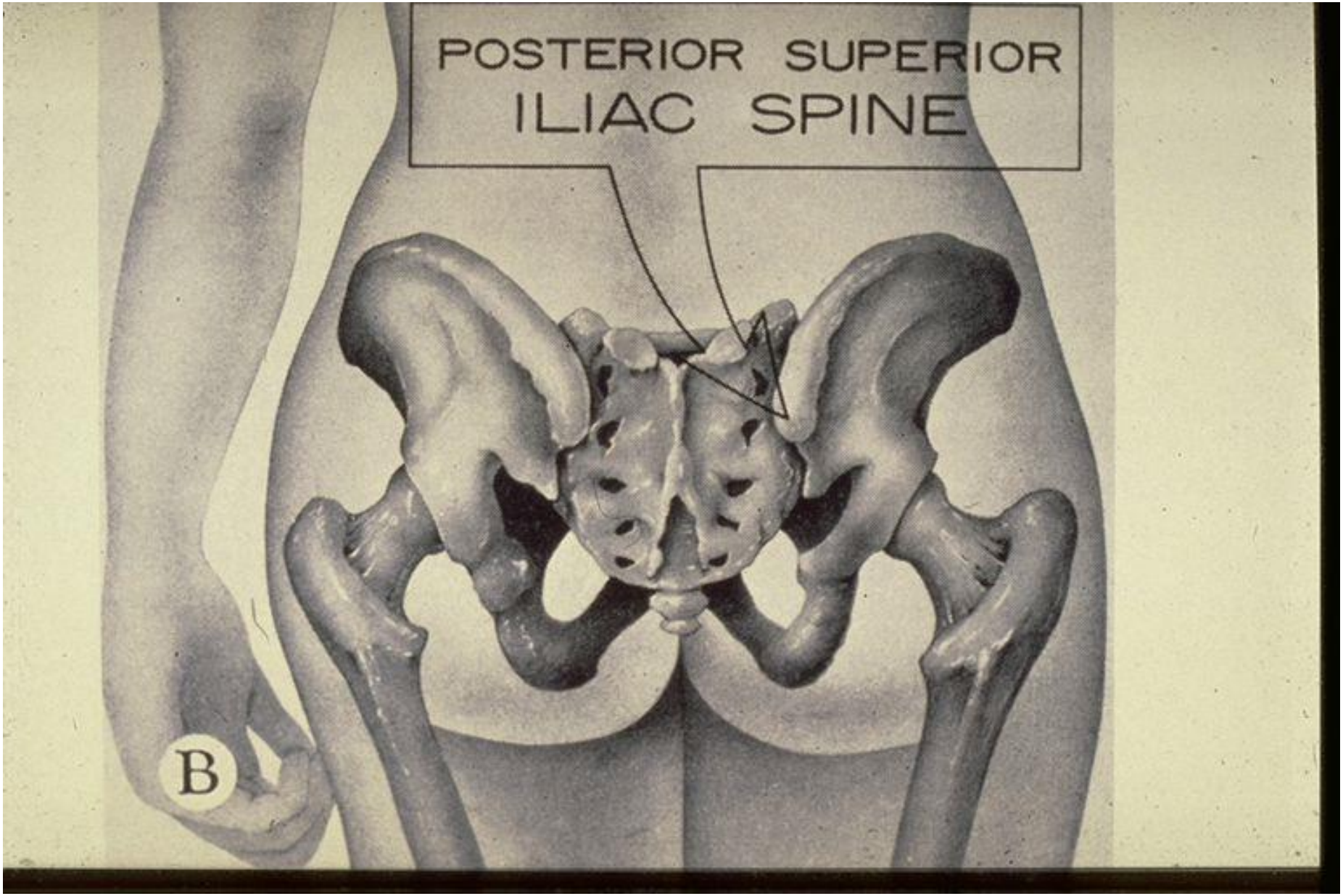


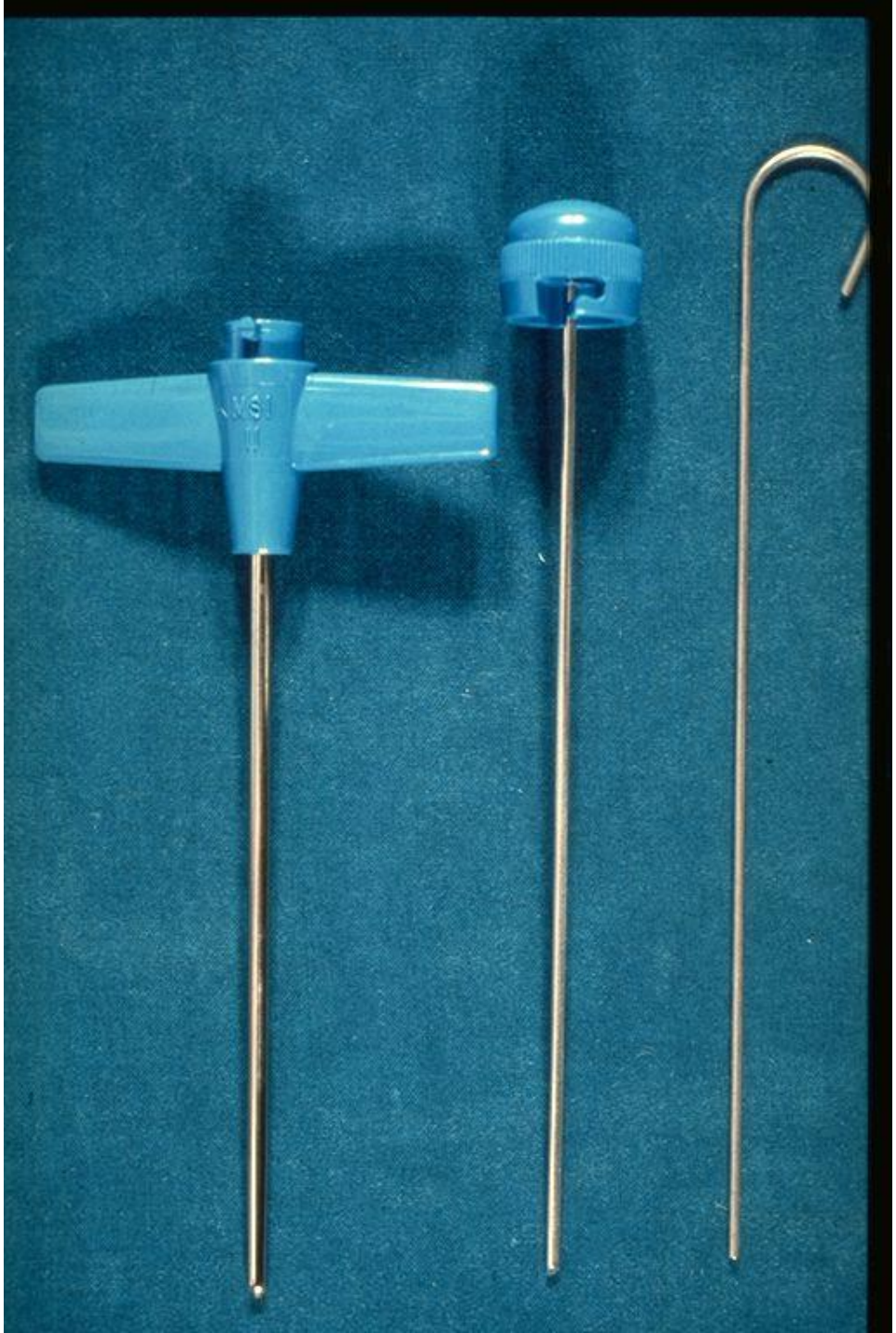
ISTITUTO DI EMATOLOGIA "L. E A. SERÀGNOLI"
UNIVERSITÀ DI BOLOGNA

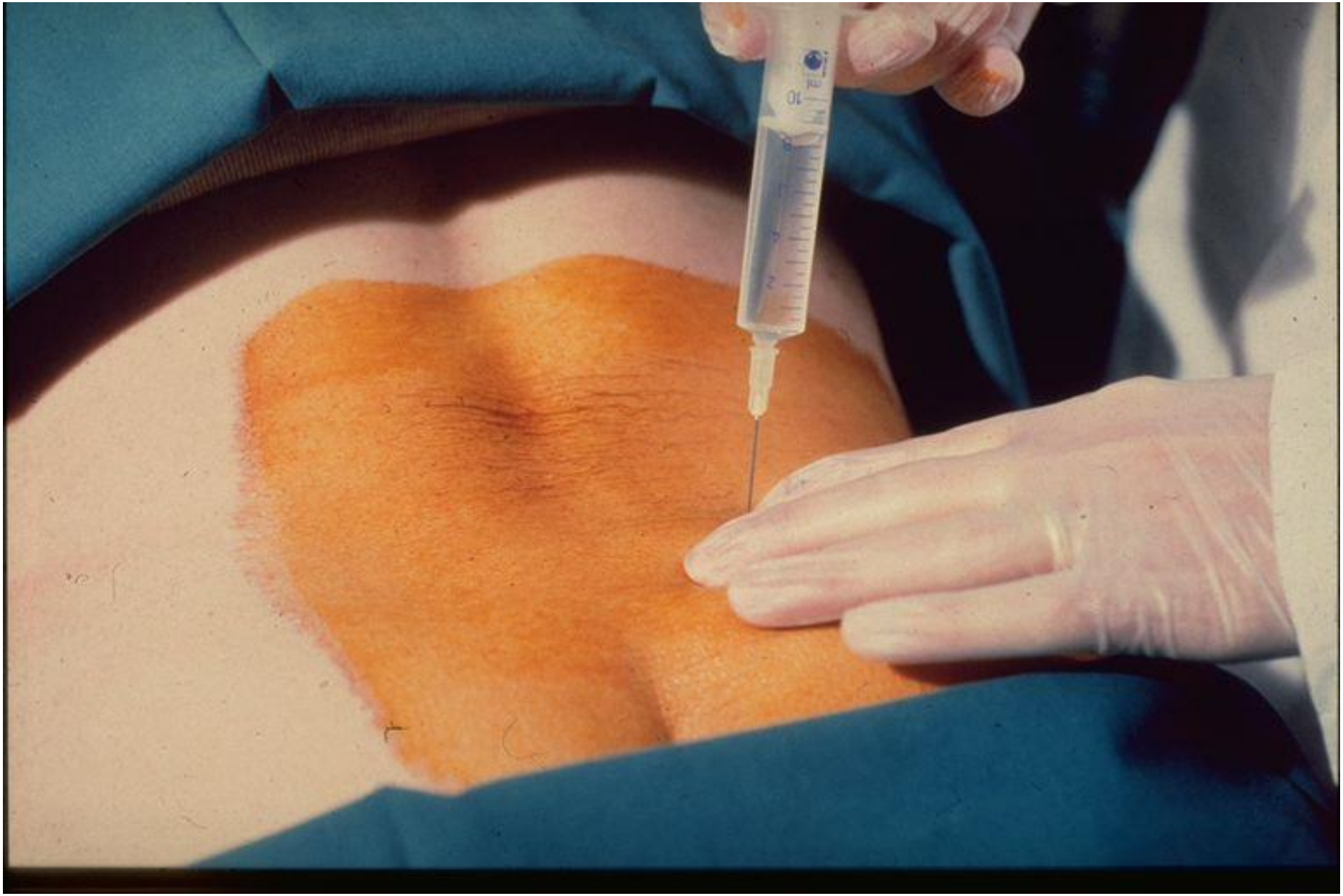


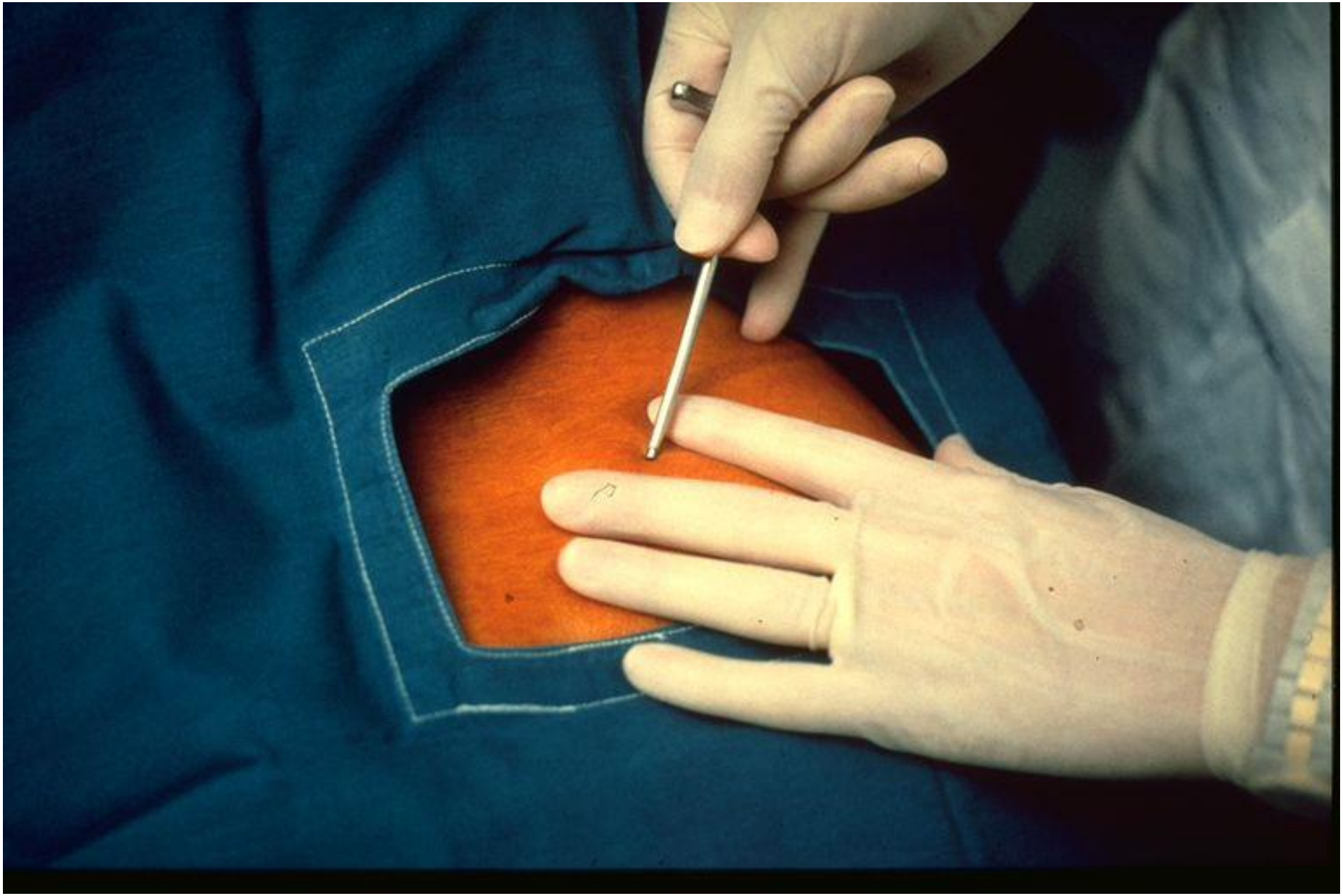
Shortening of telomere length

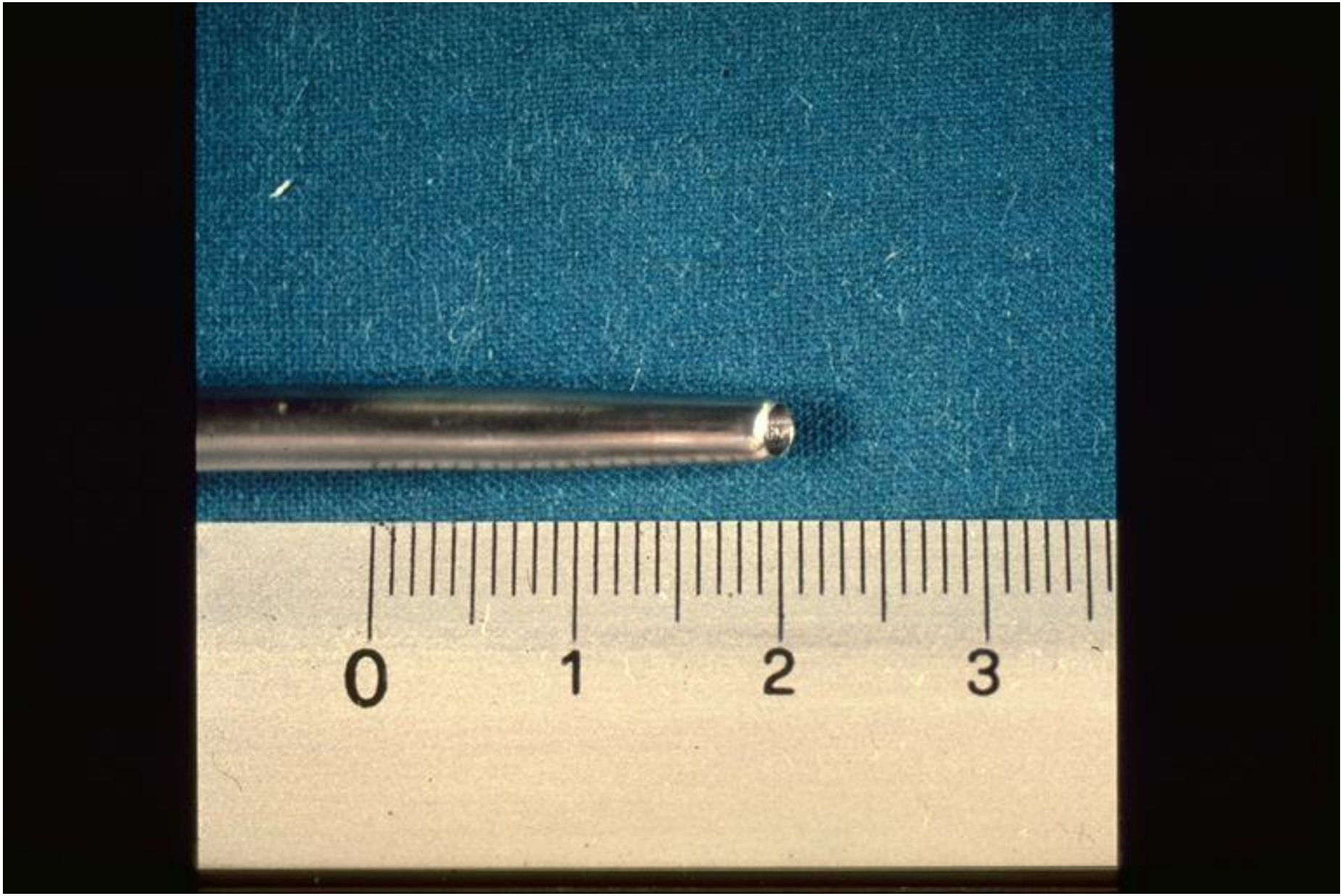
Absolute No. of stem cells and quiescent progenitors

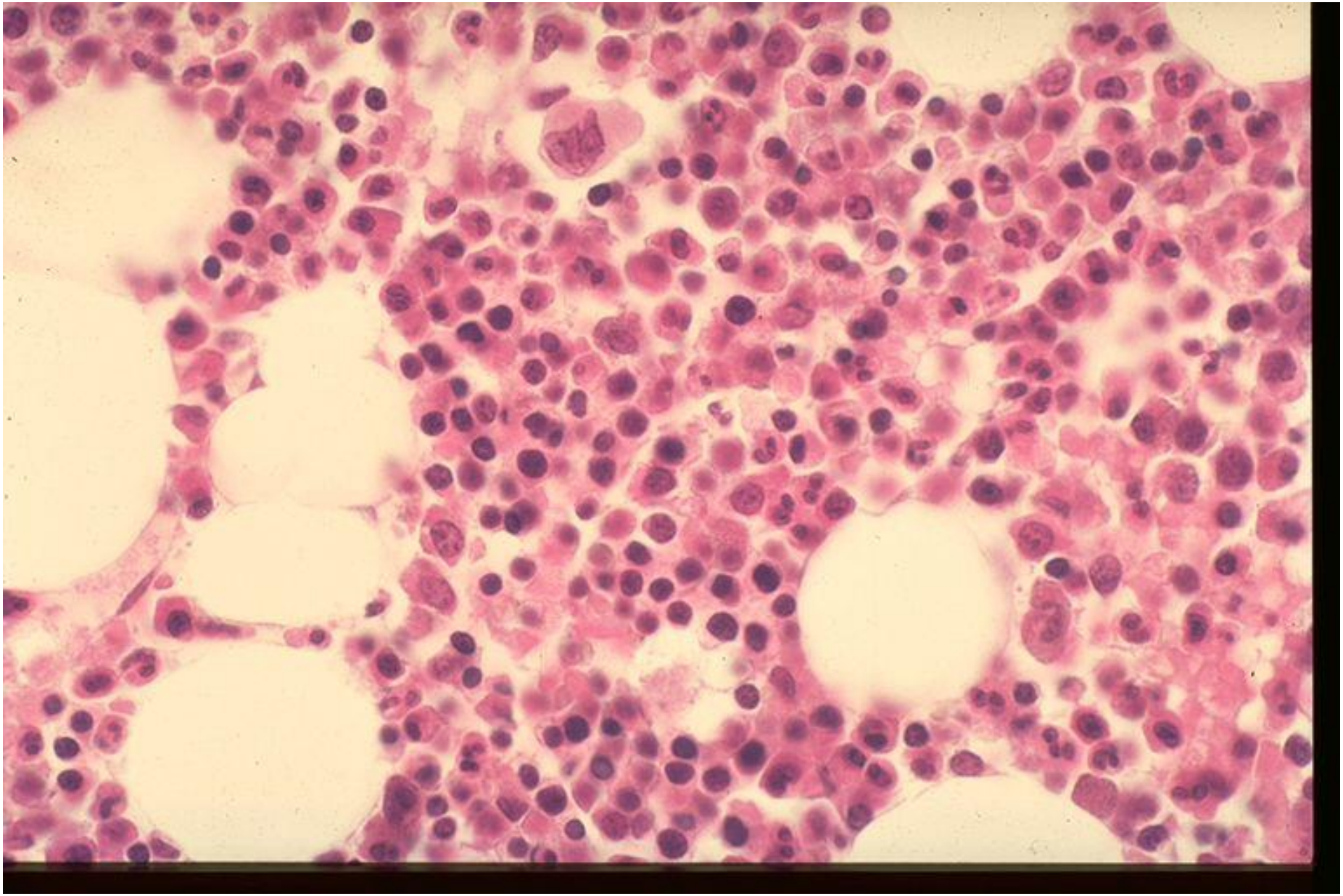

POSTERIOR SUPERIOR
ILIAC SPINE
B

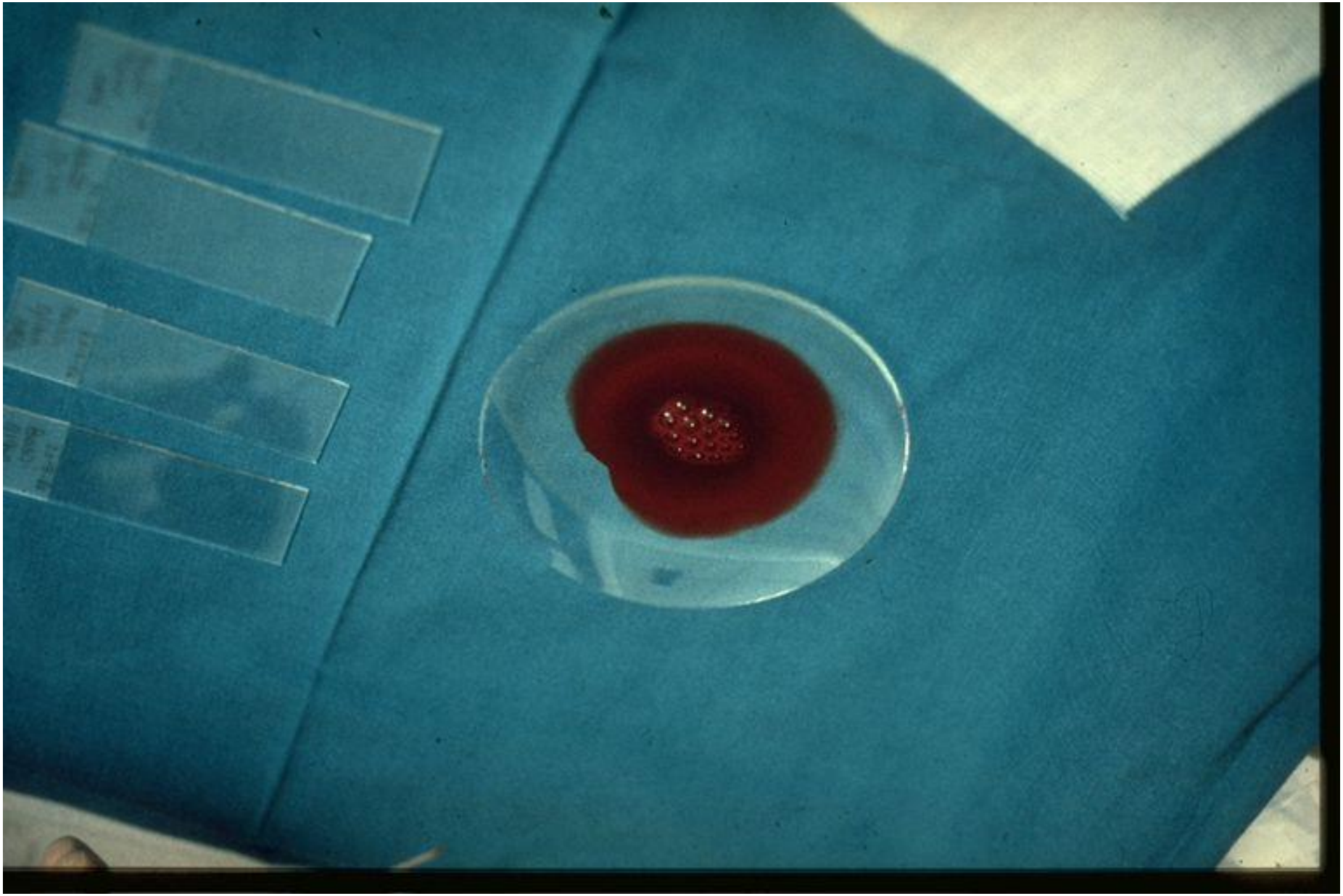


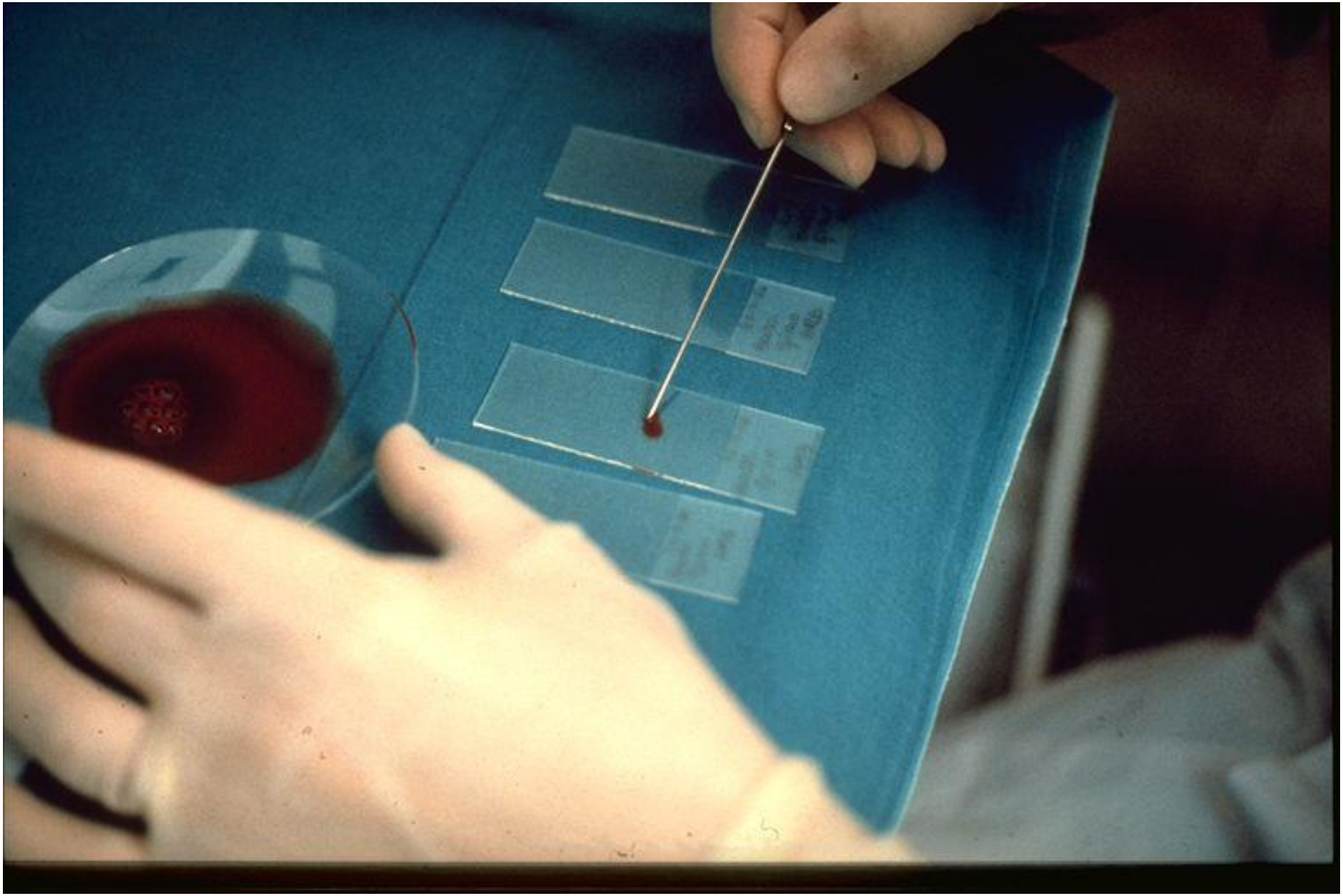


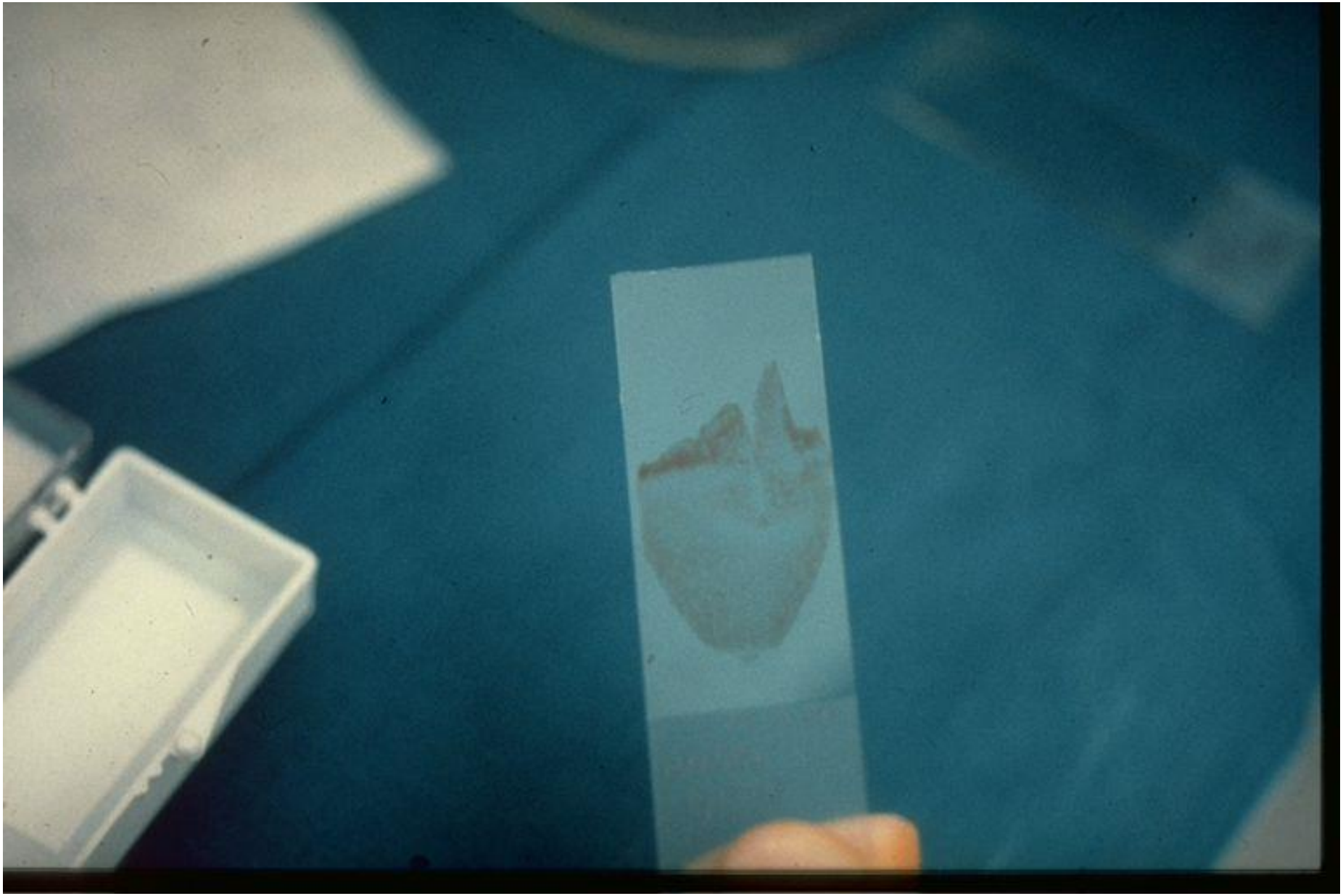


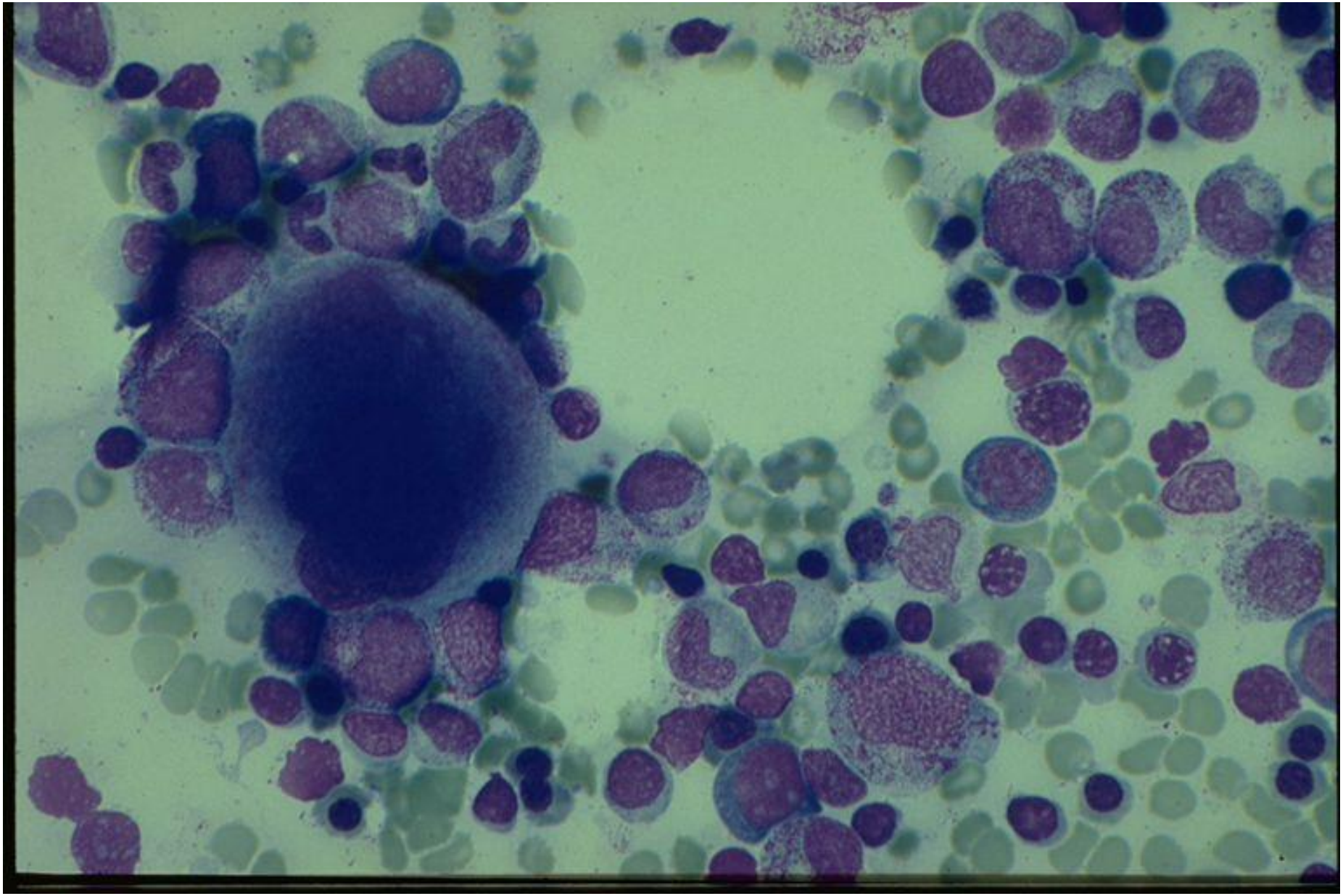

0
1
2
3

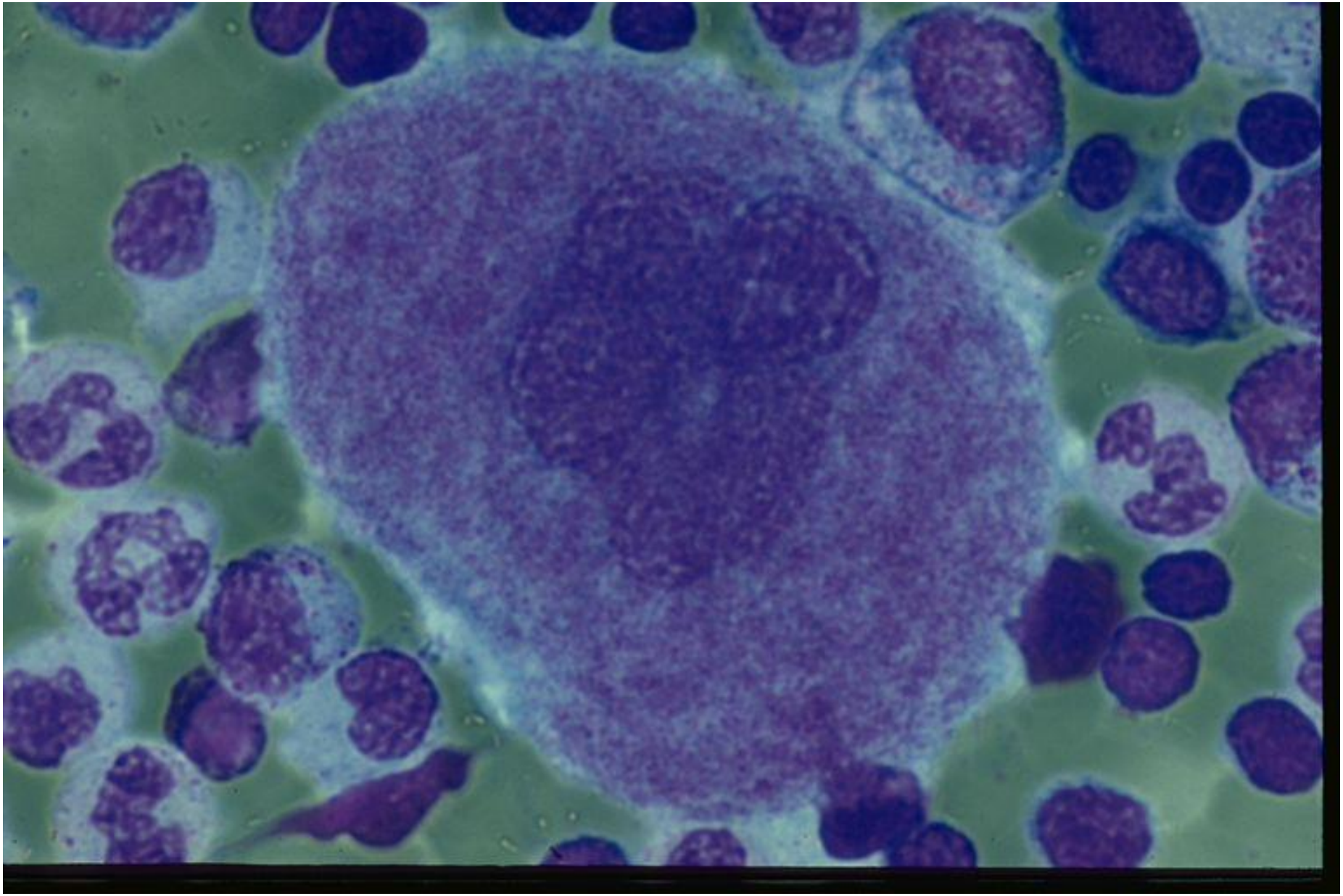


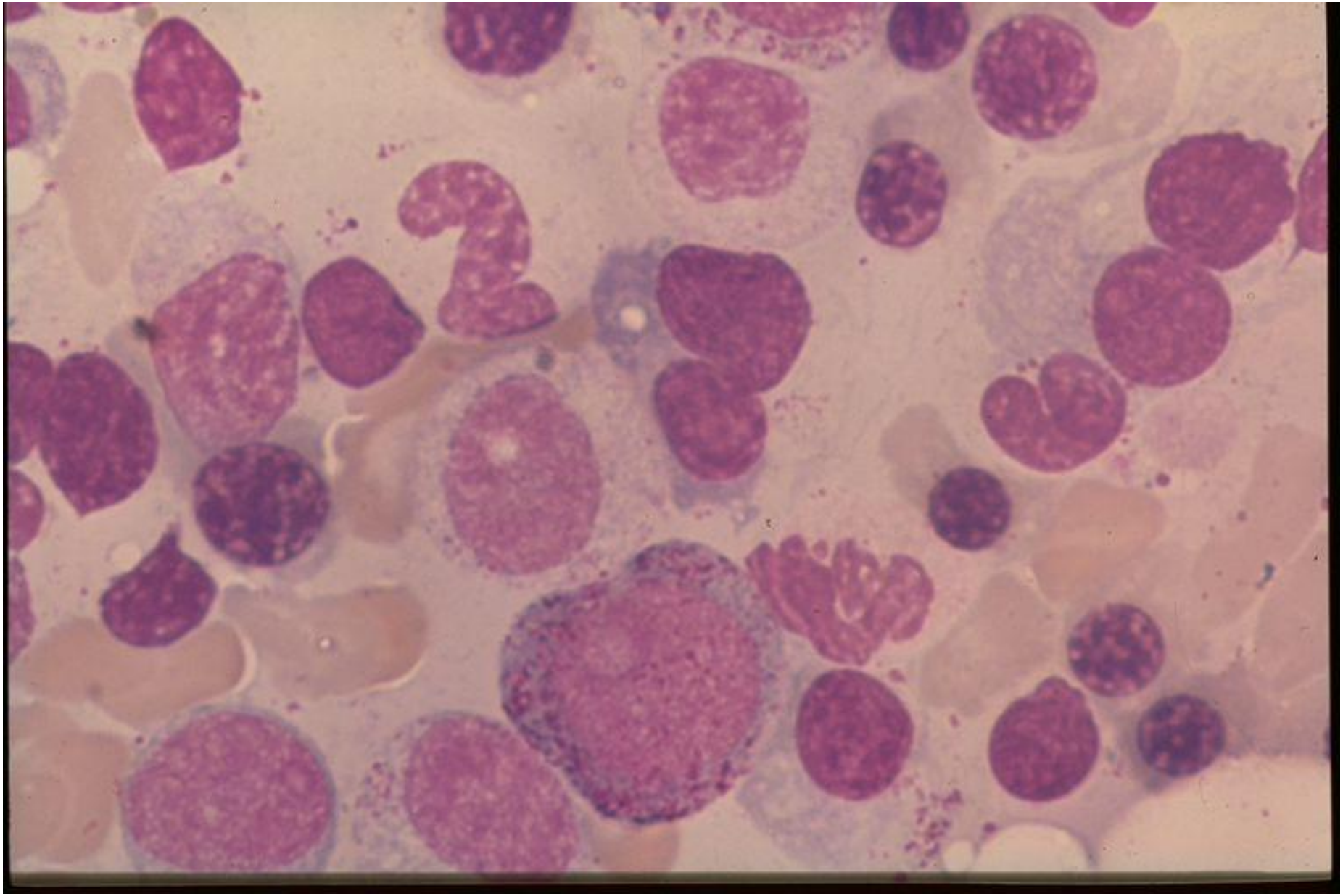










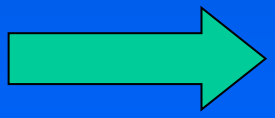


MICROAMBIENTE MIDOLLARE

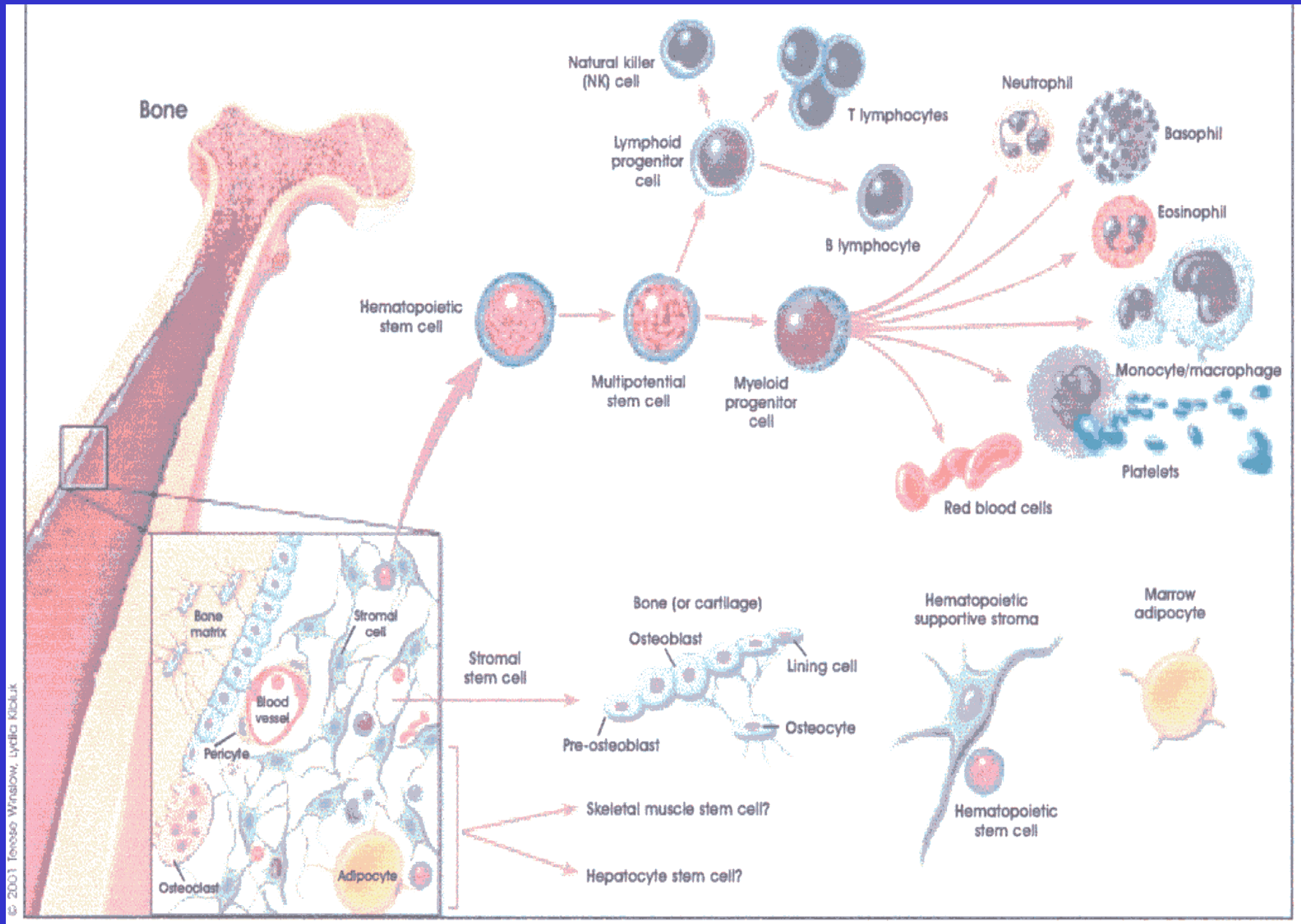
Componente cellulare

Componente vascolare
Cellule reticolari
Struttura nervosa
Adipociti, fibroblasti, Macrofagi
Cellule stam. mesenchimali

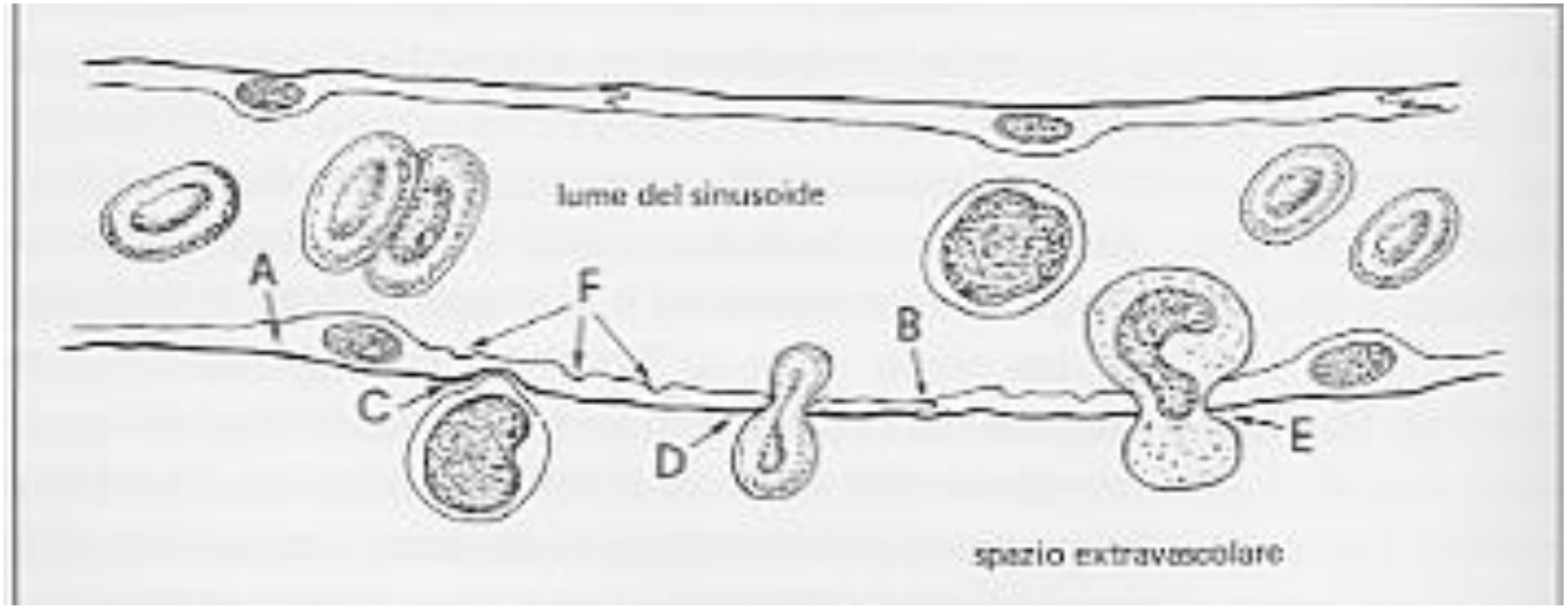
Matrice extracellulare



Fibre reticolari/collagene
Prolungamenti cell. reticolari
Proteine d'adesione
proteoglicani


Bone
Natural killer (NK) cell
T lymphocytes
Lymphoid progenitor cell
B lymphocyte
Neutrophil
Basophil
Eosinophil
Monocyte/macrophage
Platelets
Red blood cells
Hematopoietic stem cell
Multipotential stem cell
Myeloid progenitor cell
Bone matrix
Stromal cell
Blood vessel
Pericyte
Osteoclast
Adipocyte
Stromal stem cell
Bone (or cartilage)
Osteoblast
Lining cell
Osteocyte
Pre-osteoblast
Skeletal muscle stem cell?
Hepatocyte stem cell?
Hematopoietic supportive stroma
Hematopoietic stem cell
Marrow adipocyte
© 2001 Teresa Winslow, Lydia Kibiuk

MIDOLLO OSSEO



IMMISSIONE IN CIRCOLO DEGLI ELEMENTI MATURI

SANGUE PERIFERICO

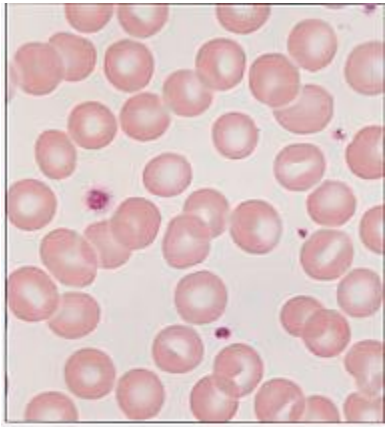


Fig. 1.62 Normal red cells: mean 8 µm in diameter with minor variations in size and shape. The majority show a central pale area of diminished staining. Platelets, 1–3 µm across, are also evident.

ERITROCITI
PIASTRINE

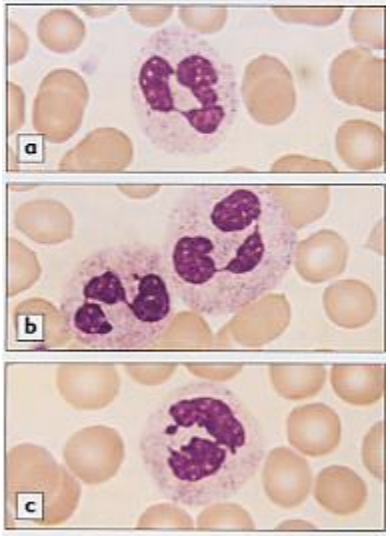


Fig. 1.65a–c Normal neutrophils: (a–c) mature forms showing typical nuclear lobe separation by fine filaments; normal segmented neutrophils may show up to five lobes; (c) a 'Barr body' is attached to a lobe of the nucleus, which is typical of a female neutrophil and results from the possession of two X chromosomes.

GRANULOCITI
NEUTROFILI

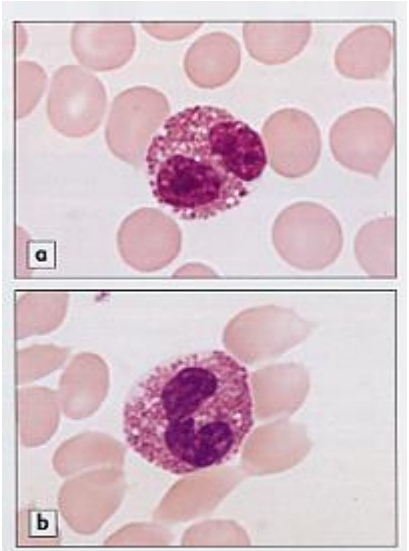


Fig. 1.67a and b Normal eosinophils: (a, b) each of these cells shows two nuclear segments and the typical coarse eosinophilic granulation of the cytoplasm.

GRANULOCITI
EOSINOFILI

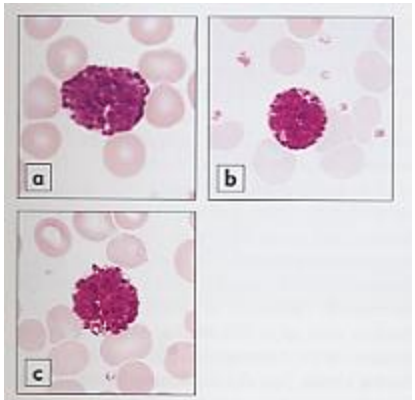


Fig. 1.68a–c Basophils: (a–c) the coarse basophilic granules of these cells often overlie the nucleus, thus obscuring the detail of its segmented structure. Only small numbers of basophils are found in the normal blood film.

GRANULOCITI
BASOFILI

SANGUE PERIFERICO

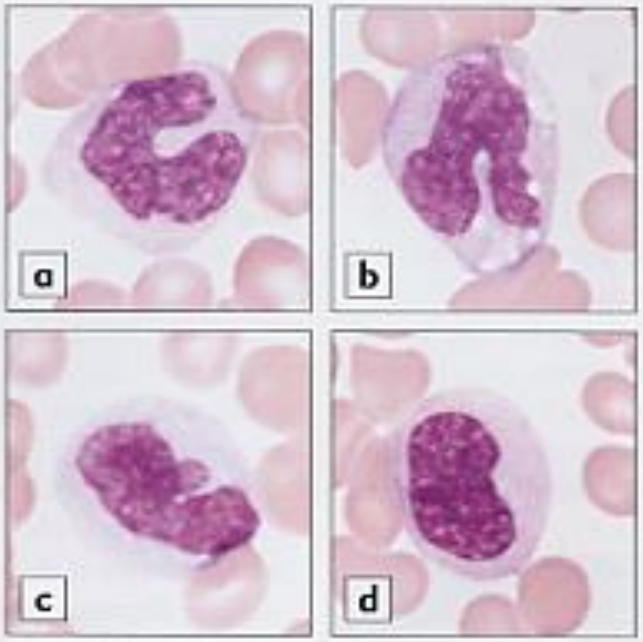


Fig. 1.69a–d Monocytes: (a–d) these cells are usually the largest white cells found in normal blood. The nucleus is usually folded or convoluted, with a moderately fine chromatin pattern. The cytoplasm typically has a grey 'ground-glass' appearance with fine azurophilic granules. Some (b) have rather prominent cytoplasmic vacuoles.

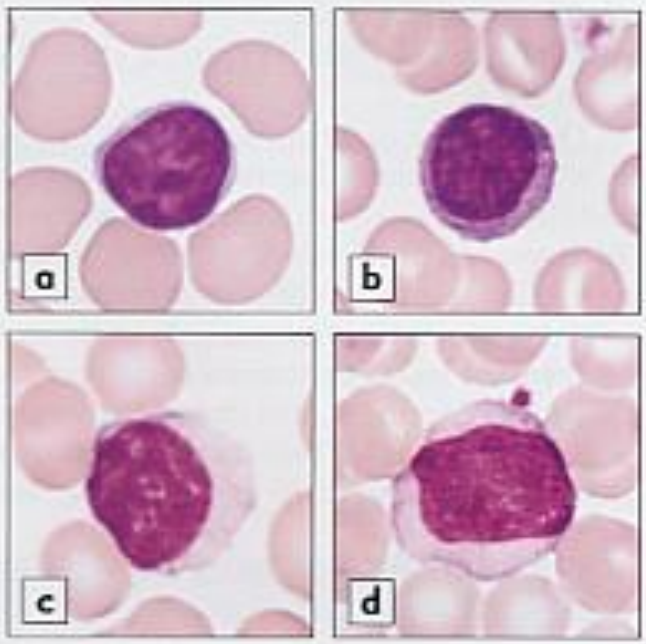
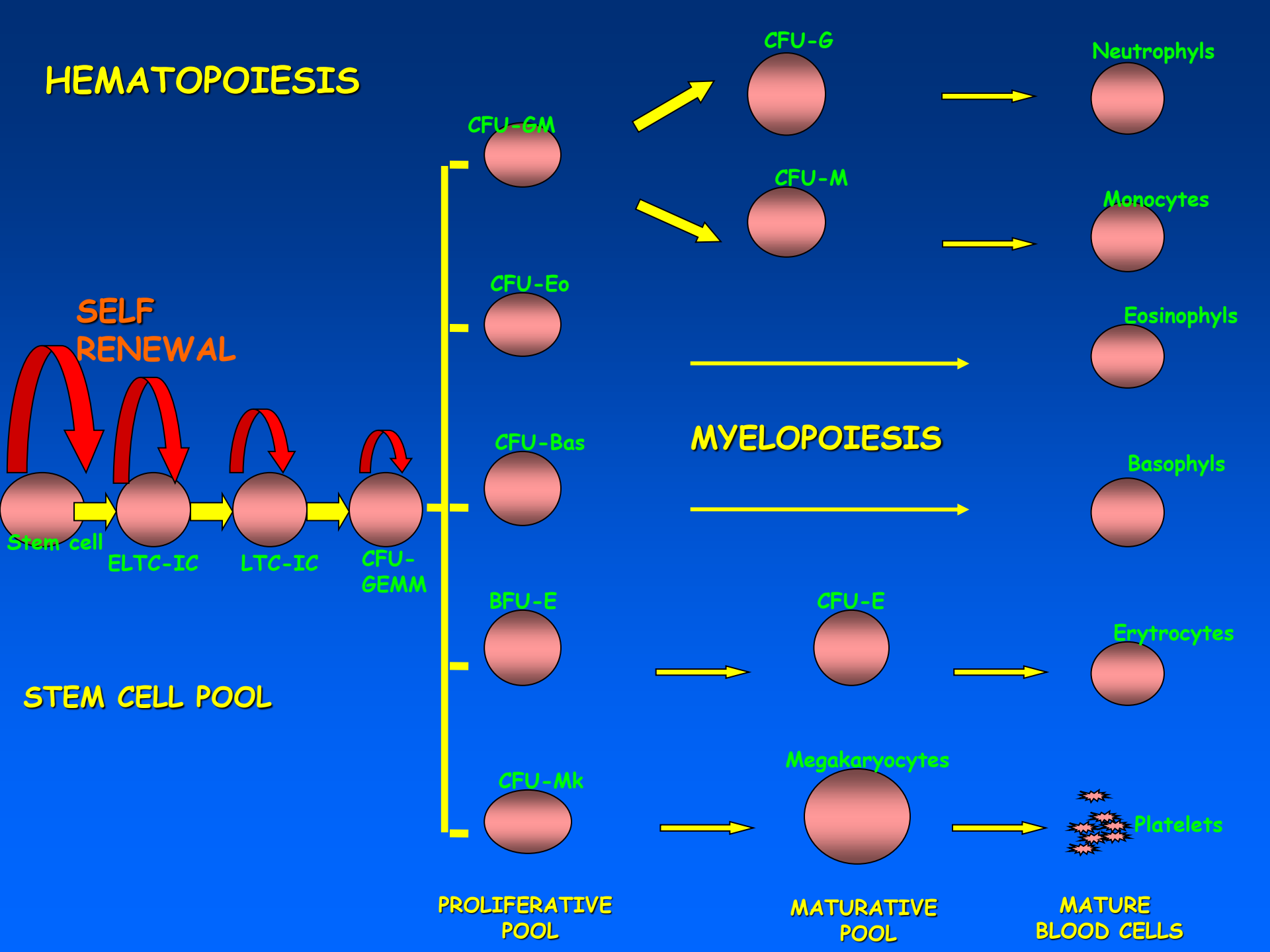


Fig. 1.70a–d Lymphocytes: (a, b) normal small lymphocytes are 7–12 μm in diameter with light blue scanty cytoplasm and a central round nucleus with a condensed amorphous chromatin pattern. (c, d) Some lymphocytes have diameters up to 20 μm, and even larger forms are found during viral and other infections.

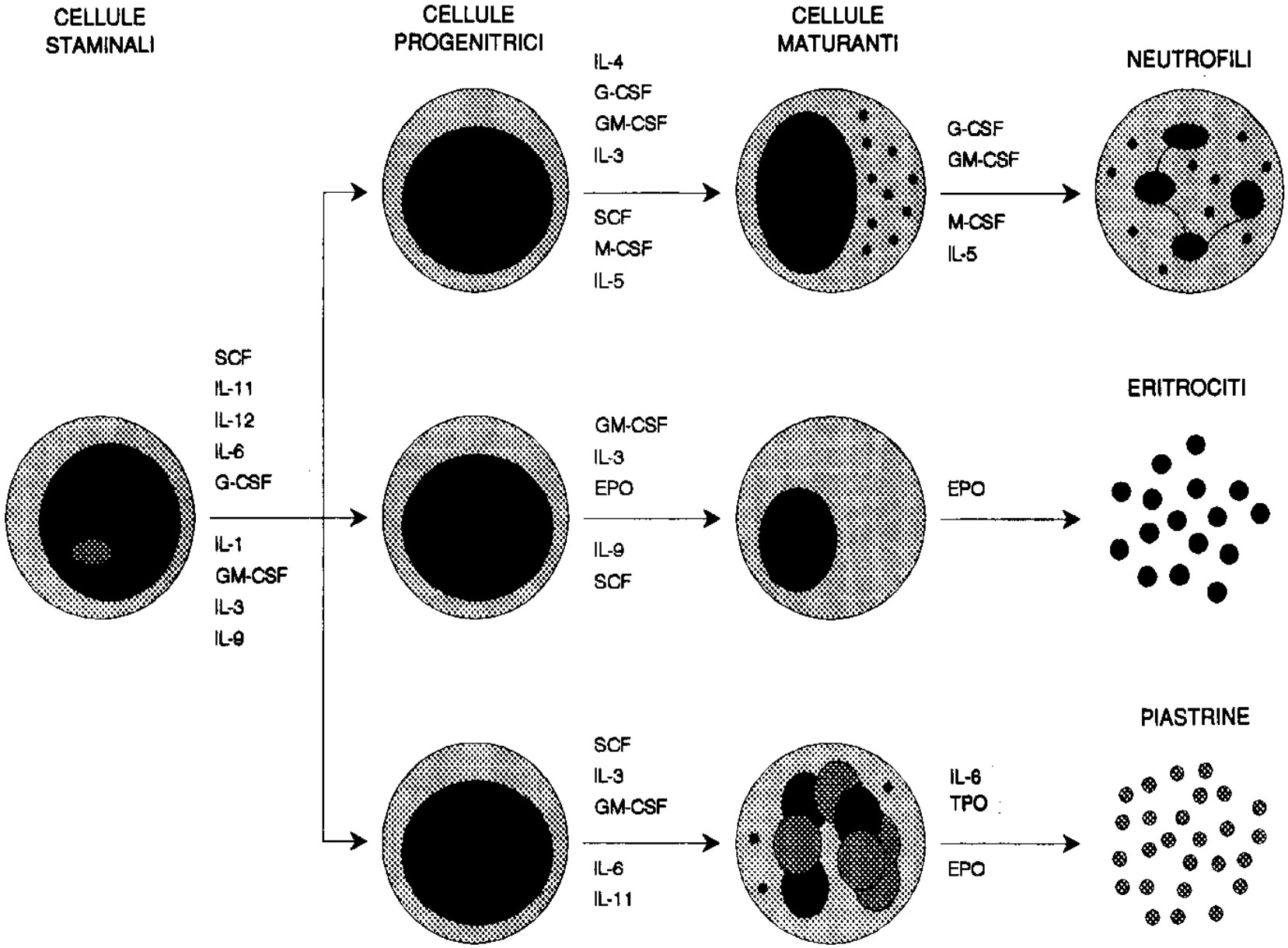
MONOCITI **LINFOCITI**

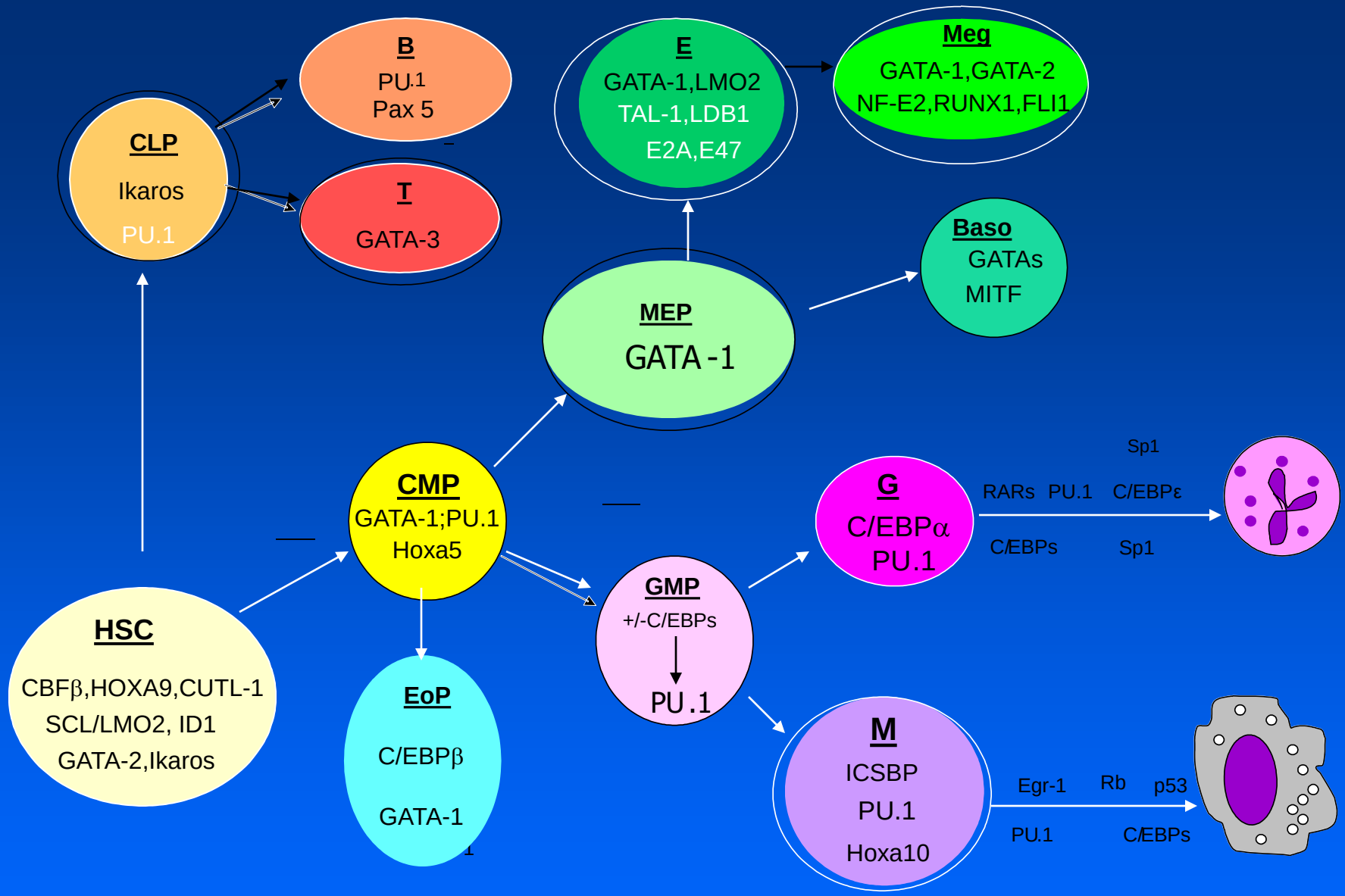
EMIVITA DELLE CELLULE DEL SANGUE

ERITROCITI	**120 giorni**
PIASTRINE	**4-5 giorni**
GRANULOCITI	**6-8 ore**
MONOCITI	**8 ore**
LINFOCITI	**VARIABILE**

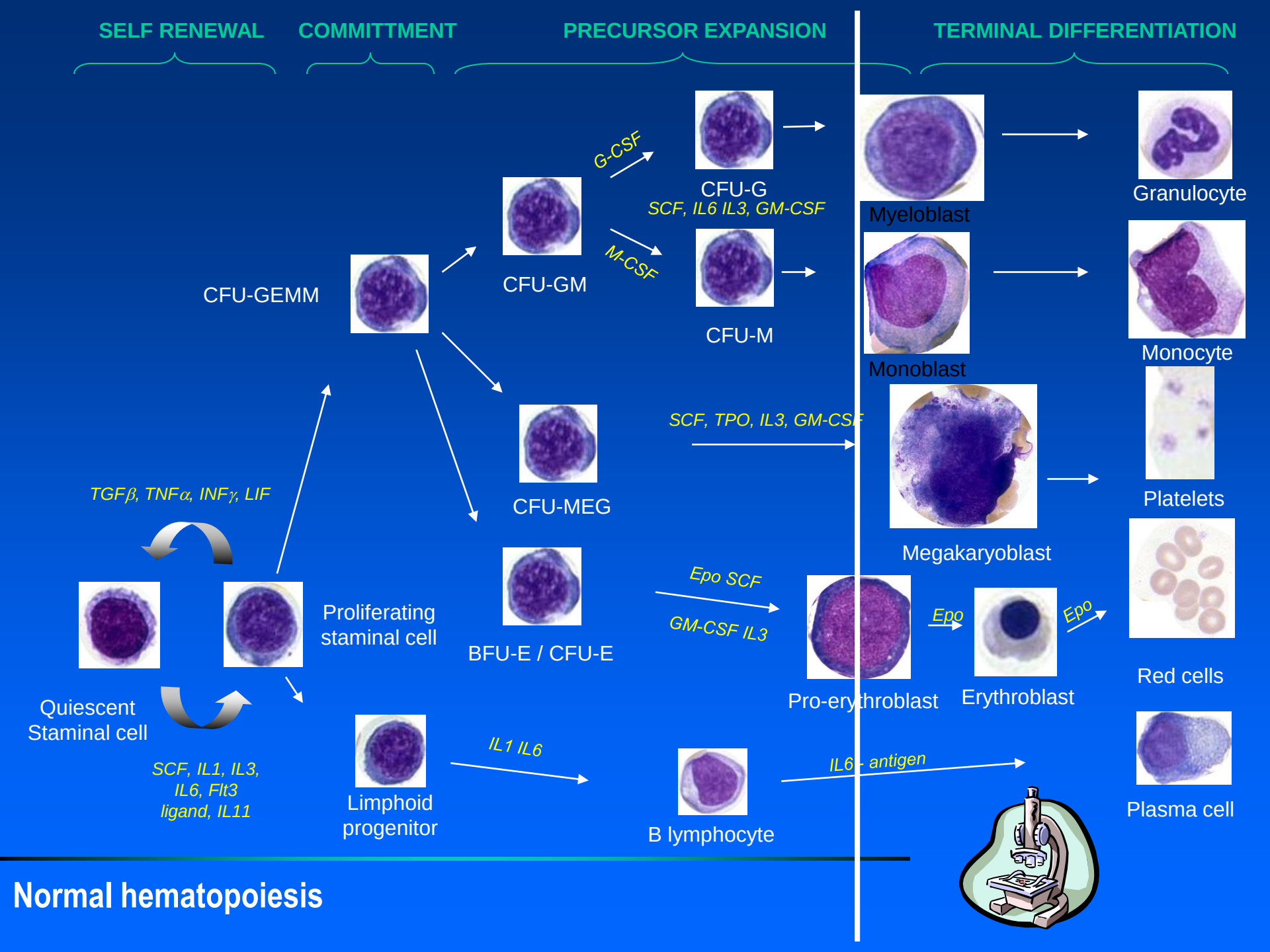

HEMATOPOIESIS
SELF RENEWAL
Stem cell
ELTC-IC
LTC-IC
CFU-GEMM
CFU-GM
CFU-G
CFU-M
CFU-Eo
CFU-Bas
BFU-E
CFU-Mk
CFU-E
Megakaryocytes
Neutrophyls
Monocytes
Eosinophyls
Basophyls
Erytrocytes
Platelets
MYELOPOIESIS
STEM CELL POOL
PROLIFERATIVE POOL
MATURATIVE POOL
MATURE BLOOD CELLS

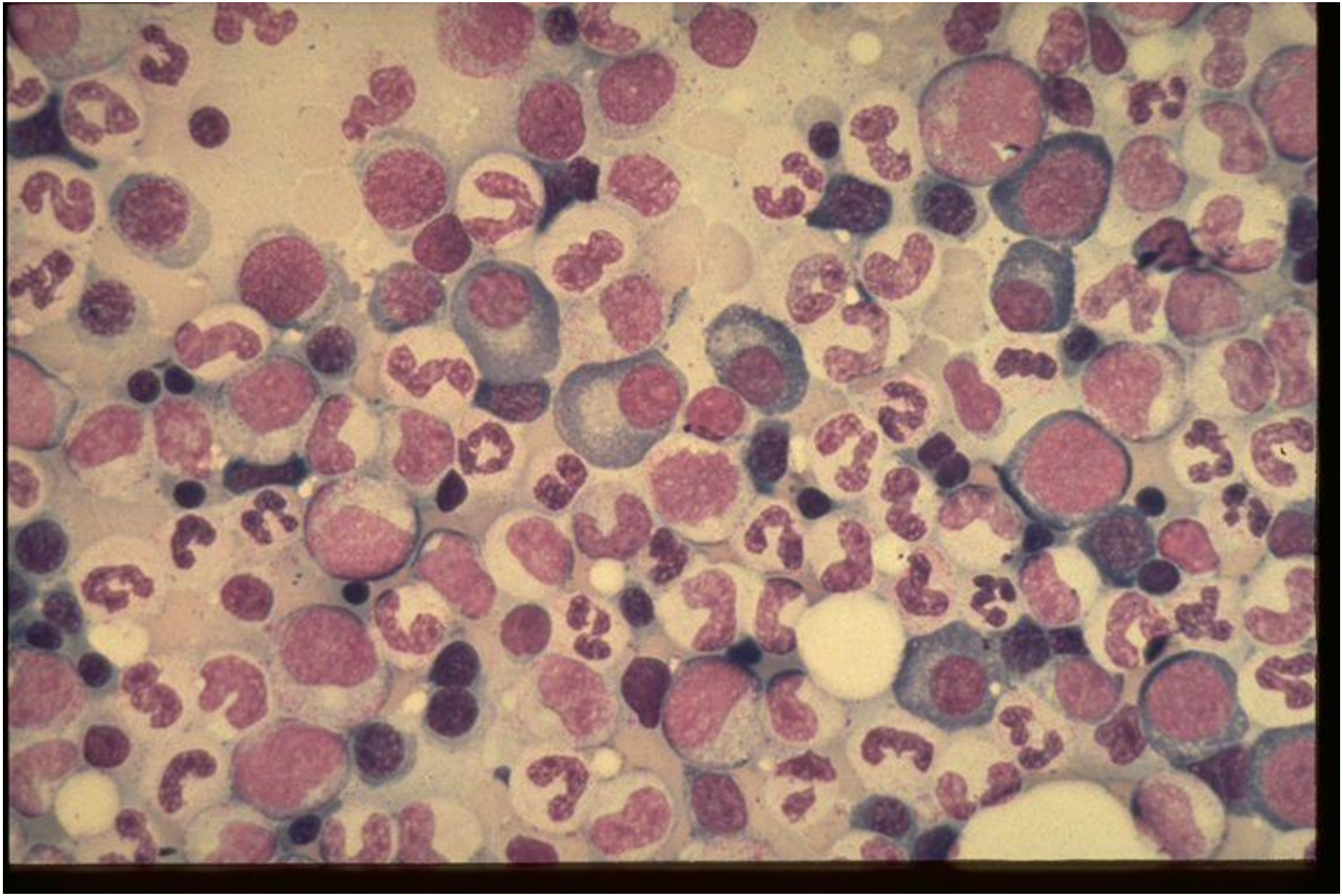
CYTOKINES REGULATION OF HAEMOPOIESIS





Fattori trascrizionali emopoietici


SELF RENEWAL
COMMITTMENT
PRECURSOR EXPANSION
TERMINAL DIFFERENTIATION
CFU-GEMM
CFU-GM
G-CSF
M-CSF
CFU-G
SCF, IL6 IL3, GM-CSF
CFU-M
Myeloblast
Granulocyte
Monoblast
Monocyte
CFU-MEG
SCF, TPO, IL3, GM-CSF
Megakaryoblast
Platelets
TGFβ, TNFα, INFγ, LIF
Proliferating staminal cell
BFU-E / CFU-E
Epo SCF
GM-CSF IL3
Pro-erythroblast
Epo
Erythroblast
Epo
Red cells
Quiescent Staminal cell
SCF, IL1, IL3, IL6, Flt3 ligand, IL11
Limphoid progenitor
IL1 IL6
B lymphocyte
IL6 - antigen
Plasma cell
Normal hematopoiesis



SELF RENEWAL | COMMITTMENT | PRECURSOR EXPANSION | TERMINAL DIFFERENTIATION

CFU-GEMM

CFU-GM

G-CSF

M-CSF

CFU-G

SCF, IL6 IL3, GM-CSF

Myeloblast

Granulocyte

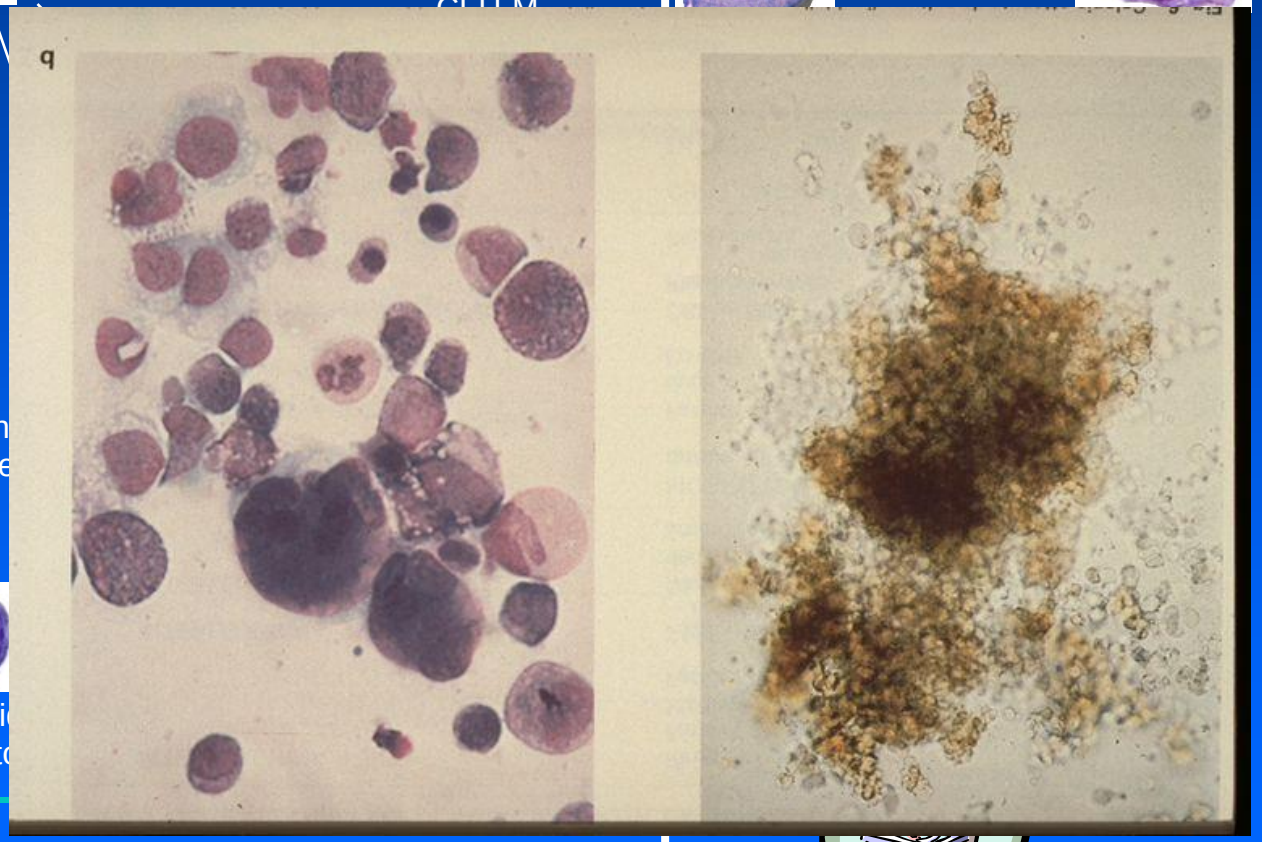
TGFβ, TNFα, INFγ, LIF

Quiescent
Staminal cell

SCF, IL1, IL3,
IL6, Flt3
ligand, IL11

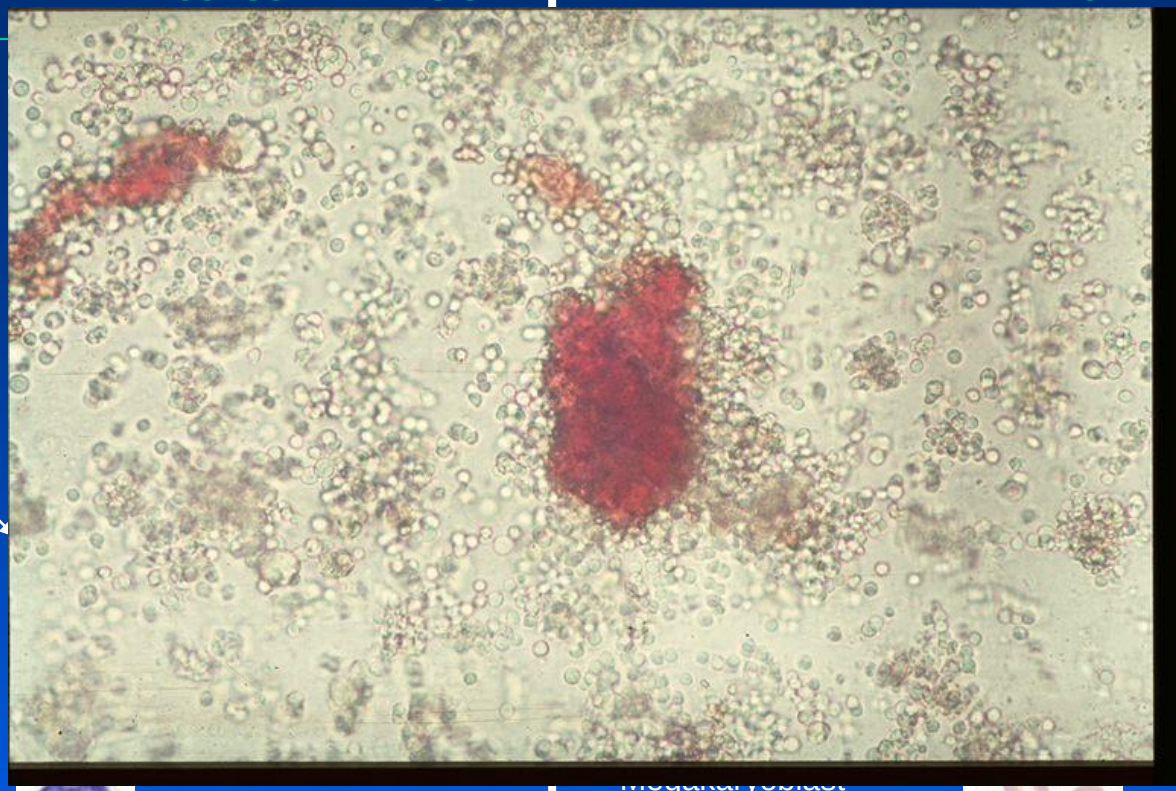
Proliferatin
staminal ce

Limphoi
progenito



Normal hematopoiesis

SELF RENEWAL | COMMITTMENT | PRECURSOR EXPANSION | TERMINAL DIFFERENTIATION



CFU-GEMM

TGFβ, TNFα, INFγ, LIF

Quiescent Staminal cell

Proliferating staminal cell

SCF, IL1, IL3, IL6, Flt3 ligand, IL11

Limphoid progenitor

IL1 IL6

B lymphocyte

IL6 - antigen

Plasma cell

BFU-E / CFU-E

Epo SCF

GM-CSF IL3

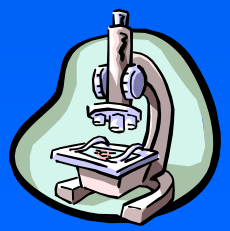
Pro-erythroblast

Epo

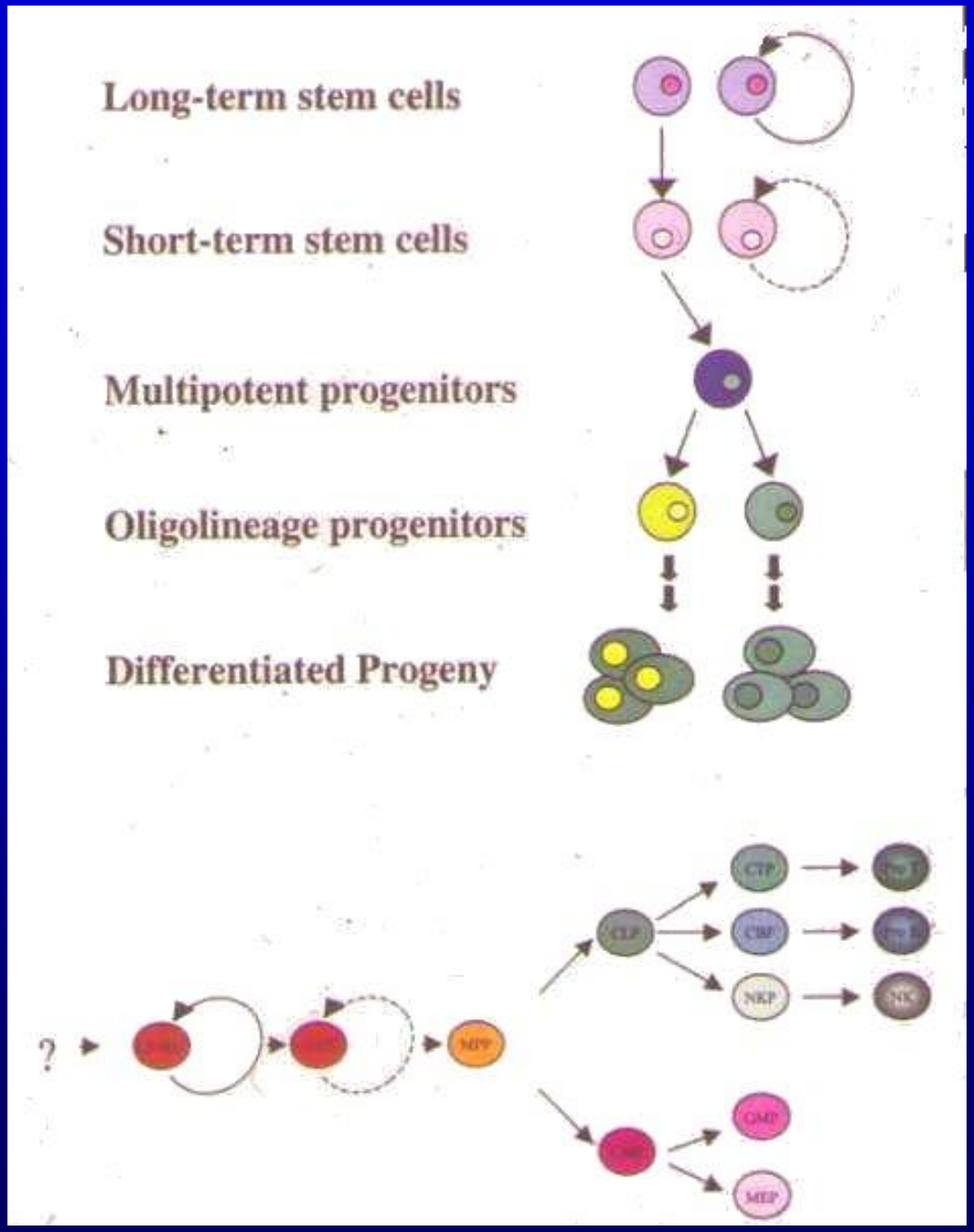
Erythroblast

Epo

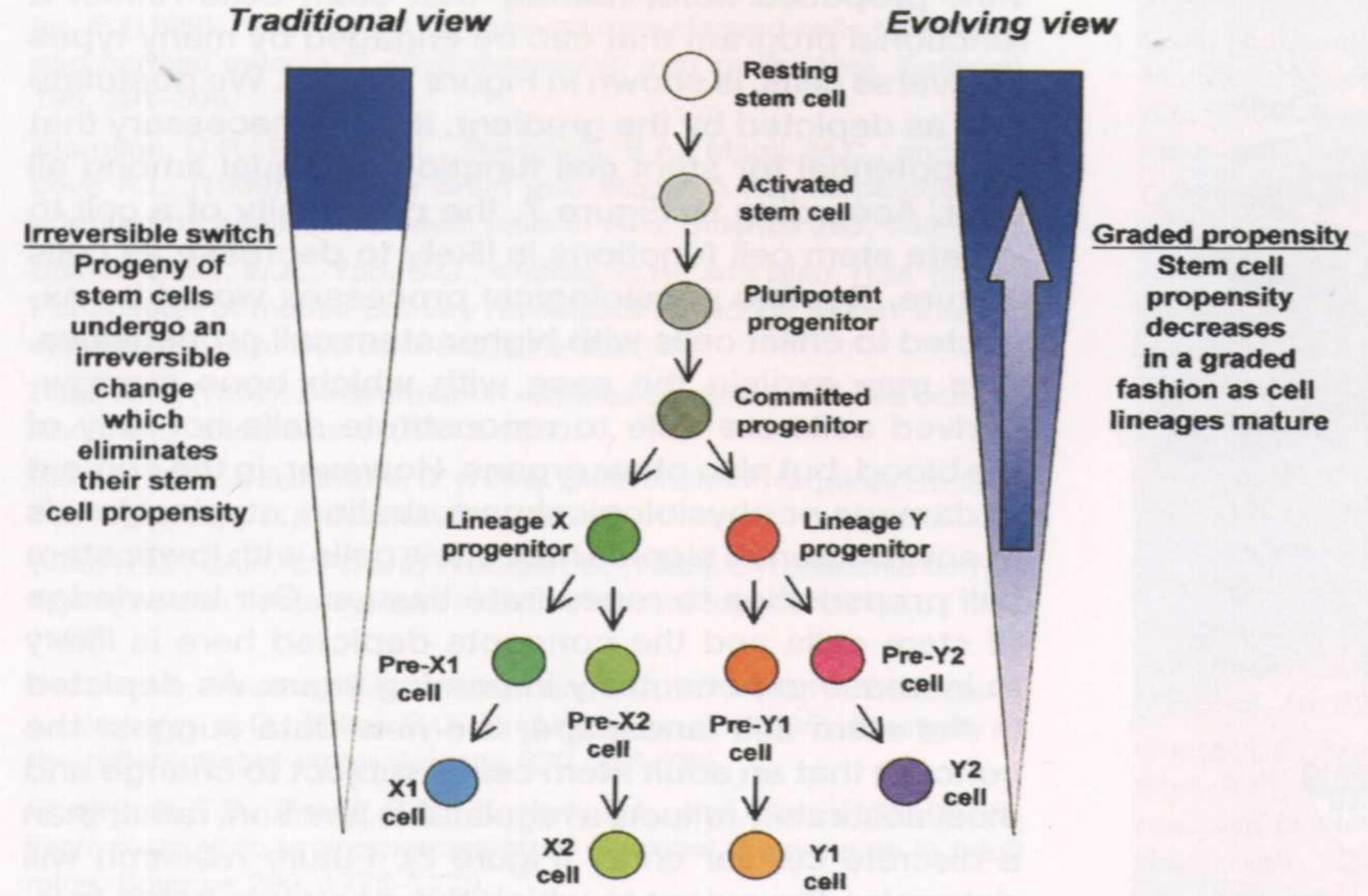
Red cells

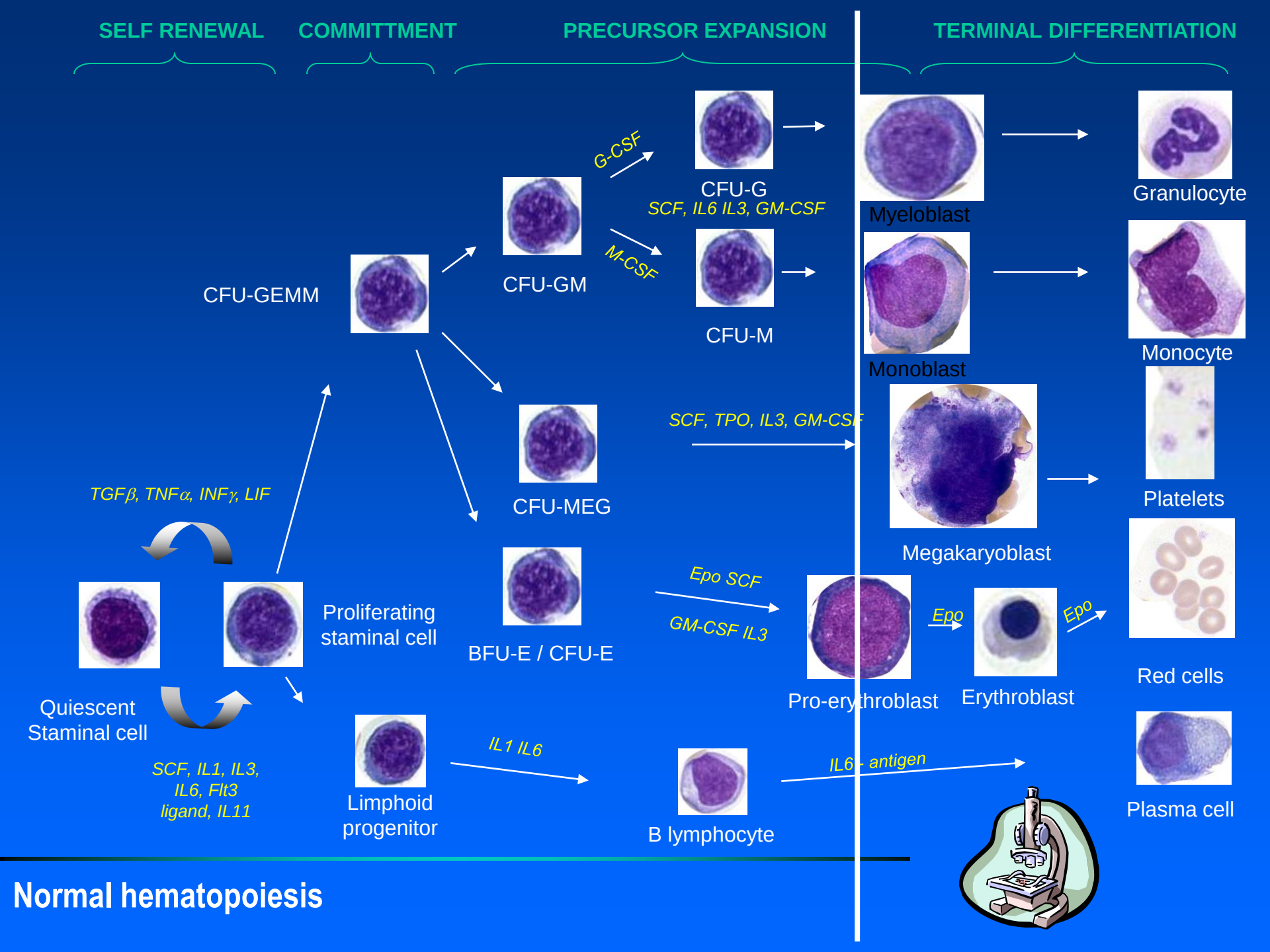


Normal hematopoiesis


Long-term stem cells
Short-term stem cells
Multipotent progenitors
Oligolineage progenitors
Differentiated Progeny
?
MPP
CLP
CMP
MEP

STEM CELL POTENTIALS




SELF RENEWAL
COMMITTMENT
PRECURSOR EXPANSION
TERMINAL DIFFERENTIATION
CFU-GEMM
CFU-GM
G-CSF
M-CSF
CFU-G
SCF, IL6 IL3, GM-CSF
CFU-M
Myeloblast
Granulocyte
Monoblast
Monocyte
CFU-MEG
SCF, TPO, IL3, GM-CSF
Megakaryoblast
Platelets
TGFβ, TNFα, INFγ, LIF
Proliferating staminal cell
BFU-E / CFU-E
Epo SCF
GM-CSF IL3
Pro-erythroblast
Epo
Erythroblast
Epo
Red cells
Quiescent Staminal cell
SCF, IL1, IL3, IL6, Flt3 ligand, IL11
Limphoid progenitor
IL1 IL6
B lymphocyte
IL6 - antigen
Plasma cell
Normal hematopoiesis

LINFOCITOPOIESI

2. HEMATOLOGY PASSPORT 2019 — 2022

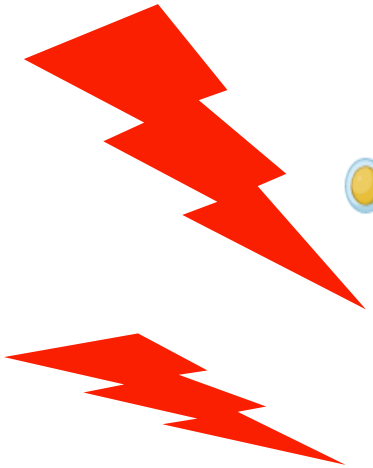
ROMA 20-21 SETTEMBRE 2019

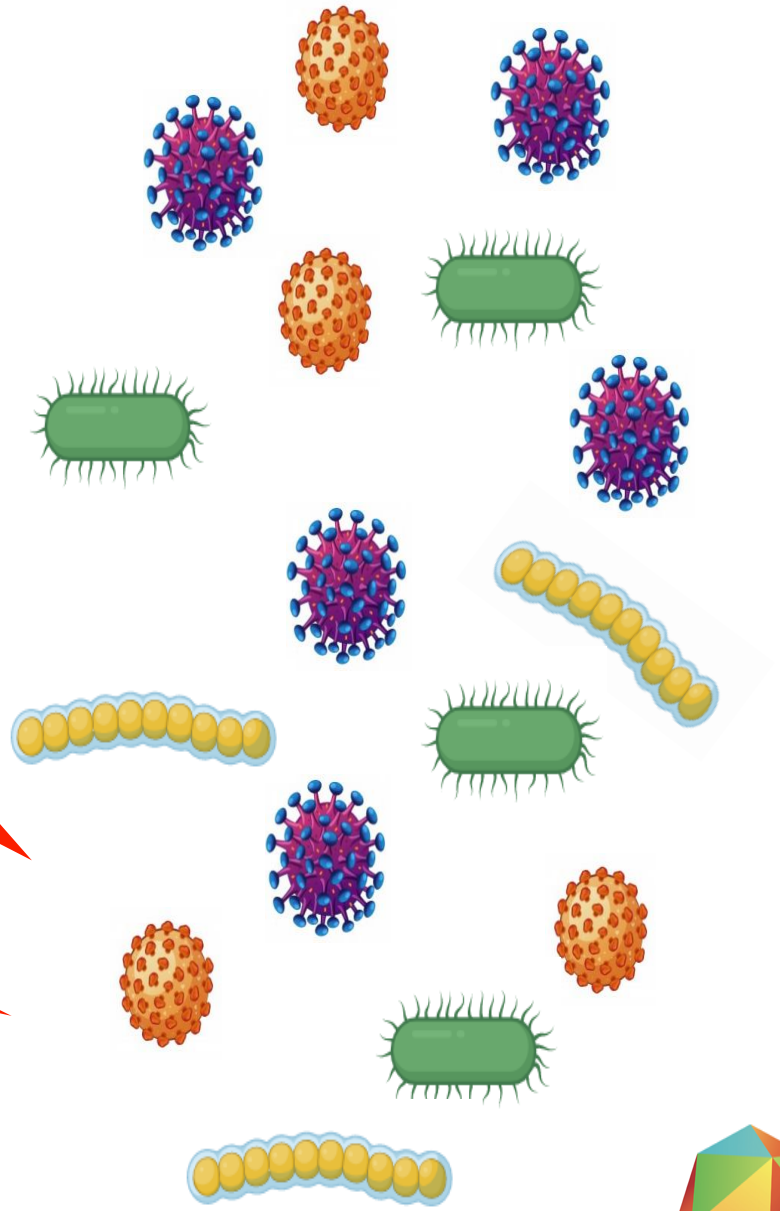
MALATTIE LINFOPROLIFERATIVE CRONICHE E MIELOMA

Adaptive immune system

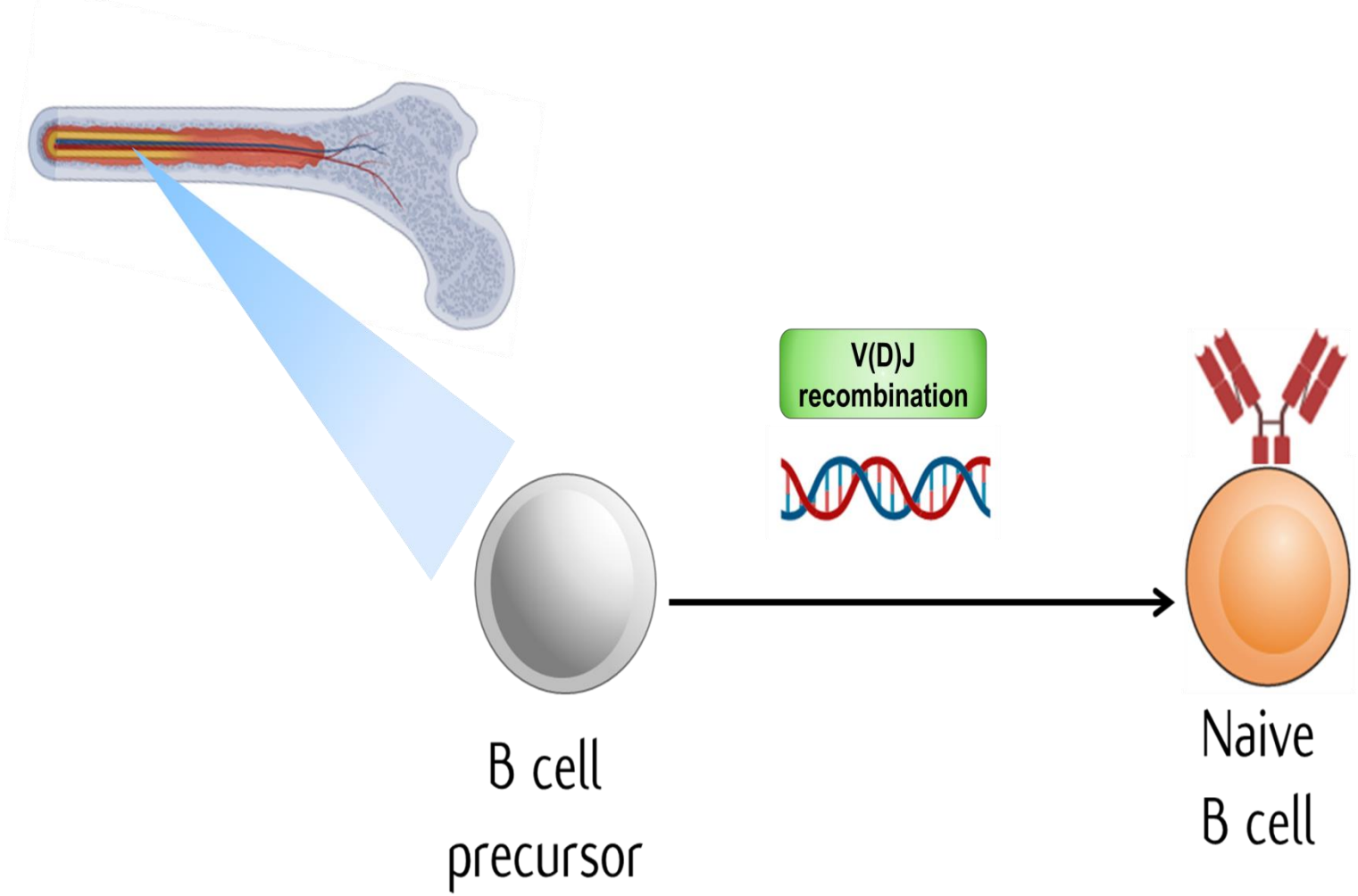
T-cell

B-cell

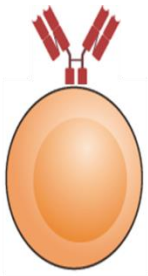




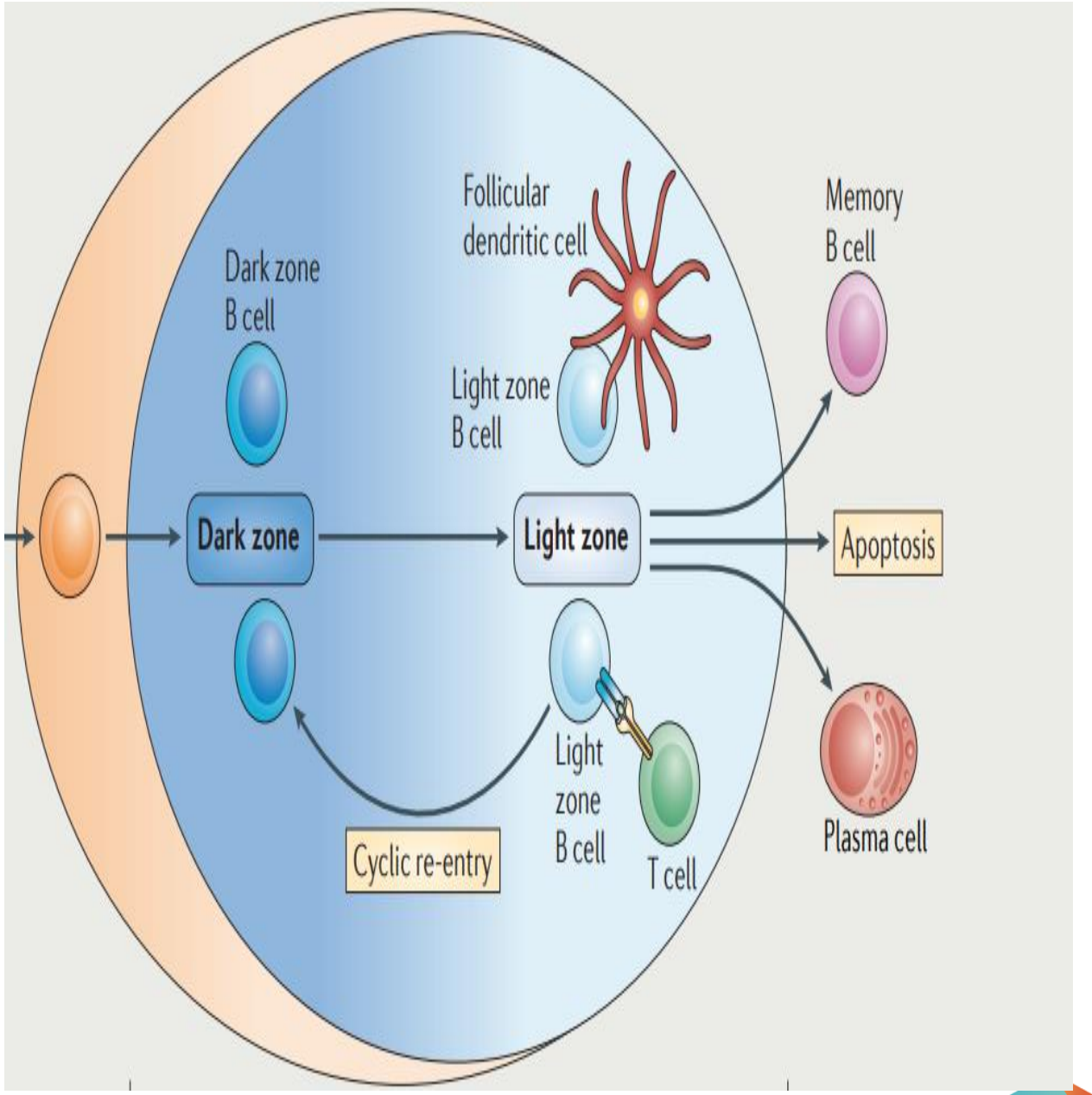
Immunoglobulin genes: V(D)J recombination



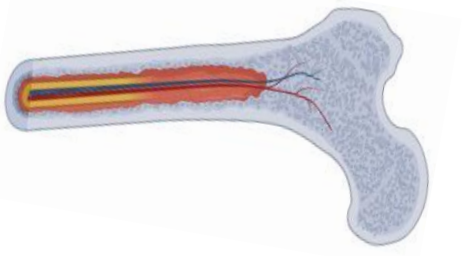
Küppers et al N Engl J Med 1999; 341:1520-1529



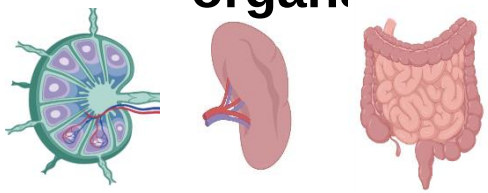
Naive
B cell


Follicular
dendritic cell
Memory
B cell
Dark zone
B cell
Light zone
B cell
Dark zone
Light zone
Apoptosis
Light
zone
B cell
T cell
Plasma cell
Cyclic re-entry

Bone marrow

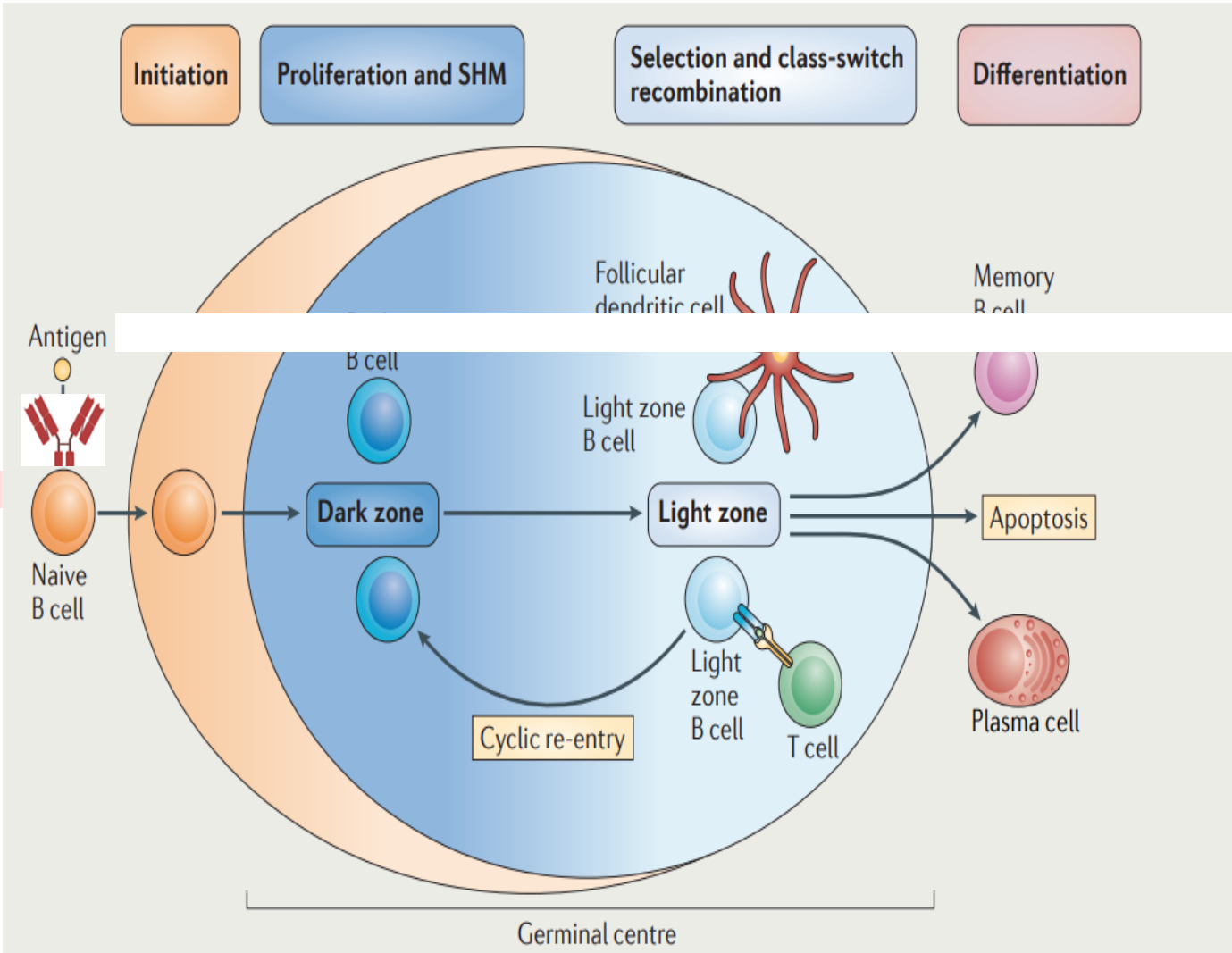


Secondary lymphoid organs



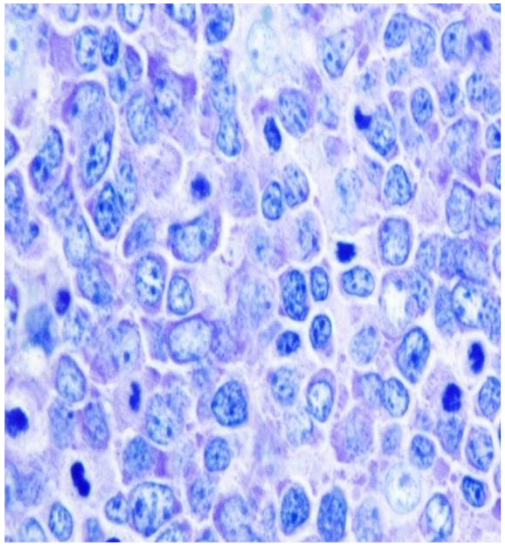
V(D)J recombination

B cell precursor



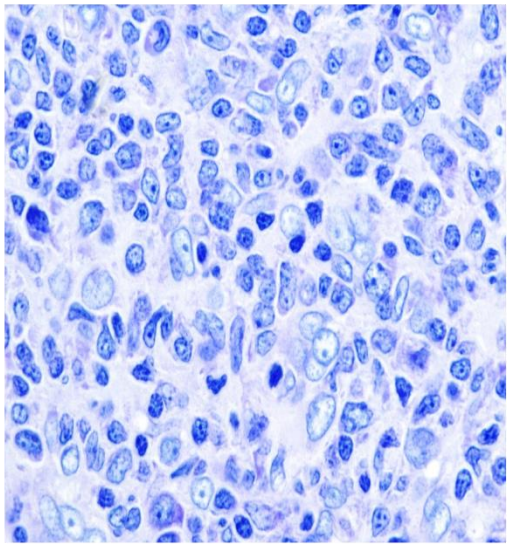
Adapted from Basso & Dalla-Favera,
Nat Rev Immunol 15, 172-184 (2015)

Dark zone



- Dark-zone B-cells (centroblasts)

Light zone

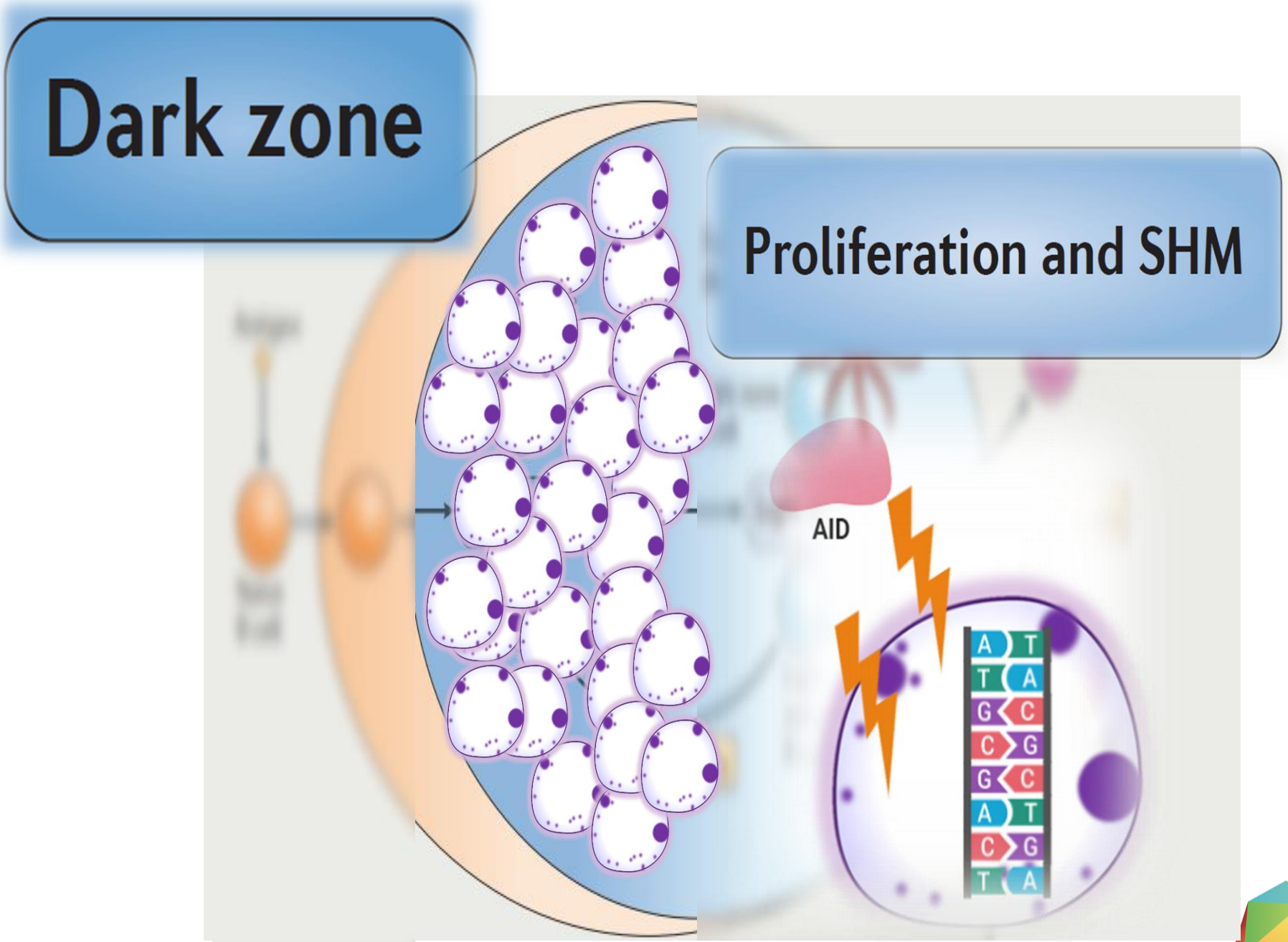


- Light-zone B-cells (centrocytes)

- T-cells

- Follicular dendritic cell

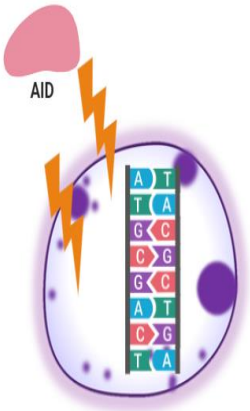
- Macrophages

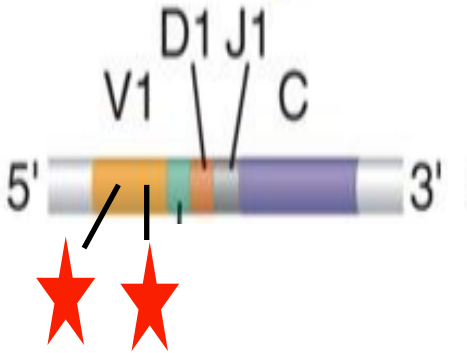


Victora & Nussenzweig. Annu. Rev. Immunol. 30, 429–457 (2012)

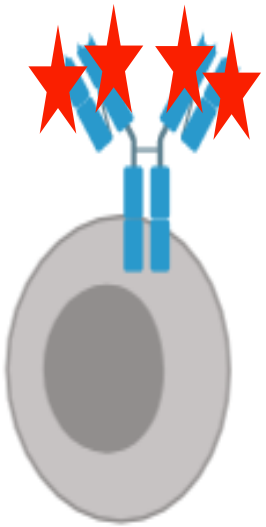
Somatic hypermutation

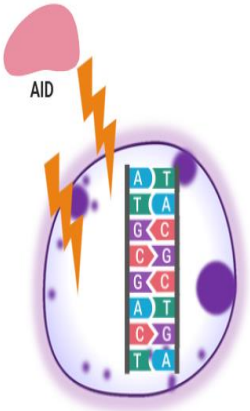
Victora & Nussenzweig. Annu. Rev. Immunol. 30, 429–457 (2012)

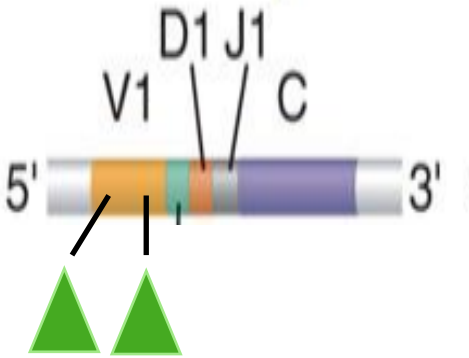




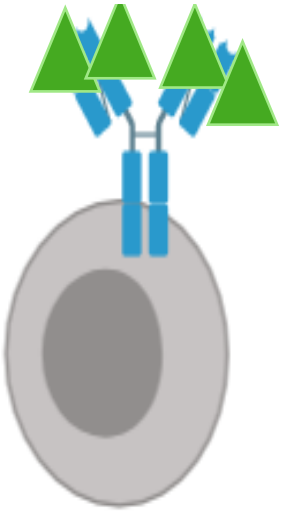
Variable region of the IG genes



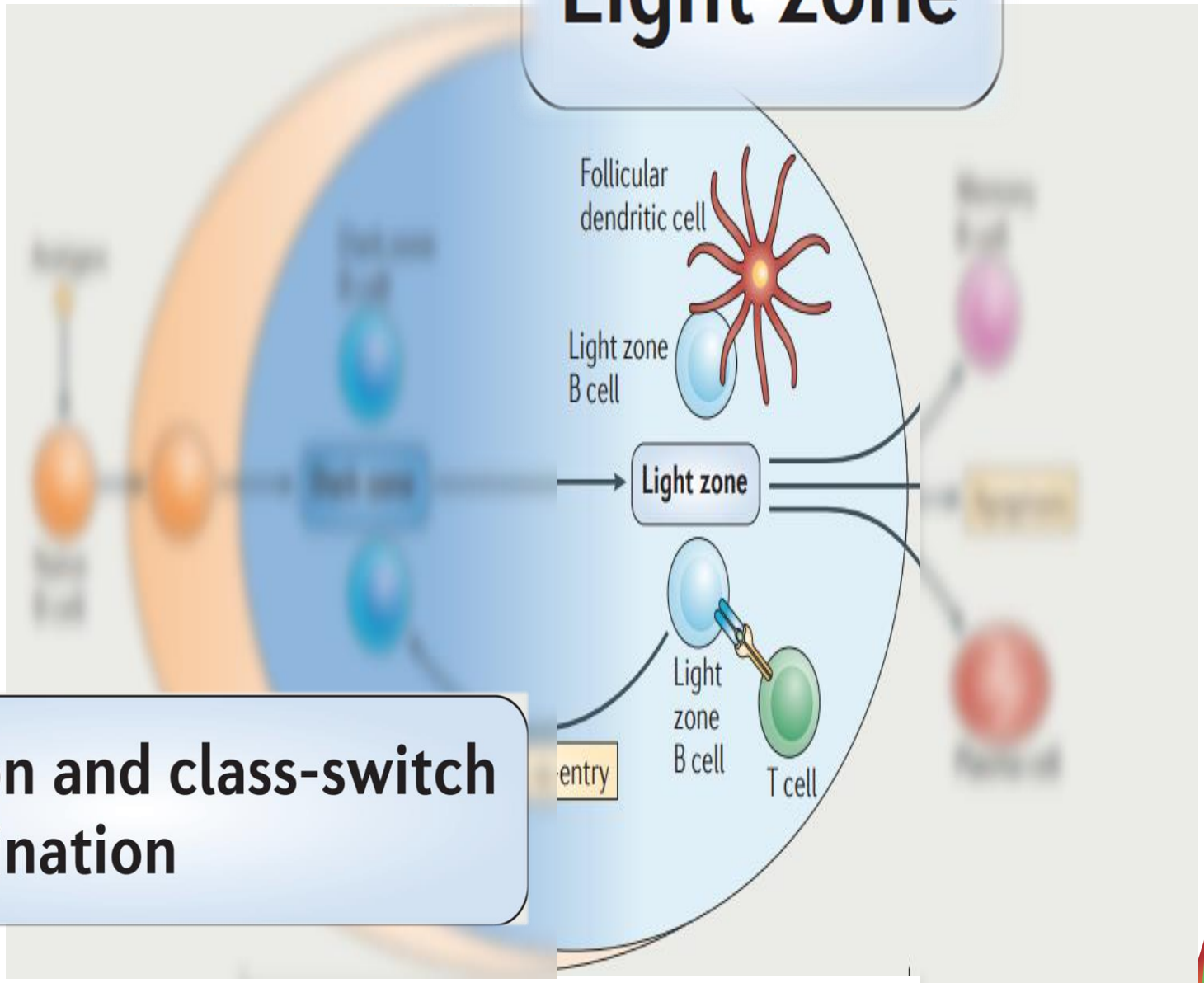




Variable region of the IG genes

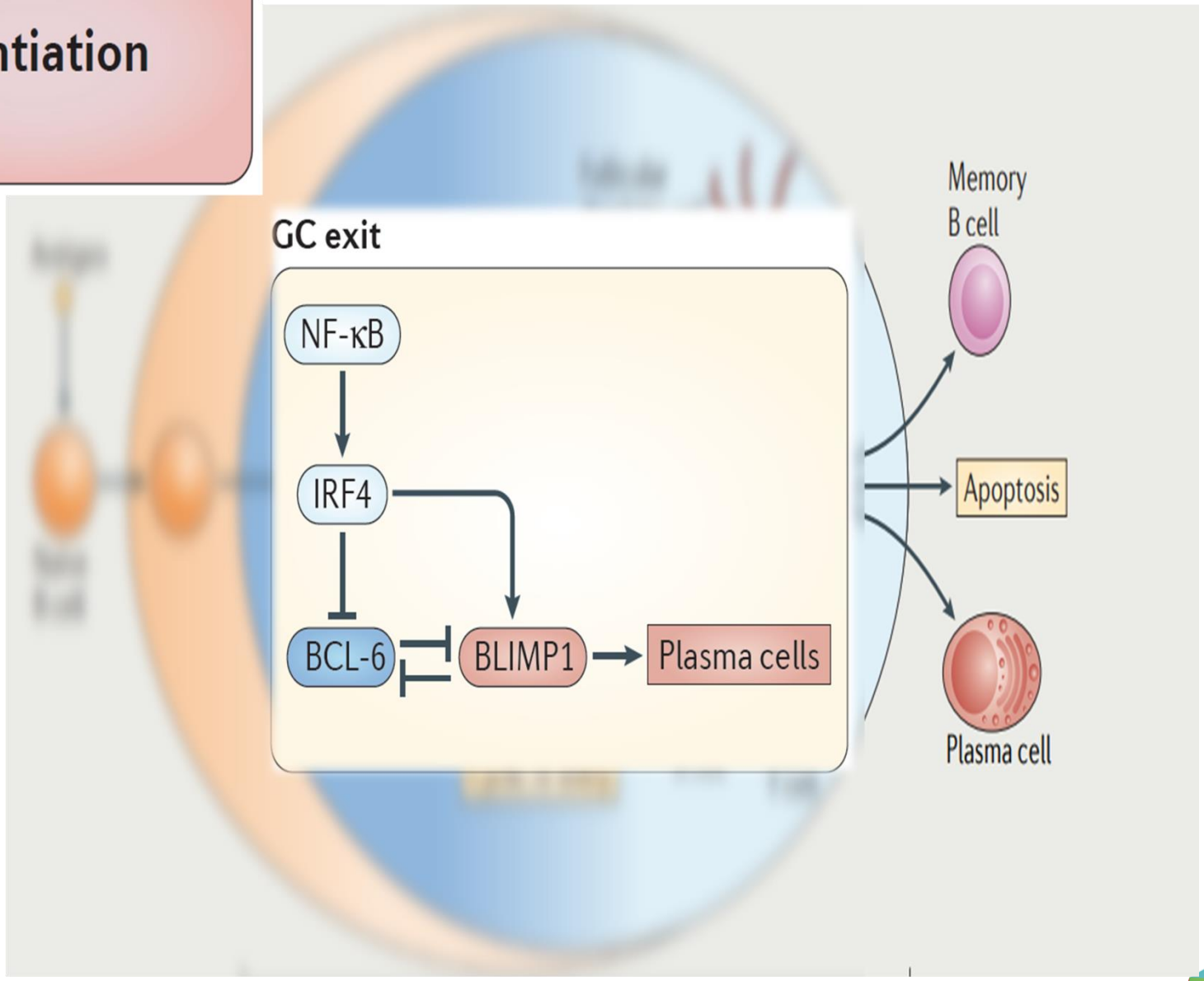


Light zone

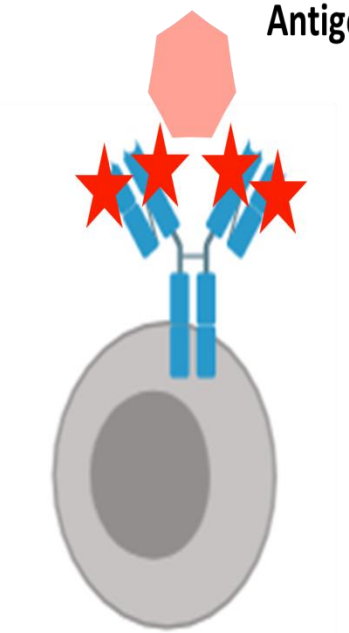


Selection and class-switch recombination

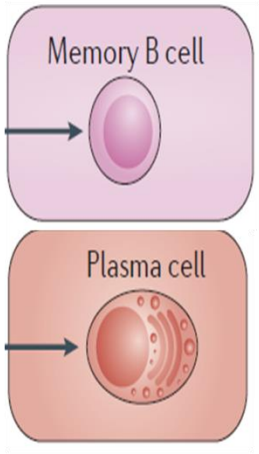
Differentiation

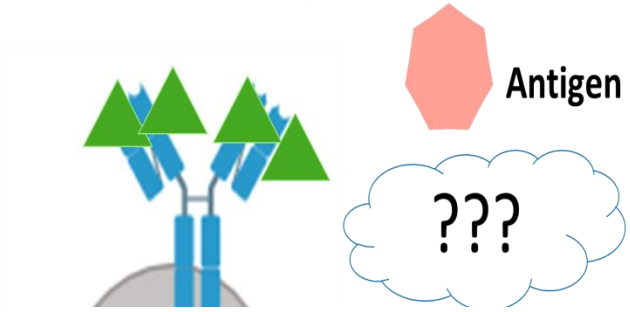


van Galen, J. C. *et al. Eur. J. Immunol.* 34, 1870–1881 (2004)

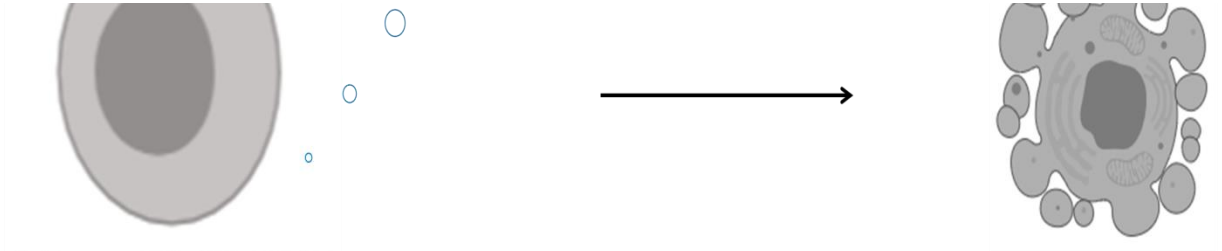


Selection



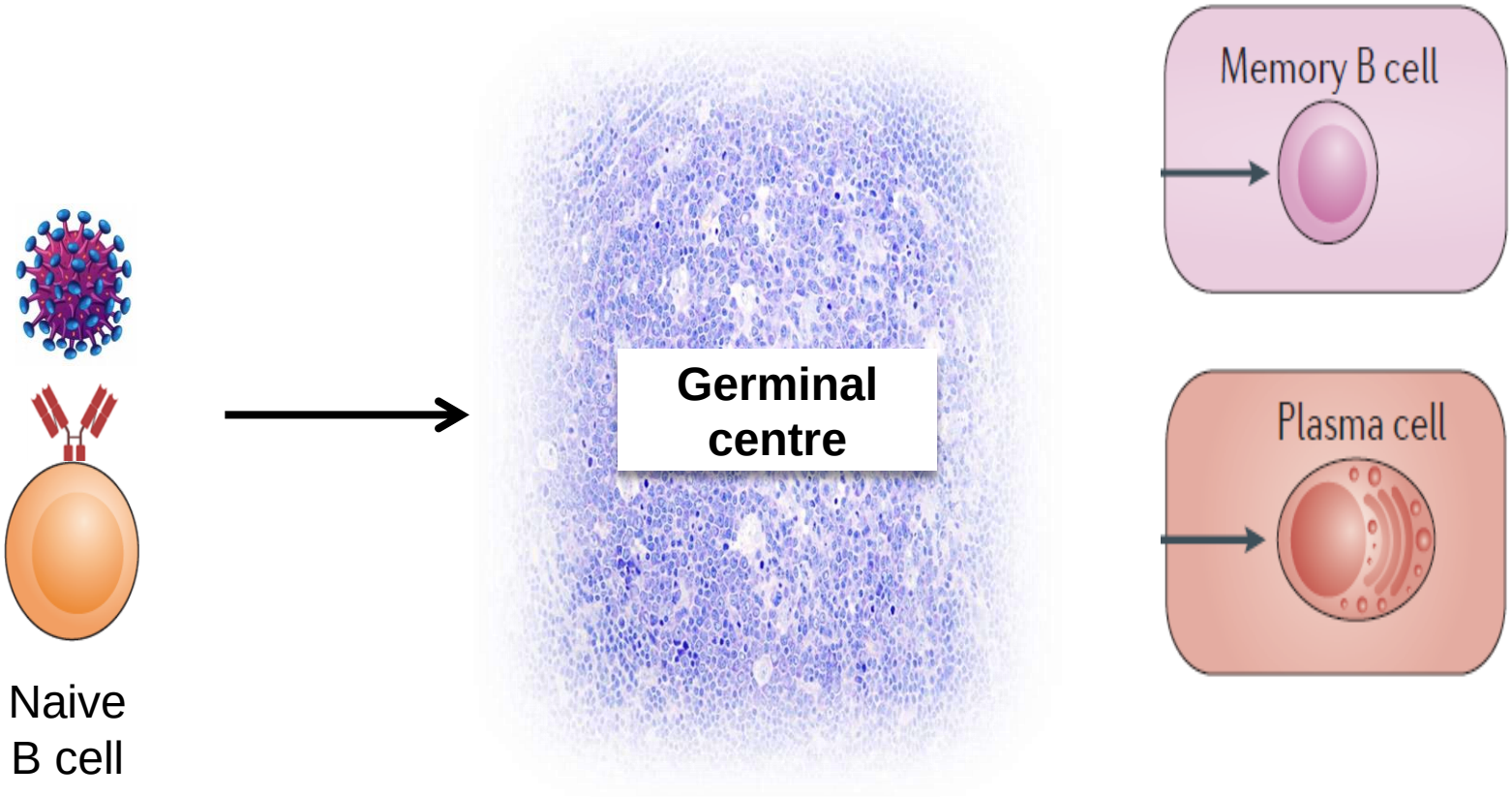


Apoptosis

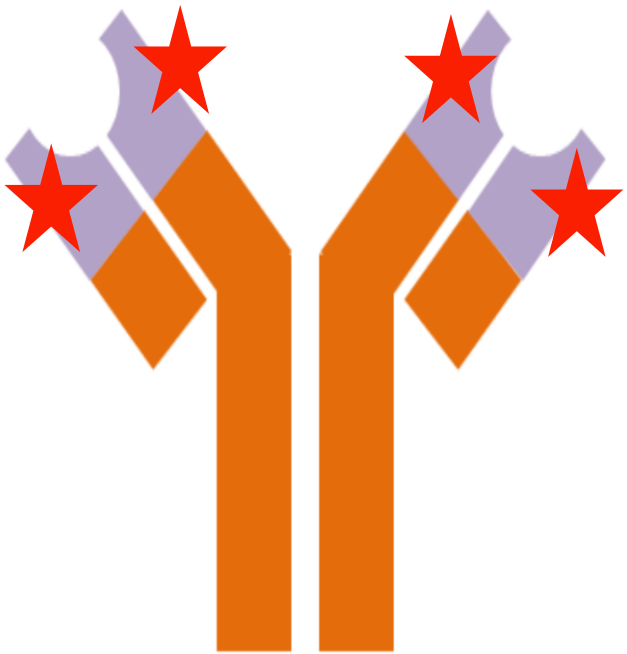


Gitlin et al. *Nature*. 509:637-640 (2014)
Muramatsu et al. *Cell*. 102:553-563 (2000)

Germinal centres: B-cell selection and differentiation



Immunoglobulin structure

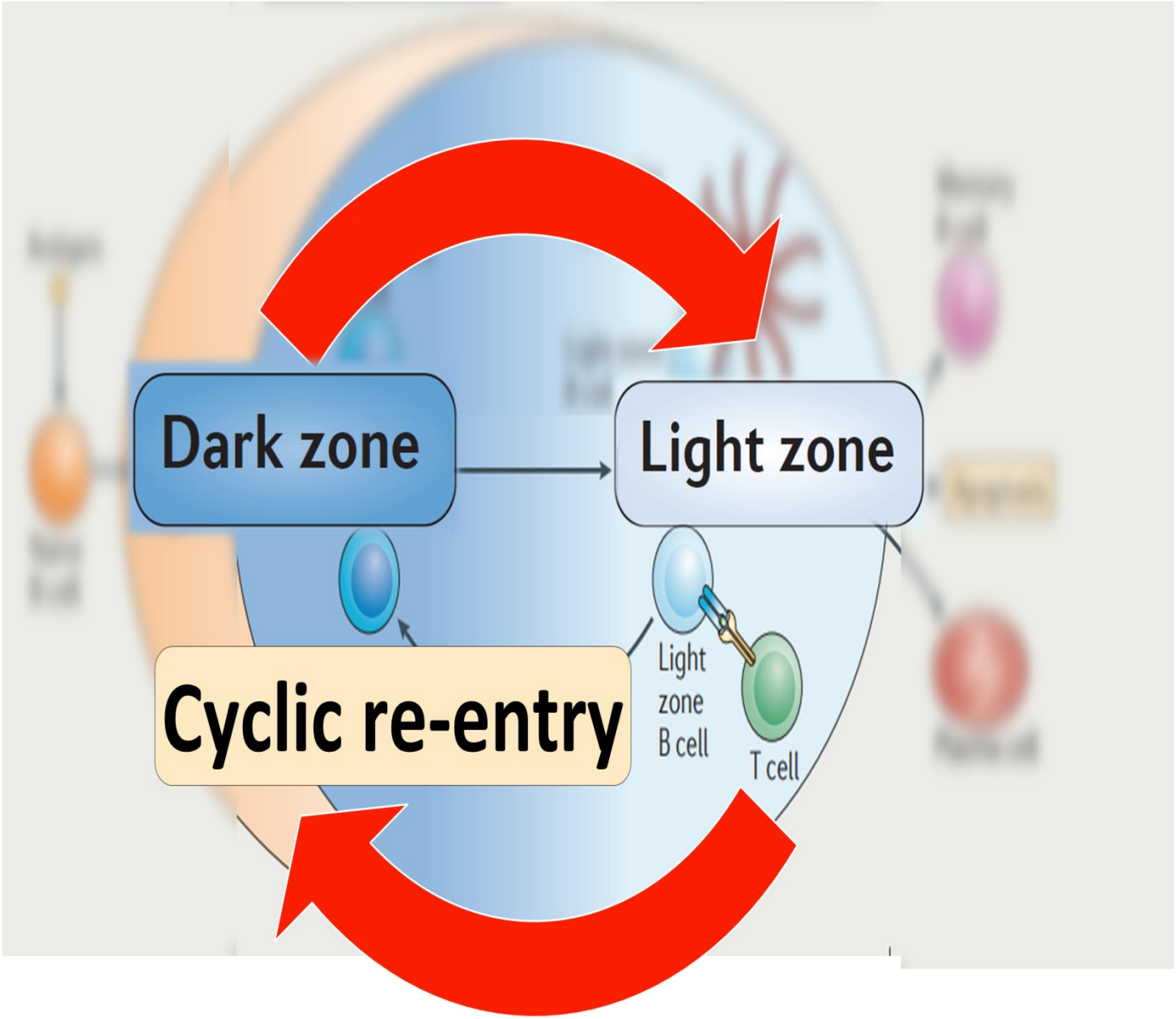


= Fv (Variable Fragment)

= Fc (Constant Fragment)

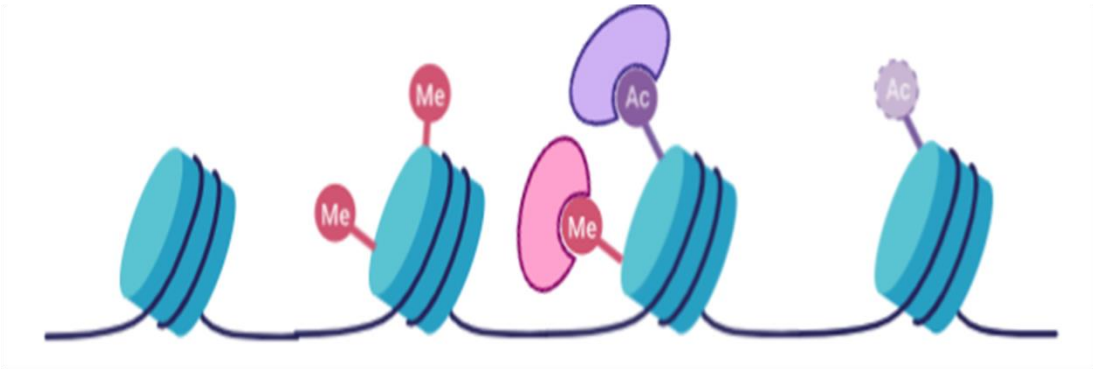
Küppers et al N Engl J Med 1999; 341:1520-1529

Hauser et al. *Immunity*. 26:655-667 (2007)



Cyclic re-entry

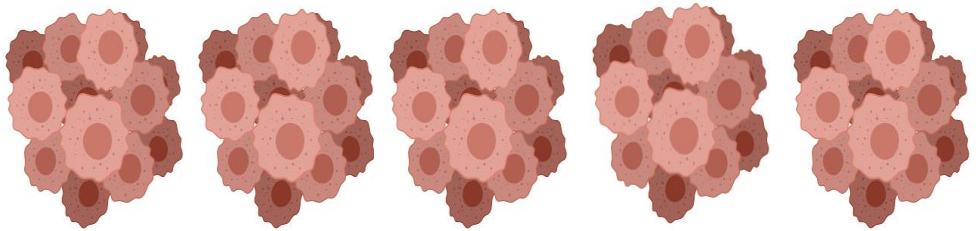
Epigenetic plasticity

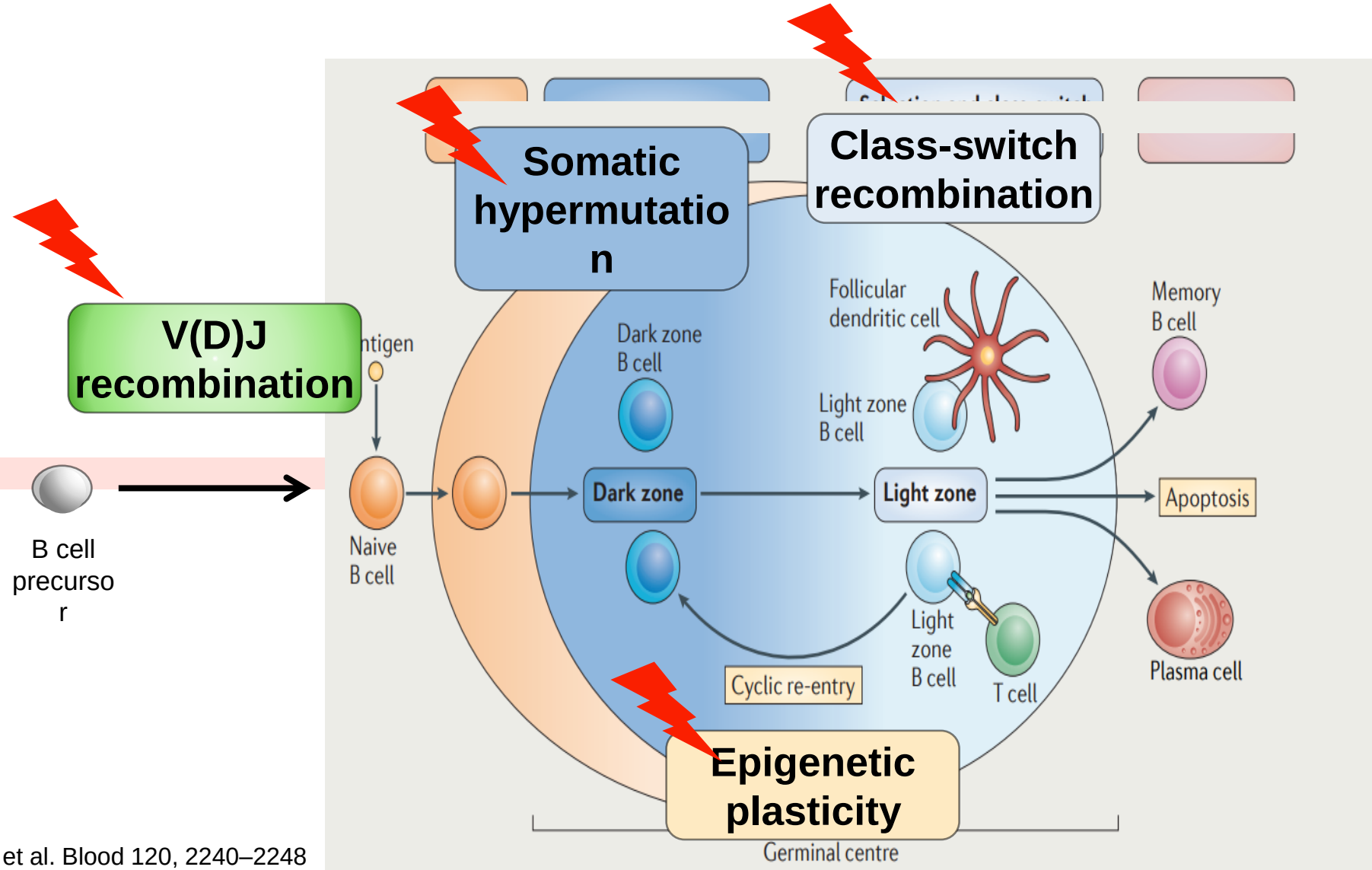


- methyltransferases (MLL2, EZH2)
- acetyltransferases (CREBBP, EP300)

van Galen, J. C. *et al. Eur. J. Immunol.* 34, 1870–1881 (2004)

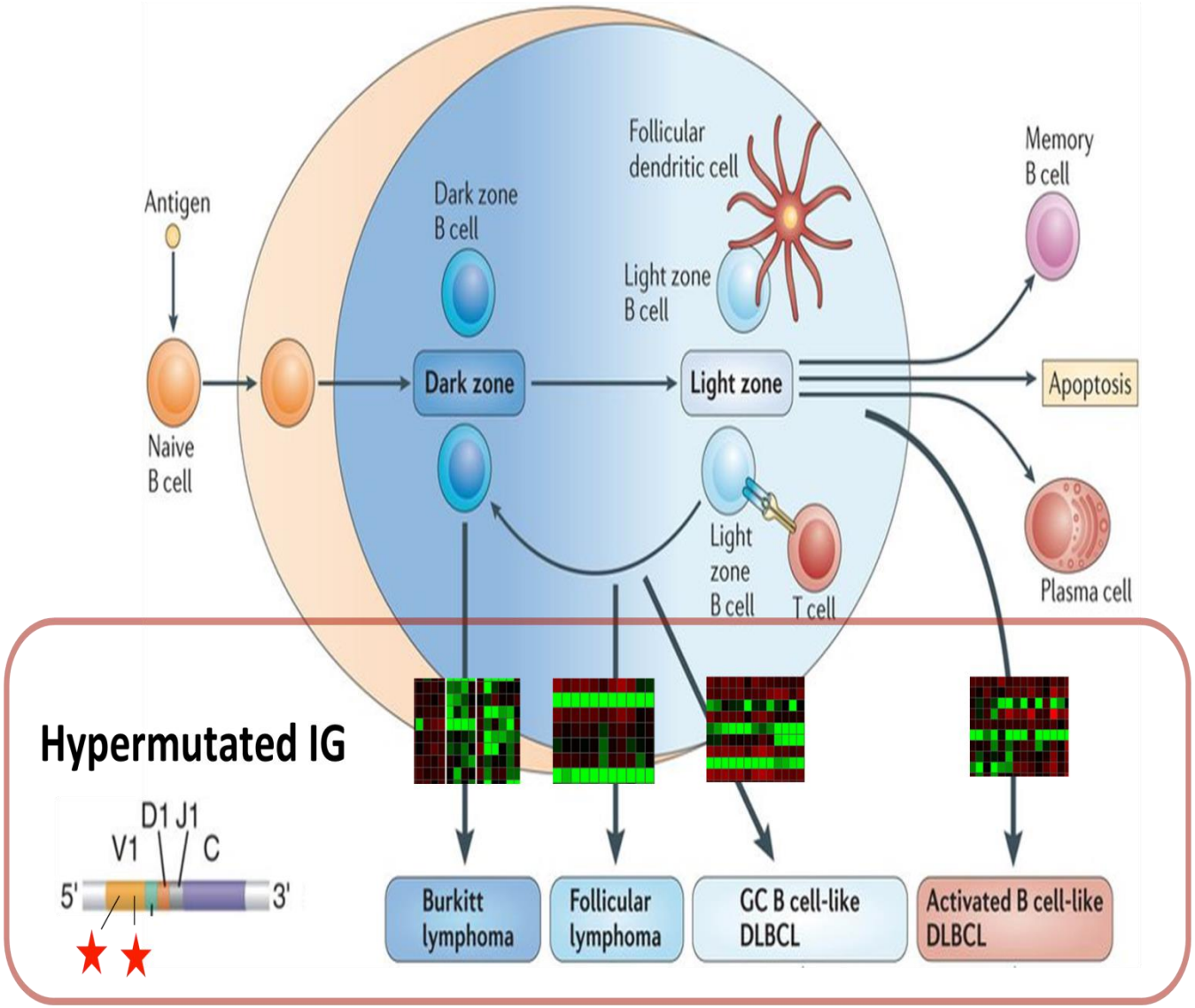
A dangerous downside…





Victora et al. Blood 120, 2240–2248 (2012)

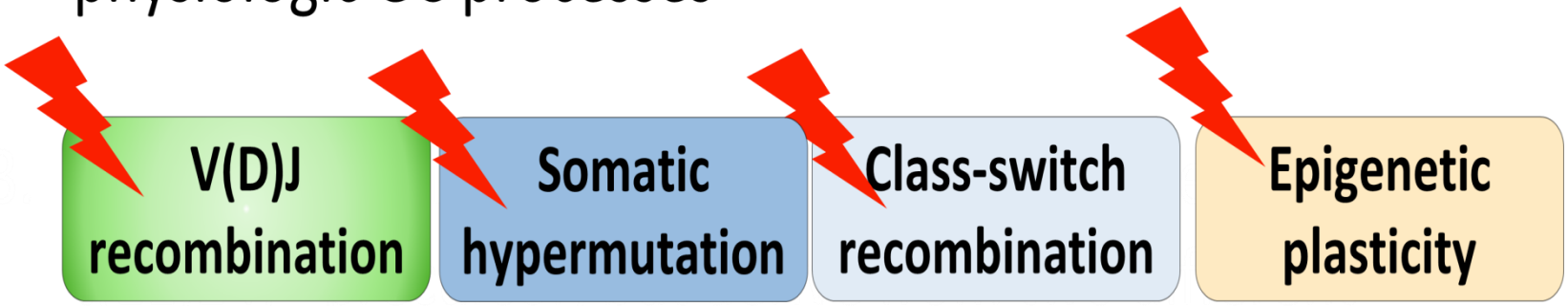
Germinal centre-derived lymphomas



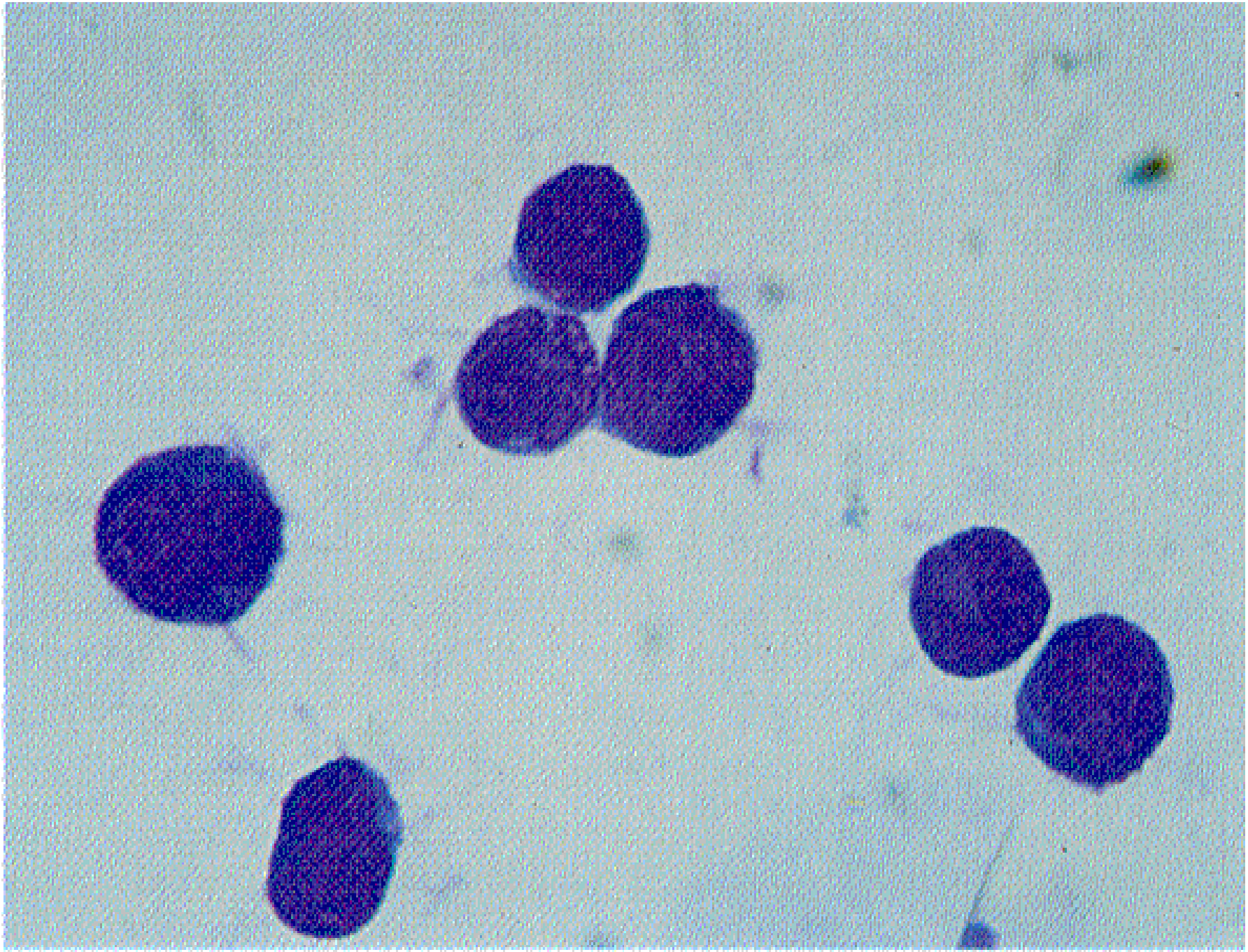
Küppers et al N Engl J Med 1999; 341:1520-1529

Key Messages

2. The genetic mechanisms involved in FL and DLBCL development are intimately connected to the physiologic GC processes



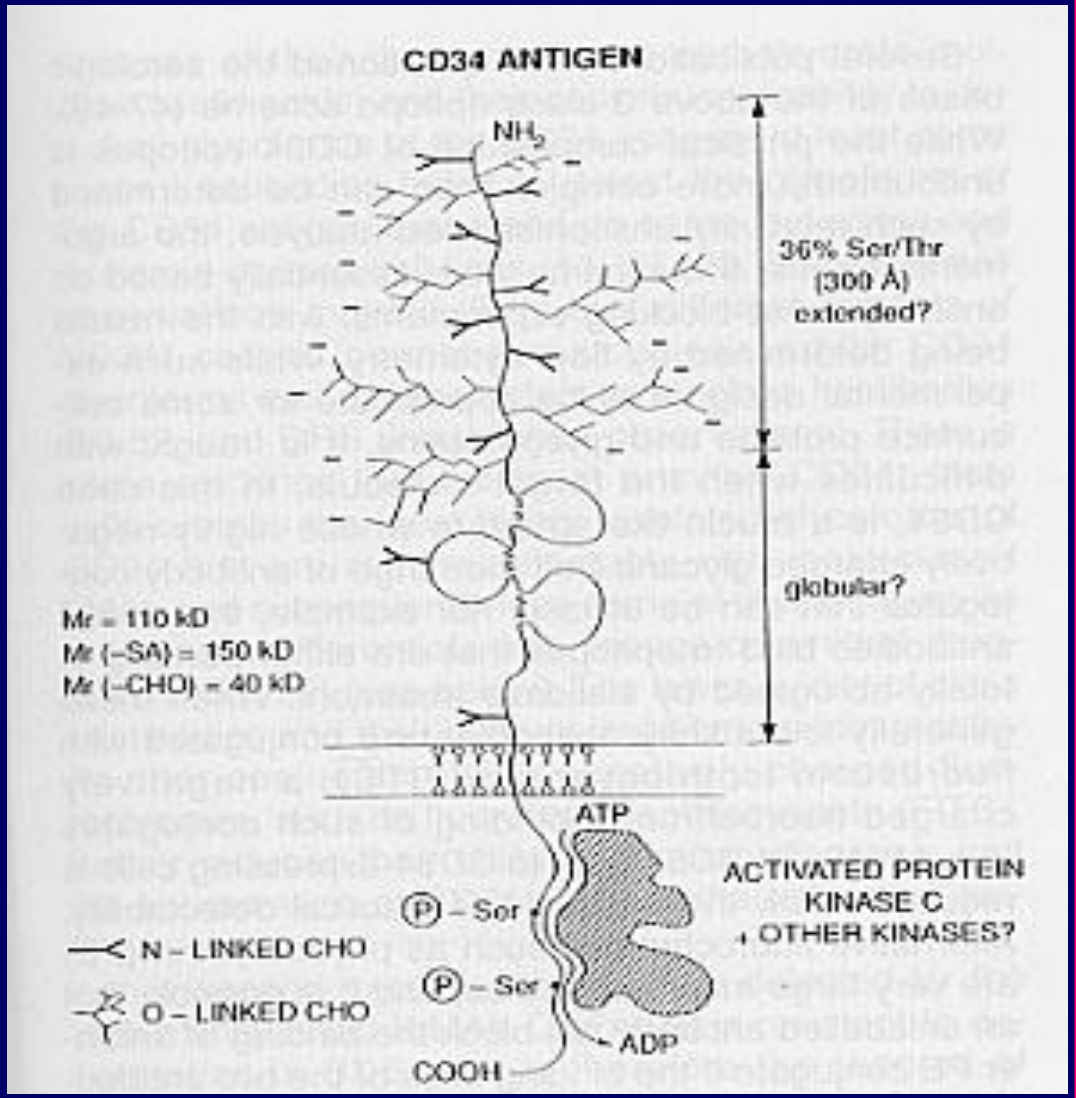
Patogenesi dei linfomi a cellule B...



SOURCES OF HEMATOPOIETIC STEM CELLS FOR TRANSPLANT

Cord Blood	0.5%
Bone Marrow	1%
Mobilized Peripheral Blood	0.1-10%

THE CD34 ANTIGEN



For more than a decade, CD34 molecule has been the best-known marker of HSC;

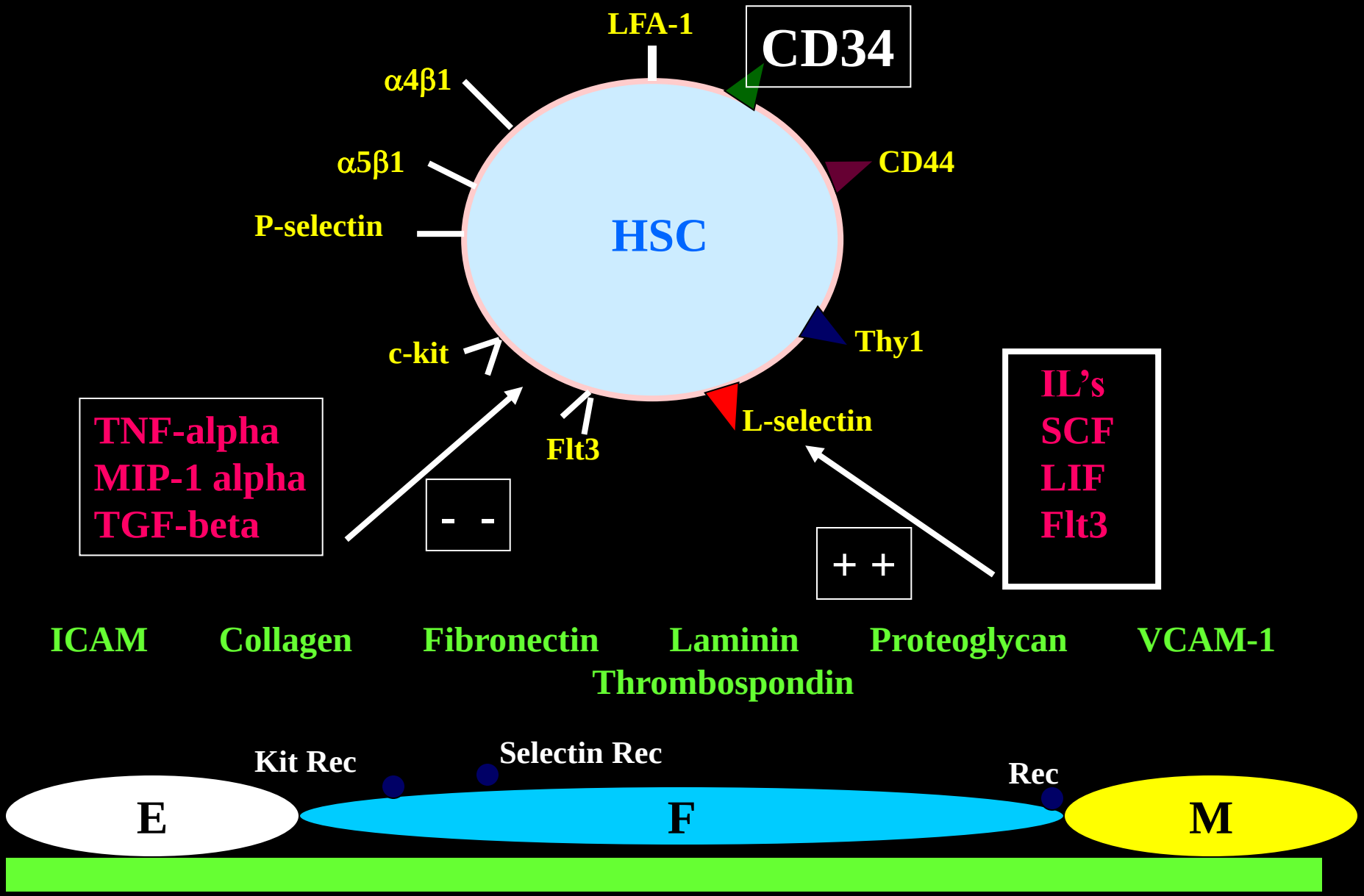
Very high academic as commercial interest;

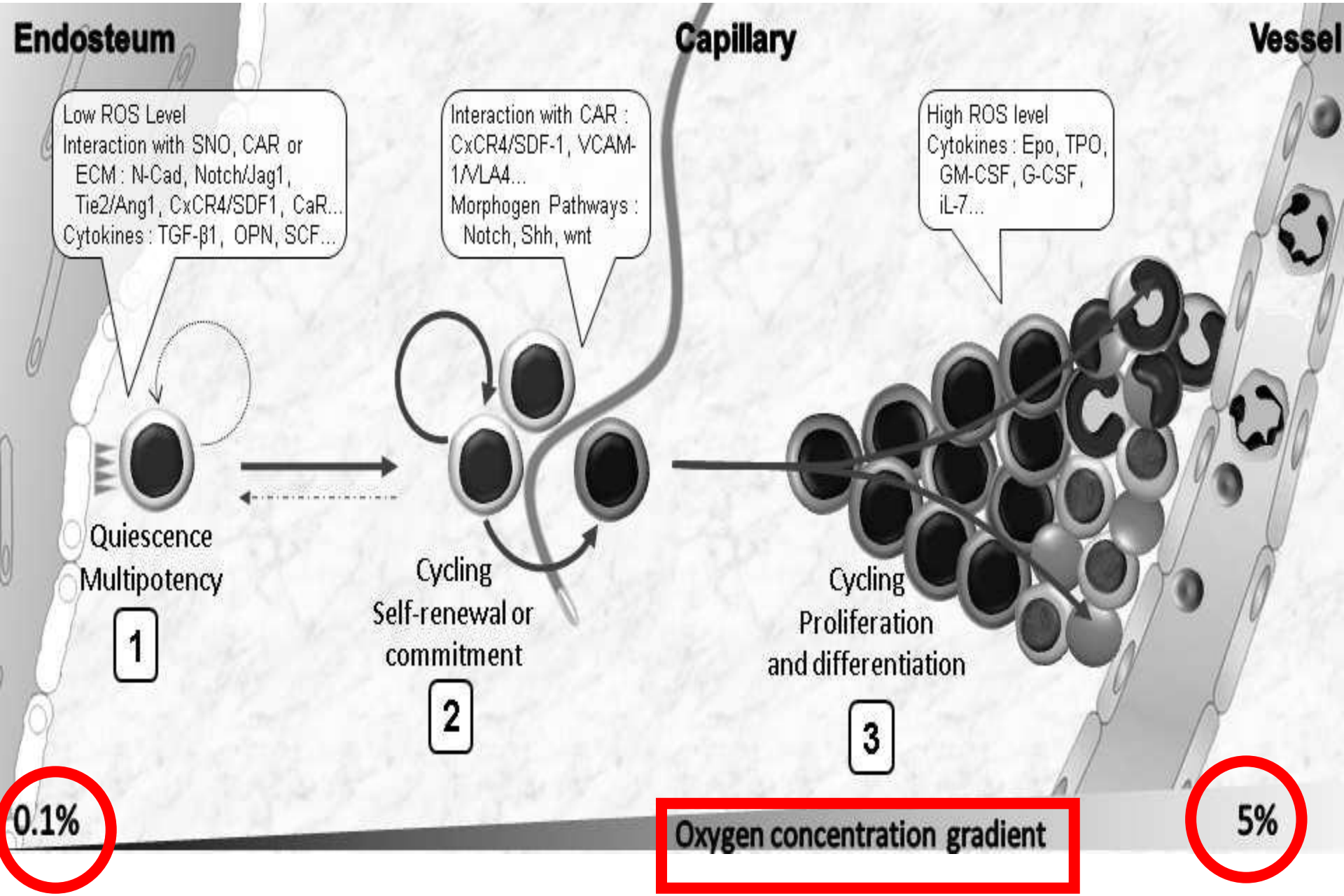
Berenson et al (1988) reported successful hematopoietic reconstitution in baboons with selected $CD34^+$ bone marrow cells.

M.W. 110-120 kDa

Chromosome localization: 1q32

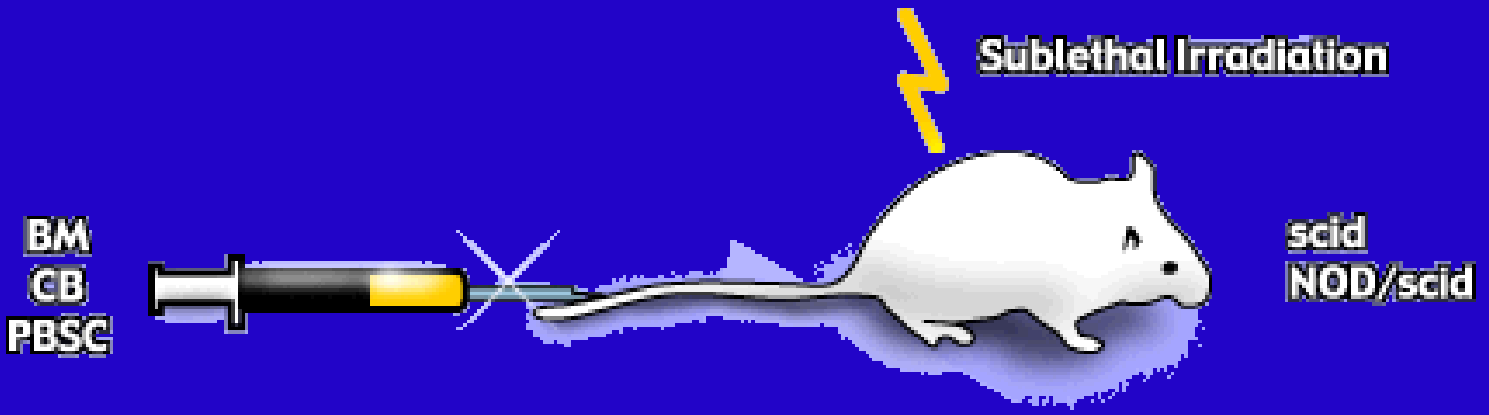
INTERACTIONS OF STEM CELLS WITH MICROENVRONMENT





Guitard...dello Sbarba et al.,Exp Hem 2010

Transplantation Assay

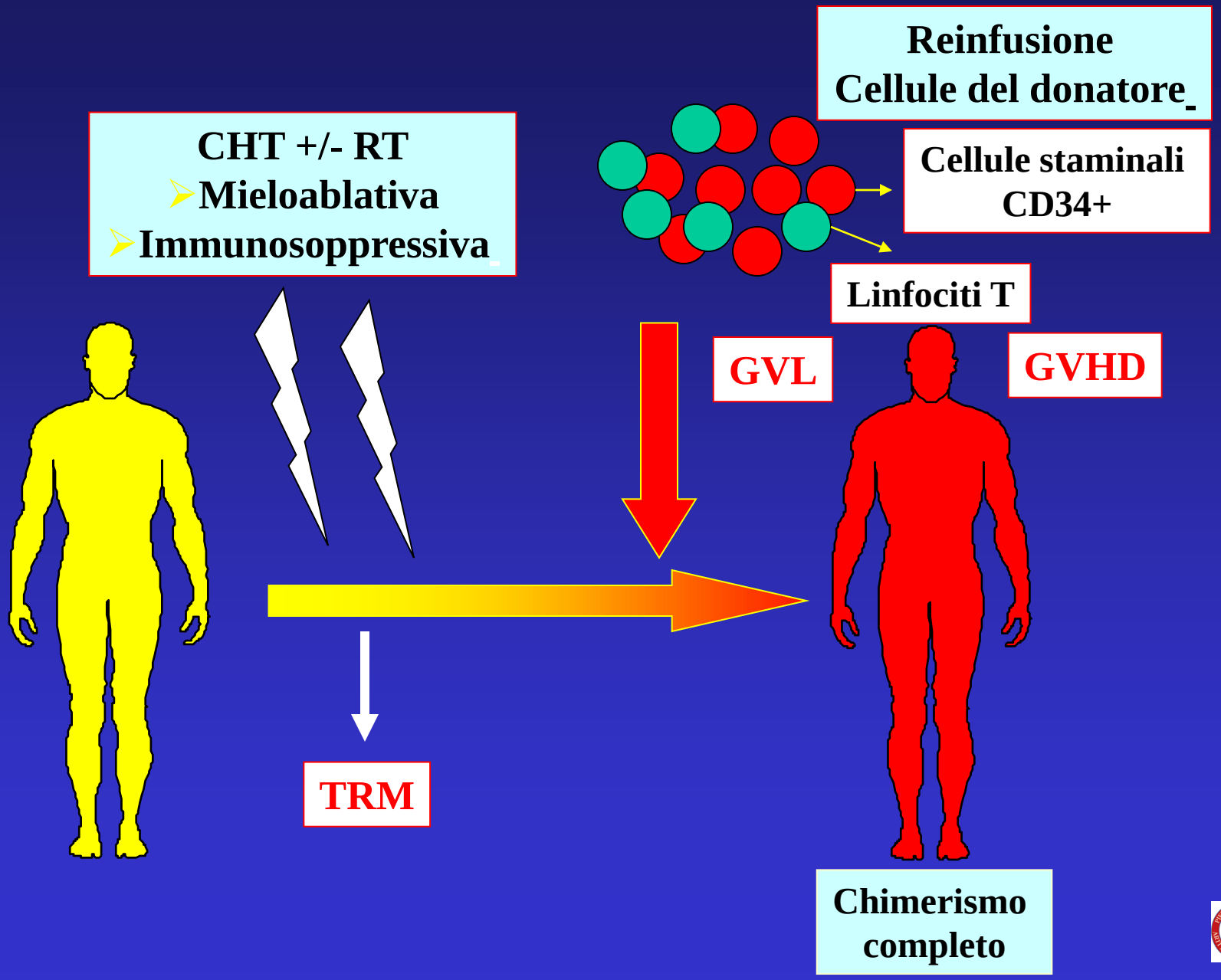


Human
Growth Factors
i.p.

≥ 4 Weeks

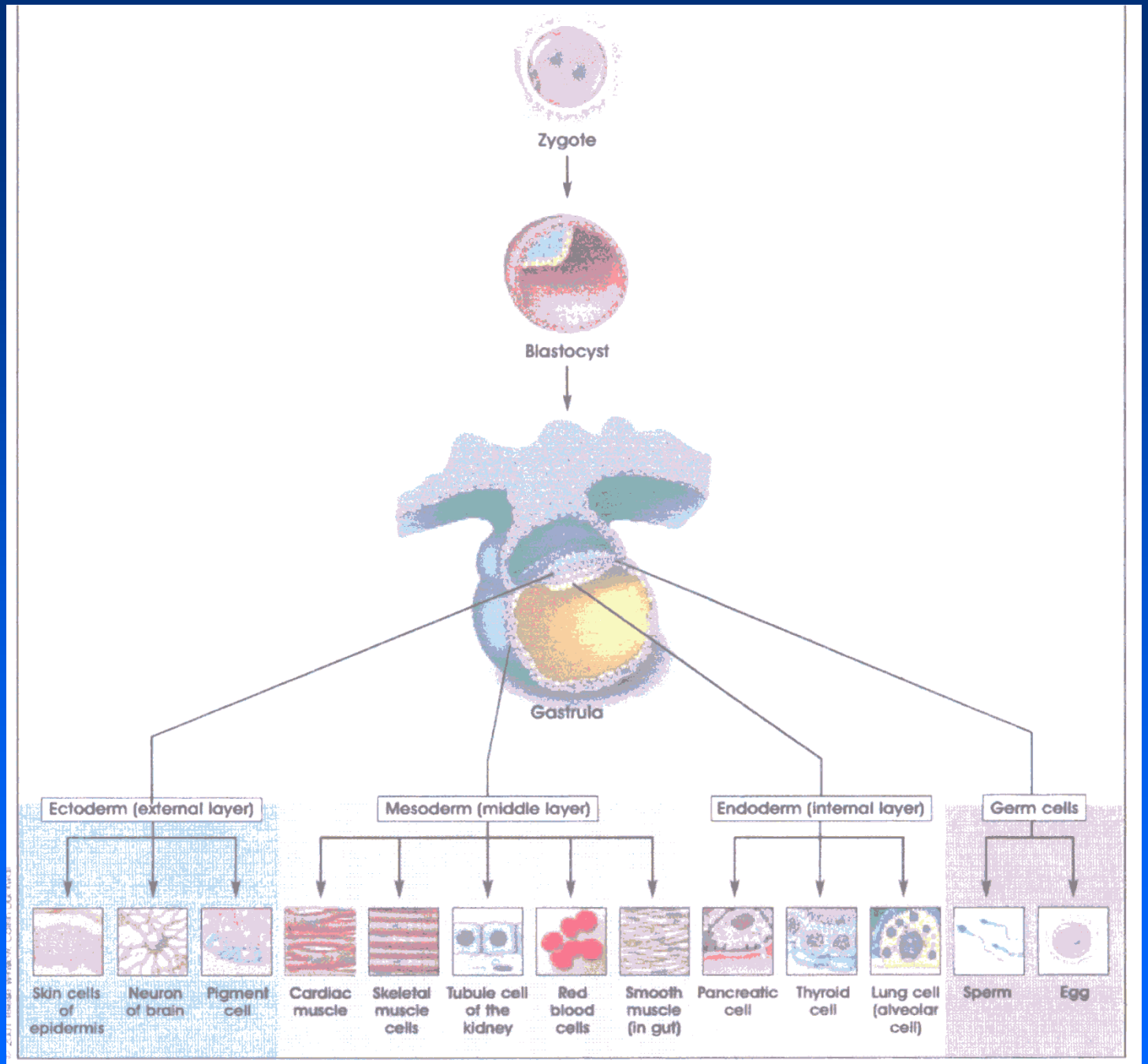
CFC/LTCIC Assays
Flow Cytometry
Southern Blot

Trapianto di CS Allogeniche



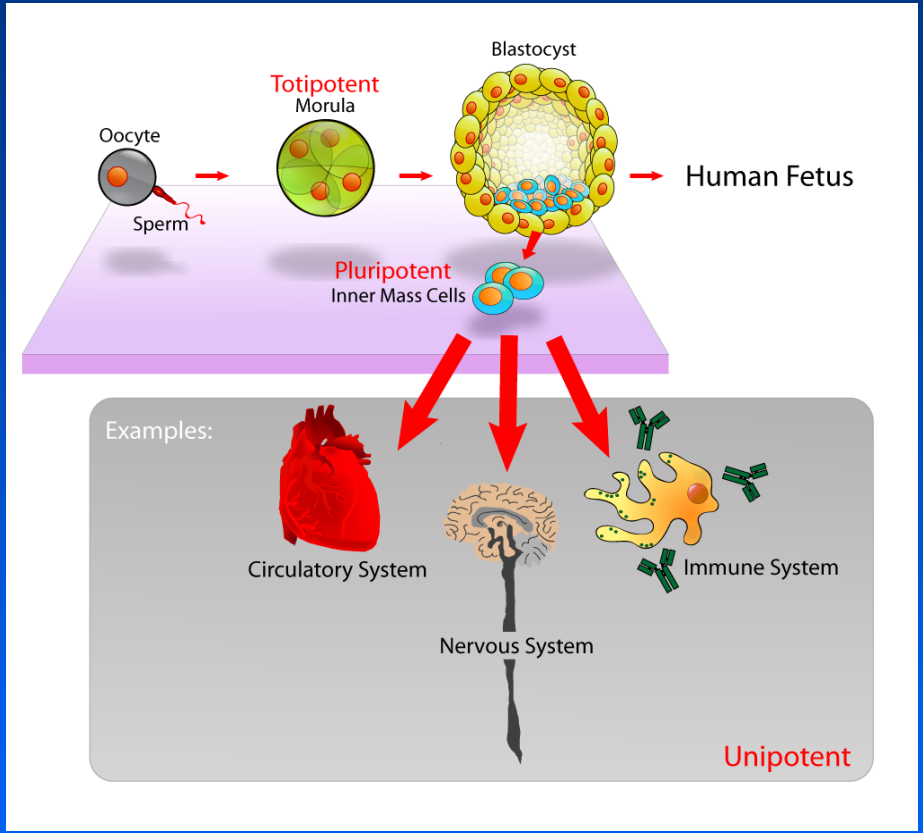
PROPRIETA' DELLA CELLULA STAMINALE EMOPOIETICA:

- capacità di "self-renewal"
- capacità di dare origine a progenitori multipotenti
- capacità di dare origine a precursori "committed" (differentiation plasticity)
- Capacità di engraftment
- Plasticità (developmental plasticity)


Zygote
Blastocyst
Gastrula
Ectoderm (external layer)
Mesoderm (middle layer)
Endoderm (internal layer)
Germ cells
Skin cells of epidermis
Neuron of brain
Pigment cell
Cardiac muscle
Skeletal muscle cells
Tubule cell of the kidney
Red blood cells
Smooth muscle (in gut)
Pancreatic cell
Thyroid cell
Lung cell (alveolar cell)
Sperm
Egg

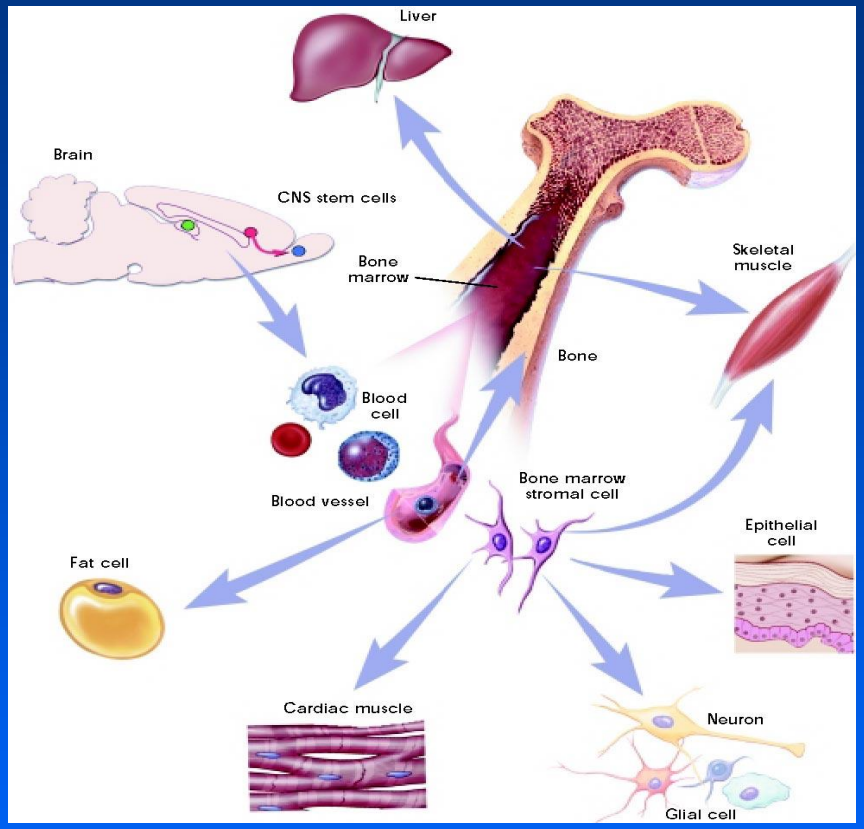
The embryonic stem cell (ES)

- **Derives from inner cell mass of blastocyst**
- **Pluripotent**
- **Give rise to endoderm, ectoderm, mesoderm tissues**



The adult stem cell

- Found throughout the body in all tissues in specific stromal niches
- Multipotent
- Partially committed with a key role in damaged tissues repair



Multi-Organ, Multi-Lineage Engraftment by a Single Bone Marrow-Derived Stem Cell

Diane S. Krause, Neil D. Theise, Michael I. Collector, Octavian Henegariu, Sonya Hwang, Rebekah Gardner, Sara Neutzel, and Saul J. Sharkis

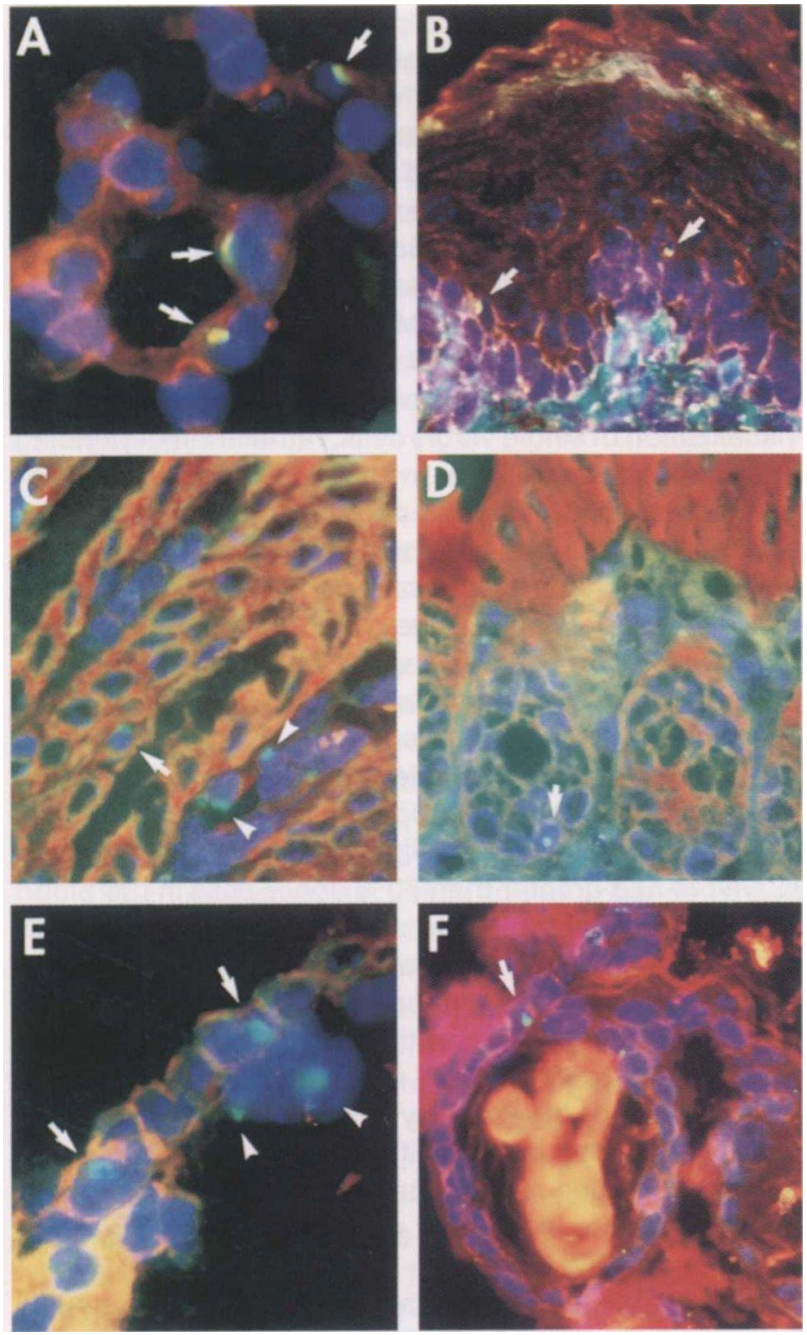
Percent Donor Engraftment of Nonhematopoietic Tissues 11 Months Post Transplant

	Bronchi	Alveoli	Esoph	Stomach	Sm. Bowel	Large Bowel	Skin	Bile Duct
M 1	3.6	14.8	0	0.5	0.3	0.2	2.6	0.4
M 2	2.3	10.3	0.4	0.5	0.4	0.1	2.4	0
M 3	3.5	18.7	2.2	0	0	0	1.2	0
M 4	2.2	10.1	2.5	0.2	0.4	0.3	1.6	2.2
M 5	0	9	0.5	0.4	1.6	0	2.7	0
Mean ± SD	2.32 ± 1.45	12.58 ± 4.07	1.12 ± 1.14	0.32 ± 0.21	0.54 ± 0.61	0.12 ± 0.13	2.1 ± 0.66	0.52 ± 0.95
Corr.[a]	3.74	20.30	1.81	0.52	0.87	0.19	3.39	0.84

The numbers shown represent the percentage of cytokeratin-positive cells within each tissue that was Y chromosome positive. For each tissue, over 150 cytokeratin-positive cells were analyzed. For stomach, small bowel, and large bowel, 10^3 to 3.4×10^3 cells were counted for each mouse.

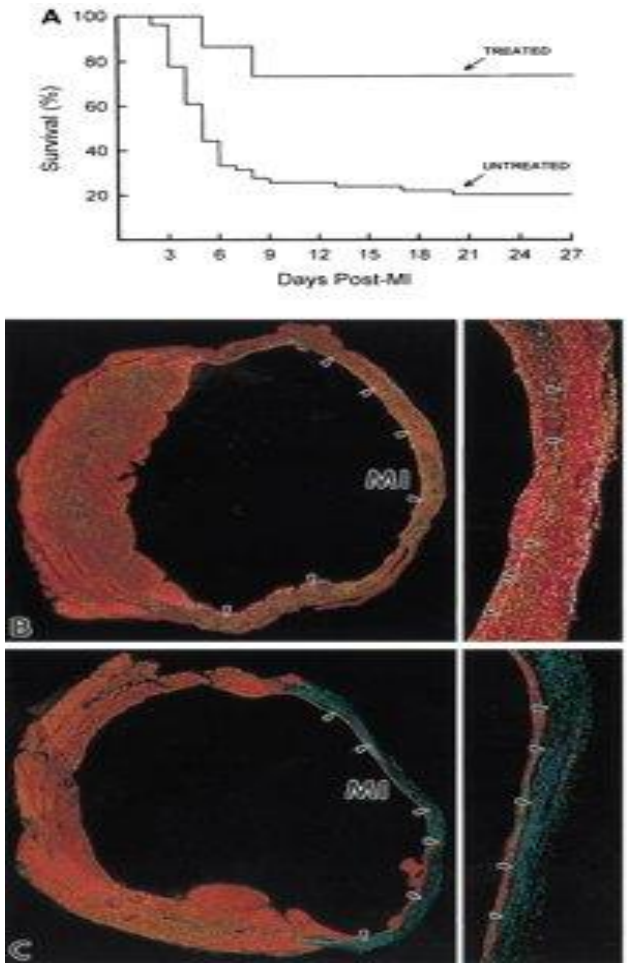
[a] The percentage of immunostained epithelial cells that are Y chromosome positive is corrected for the male control for each tissue.

Fluorescence microscopic images of (A) lung, (B) esophagus, (C) stomach, (D) colon, (E) bile duct cyst, (F) skin.

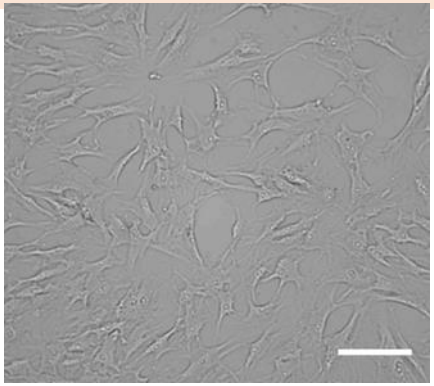


Mobilized bone marrow cells repair the infarcted heart, improving function and survival.

Donald Orlic, Jan Kajstura, Stefano Chimenti, Federica Limana, Igor Jakoniuk, Federico Quaini, Bernardo Nadal-Ginard, David M. Bodine, Annarosa Leri, and Piero Anversa.



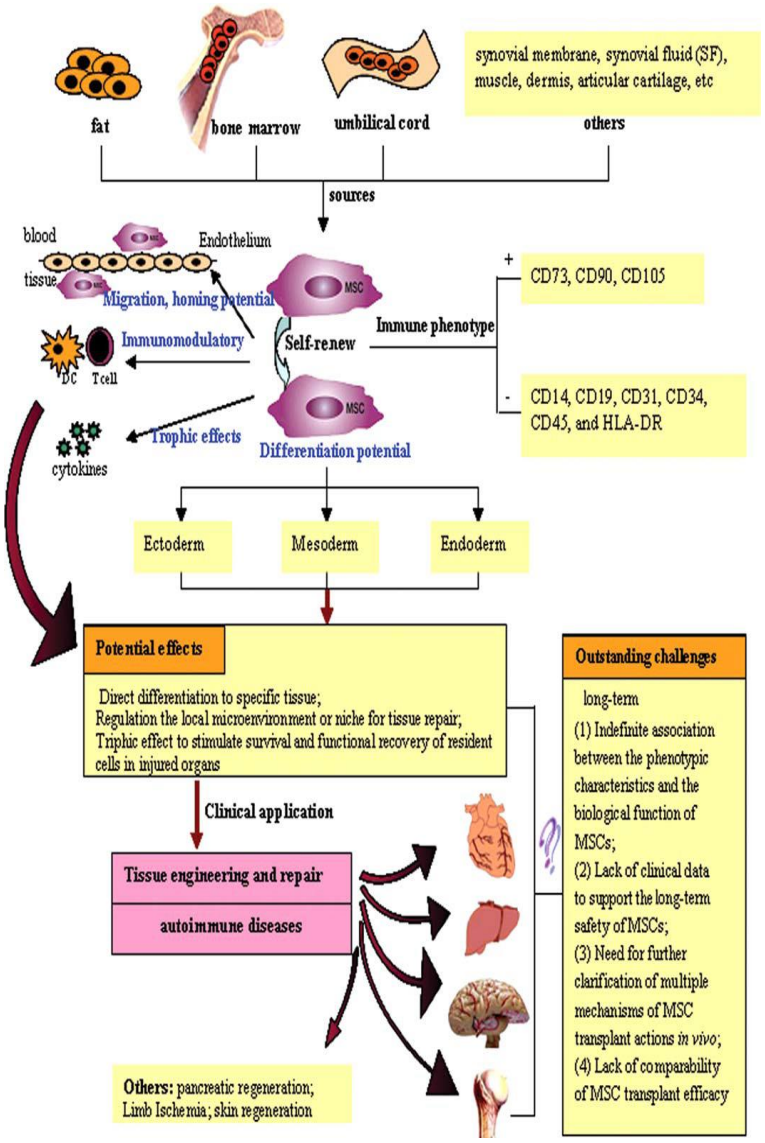
Mesenchymal Stem Cells



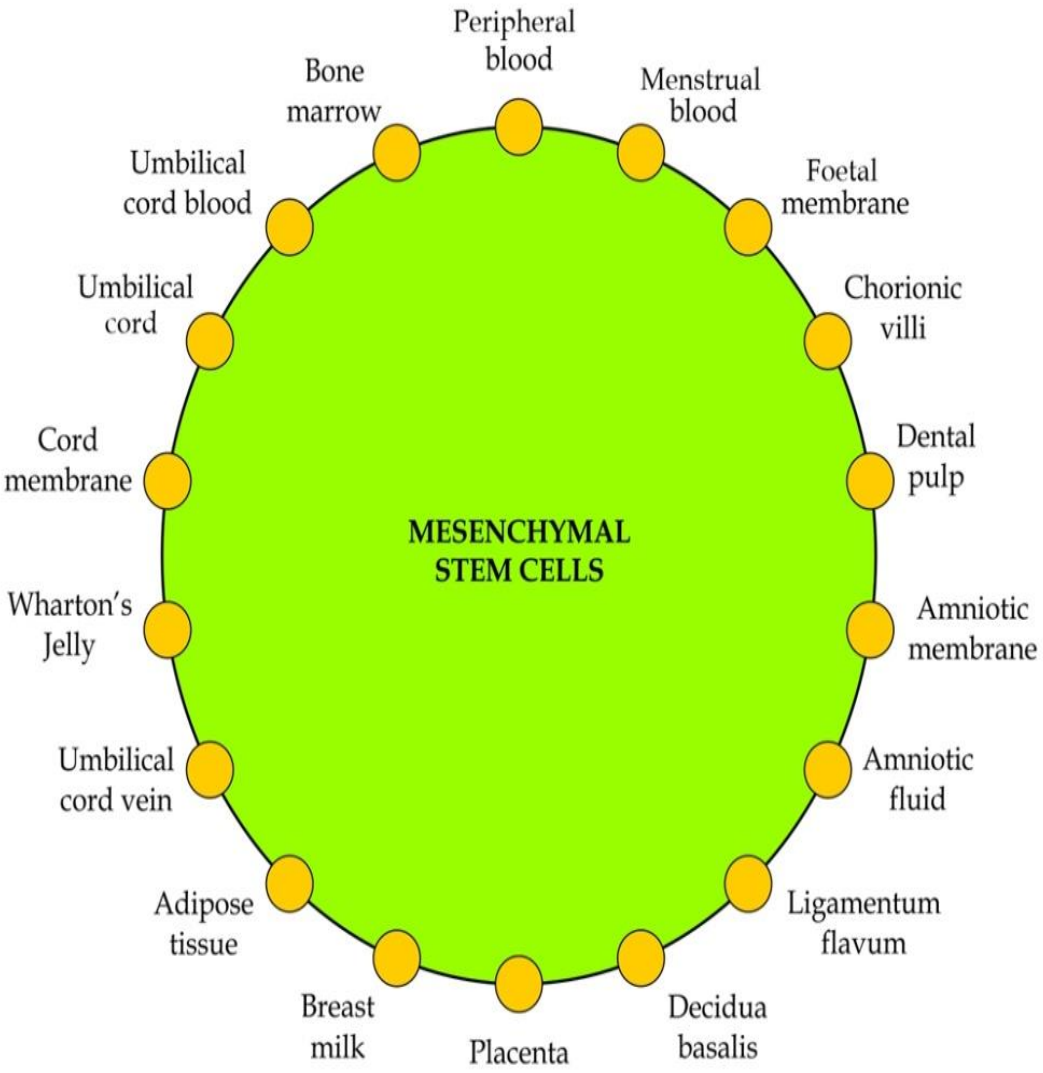
MSCs are multipotent stromal cells

Phenotype:

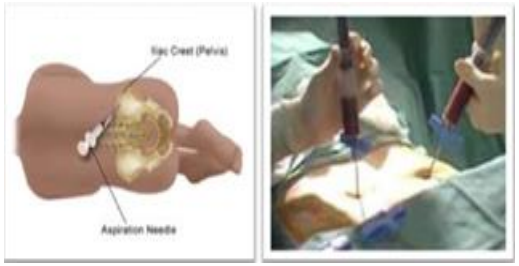
Positive (≥95% +)	**Negative (≤2% +)**
CD105	CD45
CD73	CD34
CD90	CD14 or CD11b
	CD79α or CD19
	HLA-DR



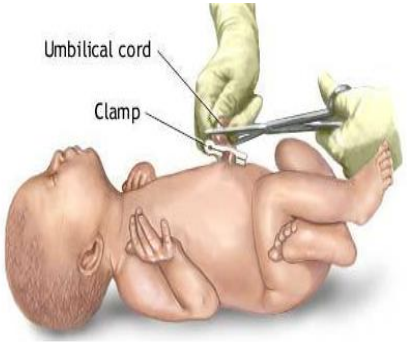
Sources of Mesenchymal Stromal Cells



Adipose Tissue



Bone Marrow



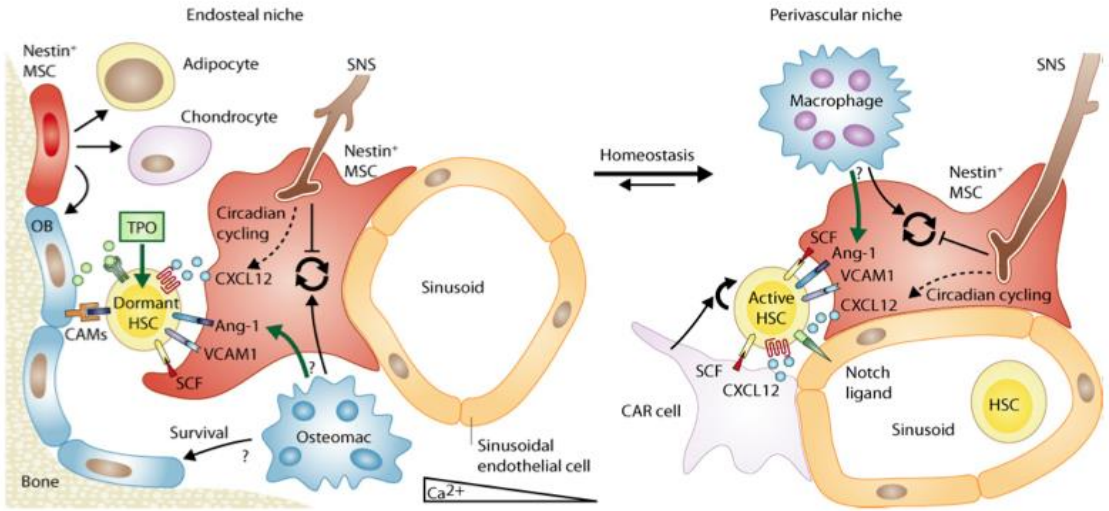
Umbilical Cord

MSC and Hematopoietic Niche

RESEARCH ARTICLE

Mesenchymal stromal cells (MSCs) induce *ex vivo* proliferation and erythroid commitment of cord blood haematopoietic stem cells (CB-CD34+ cells)

Simone Perucca[1,2], Andrea Di Palma[1,2], Pier Paolo Piccaluga[3,4], Claudia Gemelli[5], Elisa Zoratti[6], Giulio Bassi[7], Edoardo Giacopuzzi[8], Andrea Lojacono[9], Giuseppe Borsani[8], Enrico Tagliafico[10], Maria Teresa Scupoli[11], Simona Bernardi[1,2], Camilla Zanaglio[1,2], Federica Cattina[1], Valeria Cancelli[1], Michele Malagola[1], Mauro Krampera[7], Mirella Marini[12], Camillo Almici[12], Sergio Ferrari[13], Domenico Russo[1]*

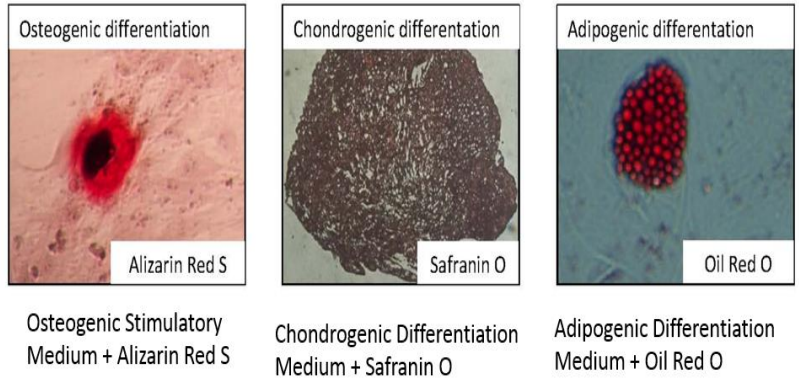


Perucca S. et al., 2017 PLoSONE 12(2)

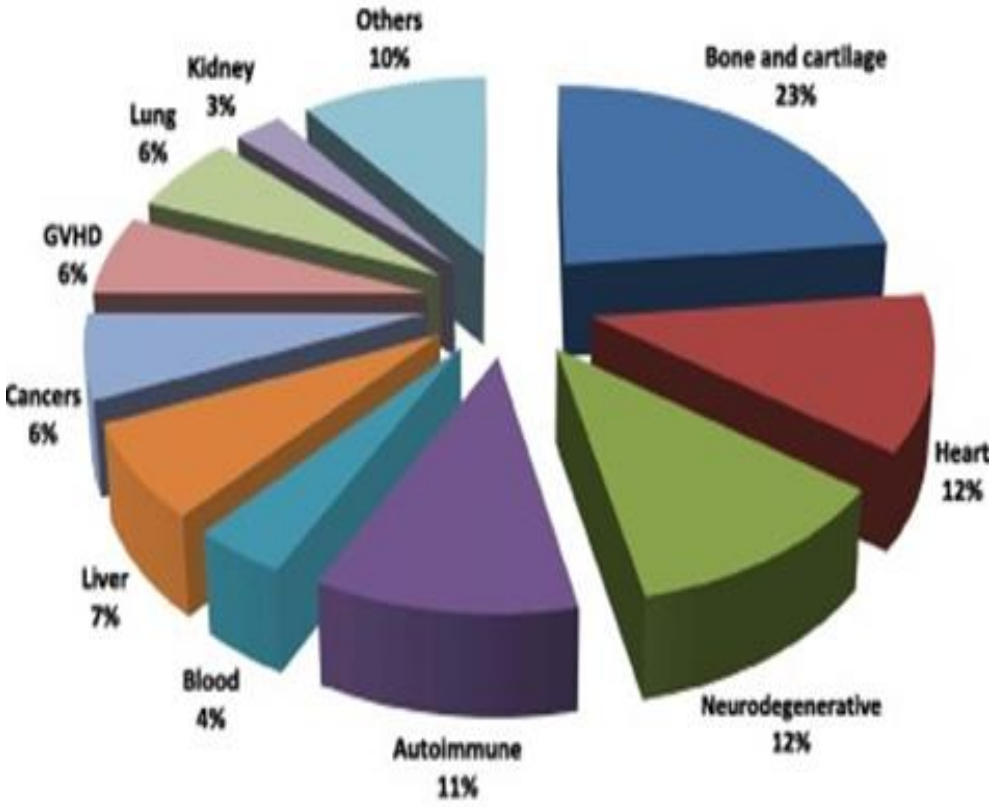
Activities of Mesenchymal Stromal Cells

In vitro/invivo effects:

- Proliferative
- Differentiatiative : osteoblasts, chondrocytes, adipocytes, myocytes
- Migration / homing to injured tissue
- Immunomodulatory
- Trophic



Clinical Trials of Mesenchymal Stromal Cells



Number of Clinical Trials (n=516) as reported on the website http://www.clinicaltrials.gov (Accessed 2015). GVHD (graft-versus-host disease).

Bone and cartilage diseases

BMT and GVHD

Cardiovascular diseases

Autoimmune diseases

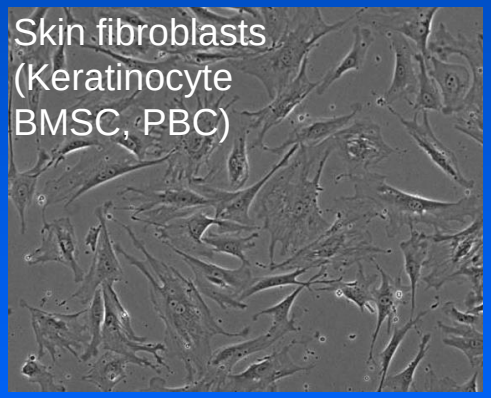
Liver diseases

Cancer

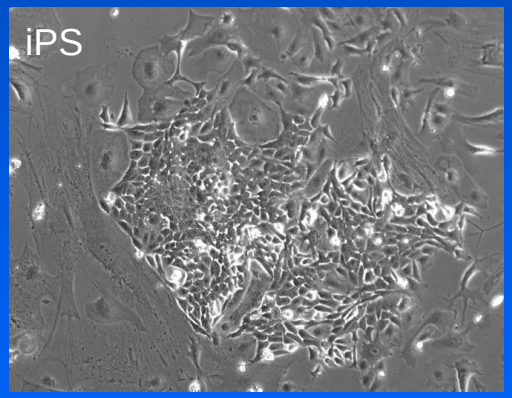
The iPS: induced Pluripotent Stem cell

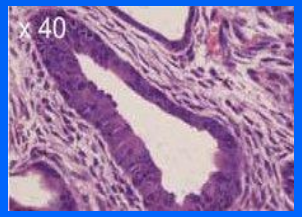
(Reprogramming of human somatic cells to pluripotency with defined factors.
Daley GQ et al., *Nature*, 2008)

- Derives from a genetic reprogramming of adult somatic cell
- Pluripotent artificial stem cell
- Different growth conditions lead to the differentiation into all possible types of specialized cells

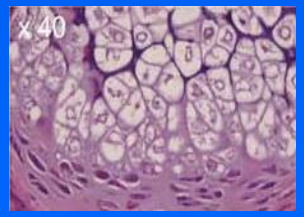


Retroviral infection with Oct4, Sox2, c-Myc and Klf4 genes

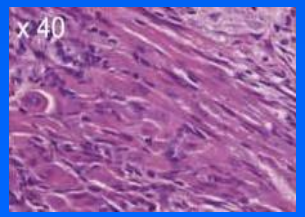




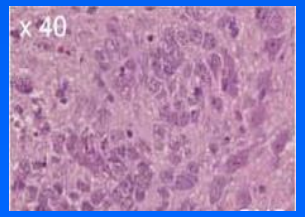
Epithelium



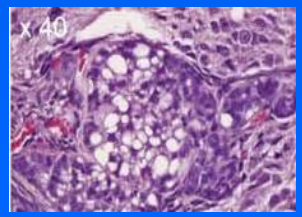
Cartilage



Muscle



CNS



Adipose