

Un esperimento è una domanda che la scienza pone alla natura, ed una misurazione è la registrazione della risposta della Natura.

(Max Planck)

Hepatic stem cells

- Liver is an organ capable of extensive regeneration

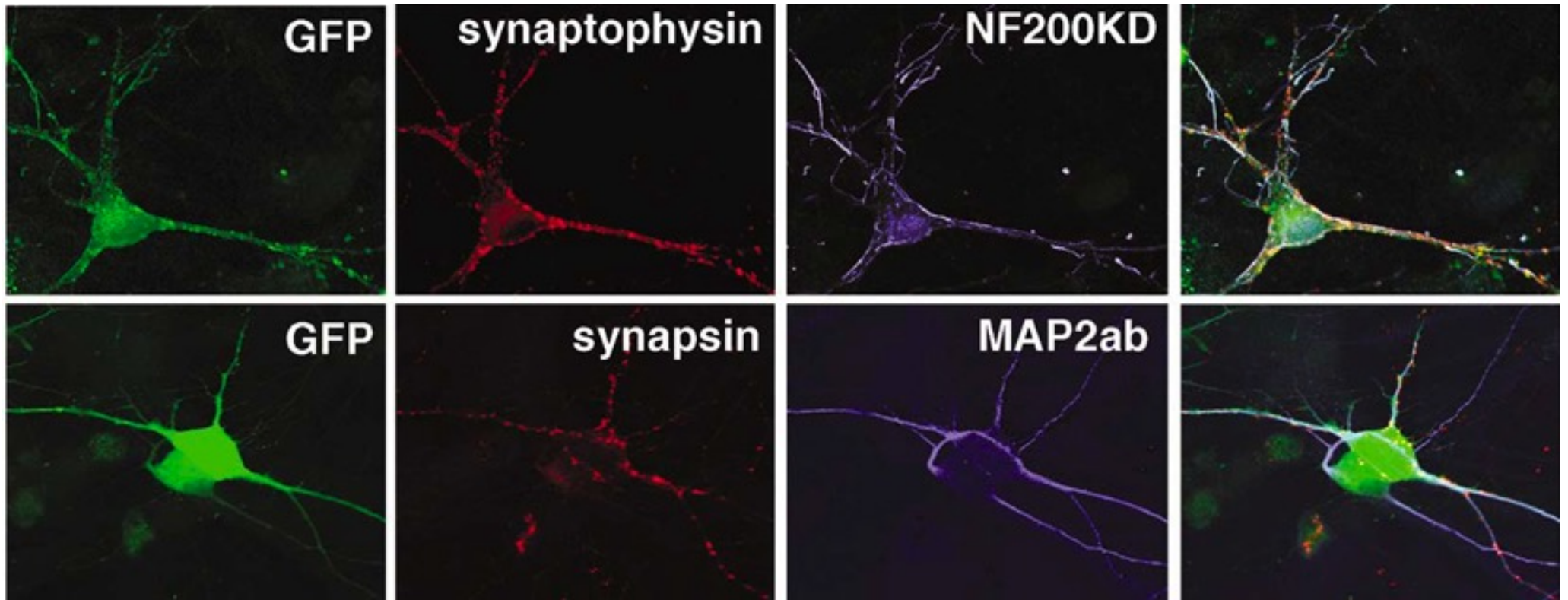
But

- The precise source of stem cells remains unclear (terminal bile ductules ?)

Neural stem cells

- Old studies in rats and songbirds (1969)
- More recent studies in mammals: neuronal progenitors exist, are capable of extensive cell division and self renewal
- Can be obtained by differential sedimentation on a gradient
- Available markers allow only 45 fold enrichment
- Neural progenitors can migrate and home to specific sites of damage or regeneration

Post natal neural cells



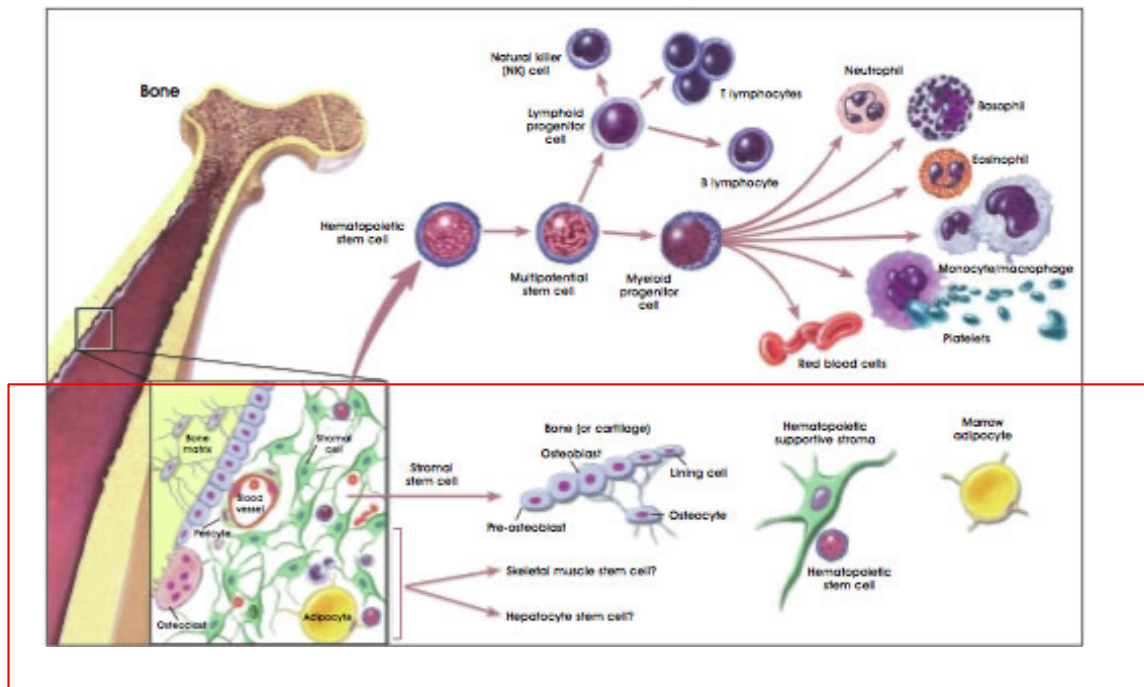
Song et al, 2002

Skeletal muscle stem cells

- Satellite cell: mononucleated cell ensheathed under the basal lamina that surrounds multinucleated muscle fibers (1961)
- Can be activated, induced to proliferate, and contribute to intact skeletal muscle fibers even after extensive tissue doublings
- Heterogeneous, no specific markers
- Are rapidly depleted in muscle of Duchenne patients

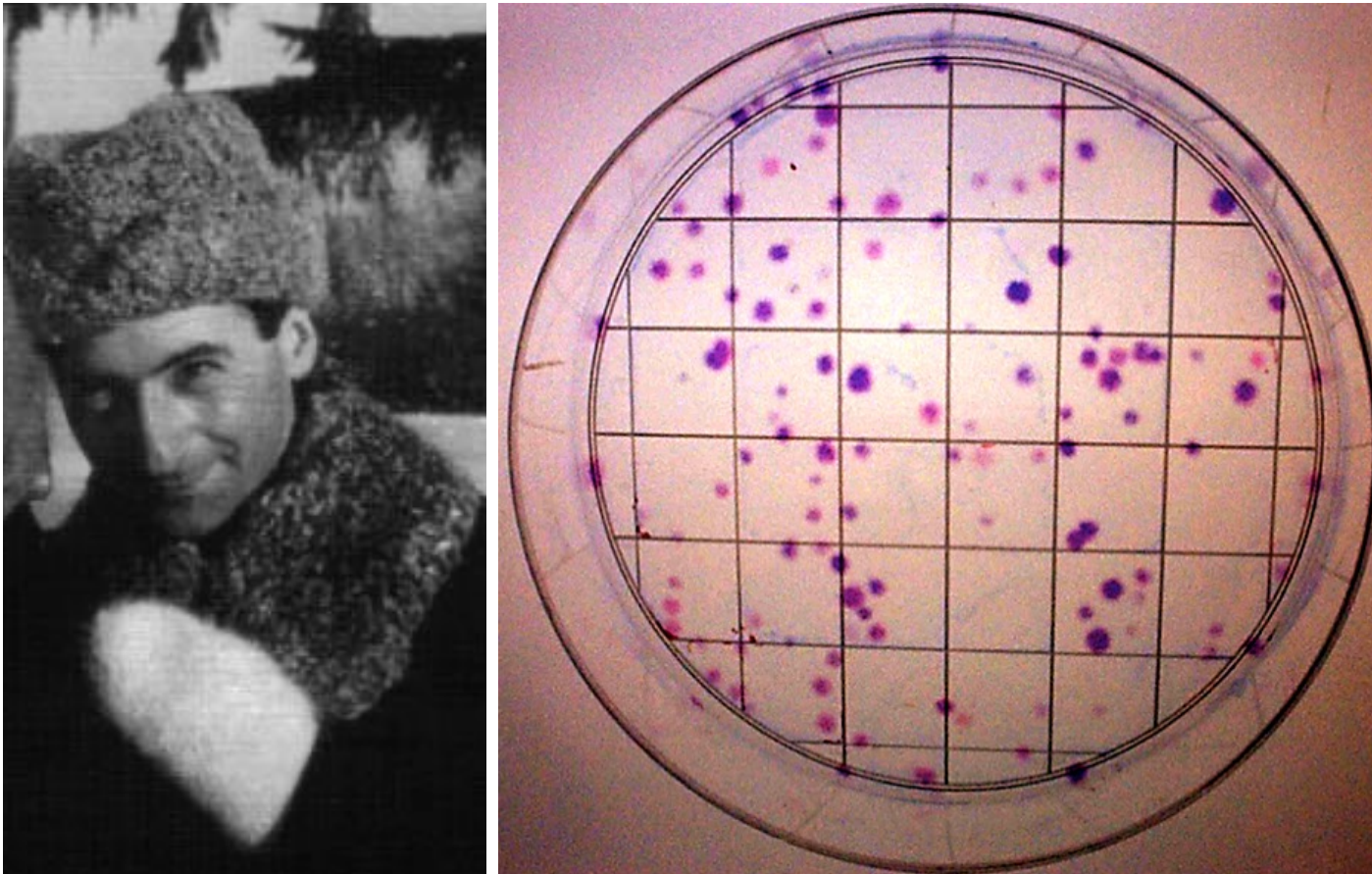
Mesenchymal stem cells

- Bone marrow-derived (non circulating fraction)
- Isolated on the basis of their adhesive properties
- Remarkable plasticity (chondrocytes, osteoblasts, adipocytes, cardiac and skeletal muscle cells, neurons, astrocytes)



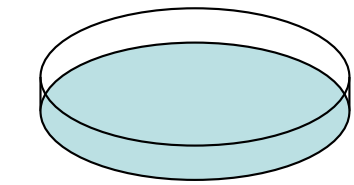
MSC properties

Alexander
Friedenstein



In bone marrow (“fibroblasts”)
Can be isolated and amplified ex vivo
transplantable
multipotent

MSC/skeletal stem cells, transplant



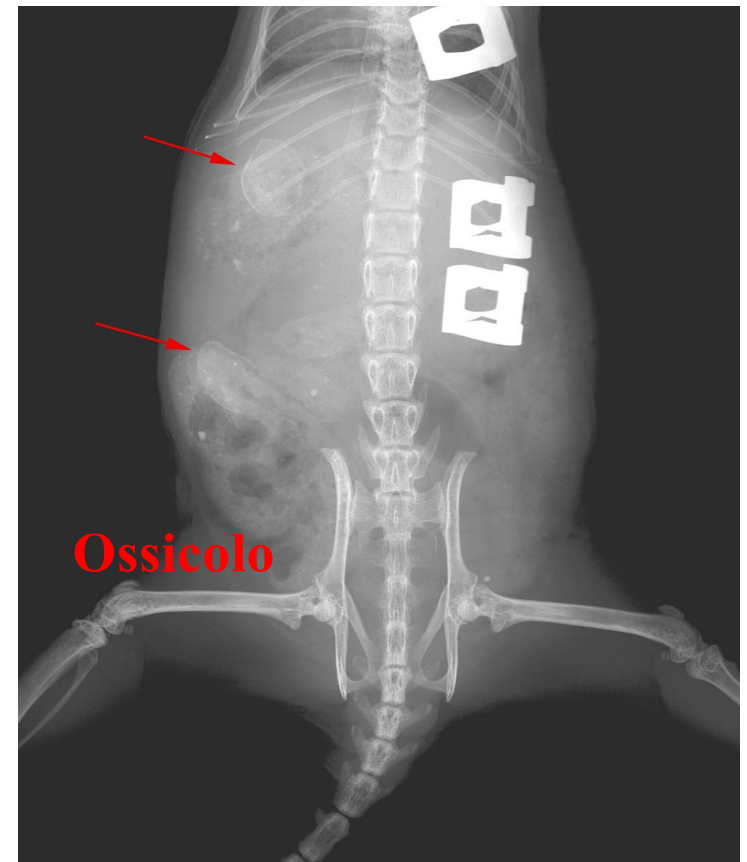
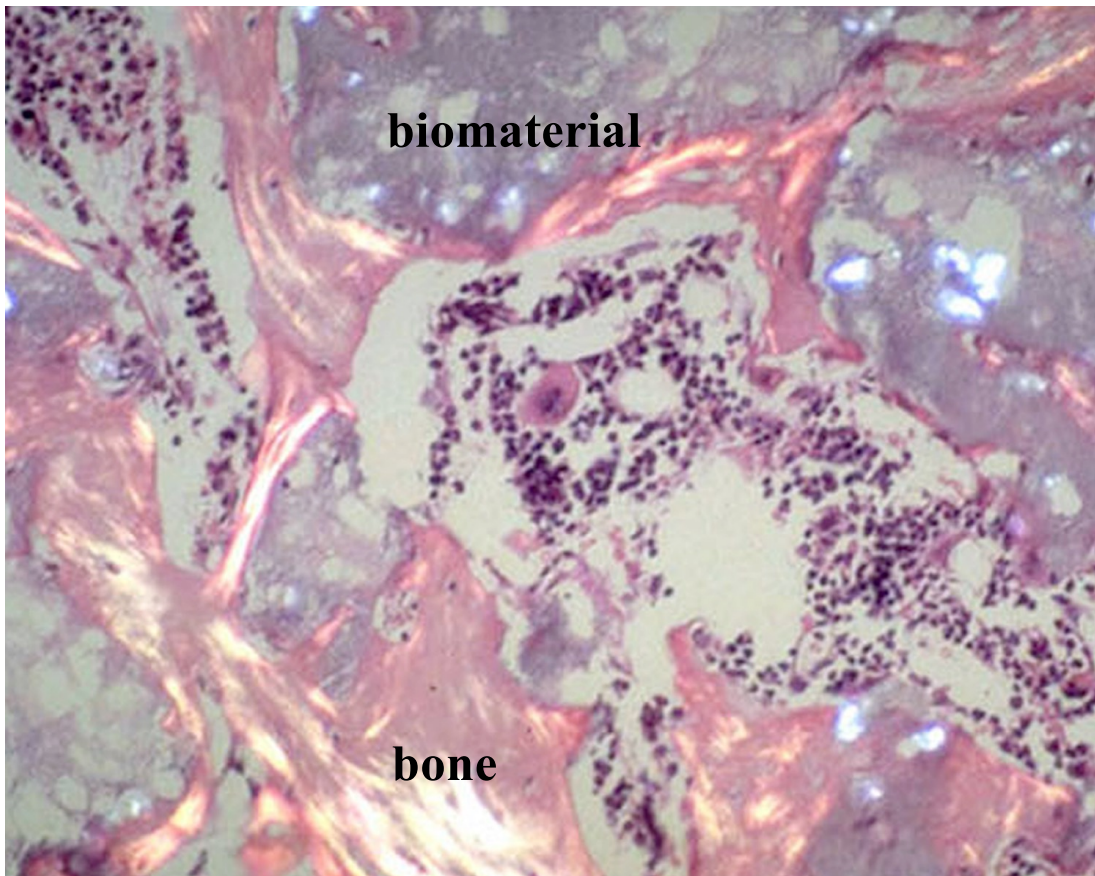
Expansion, ex vivo



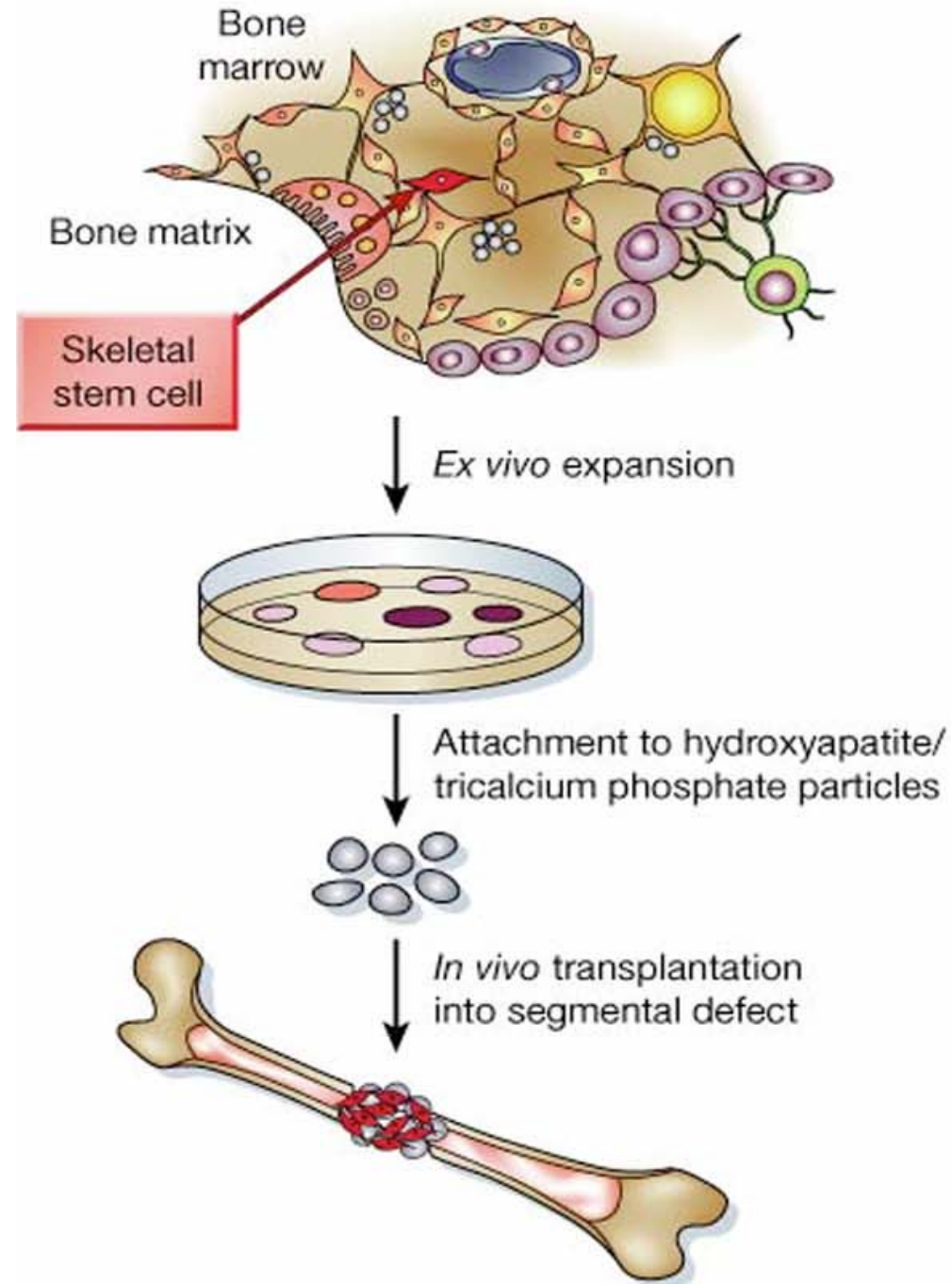
biomaterial



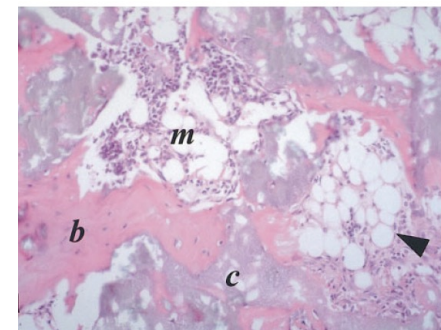
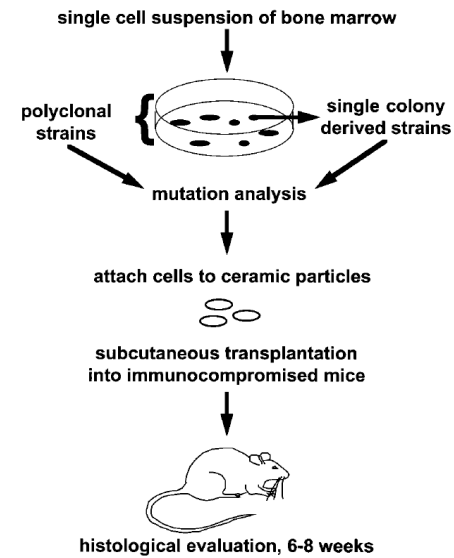
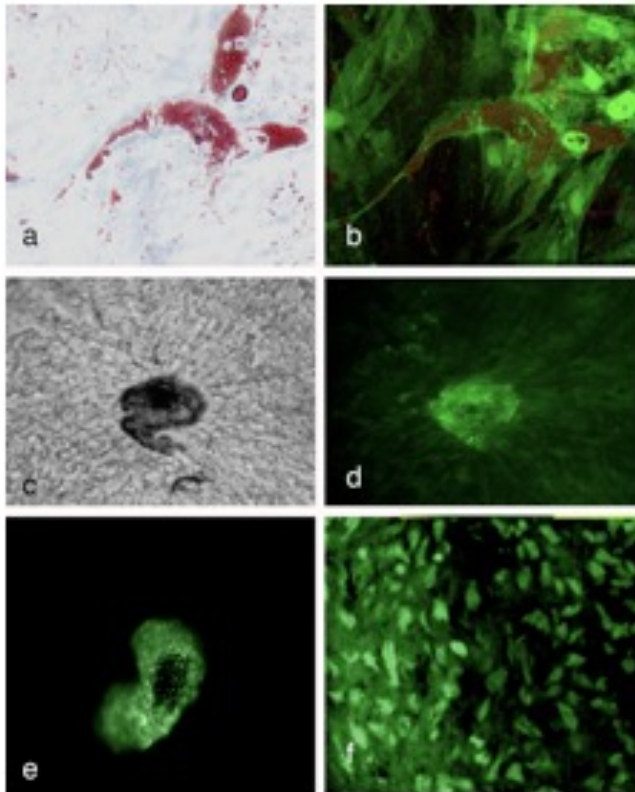
SCID mouse



MSC in therapy



Mesenchymal stem cells pluripotency



Spaces separating newly formed bony structures are occupied by hematopoietic marrow (m), in which all hematopoietic lines are detected (meg, megakaryocyte). Adipocytes are readily recognizable in the ectopic marrow (arrowheads).



Animal models

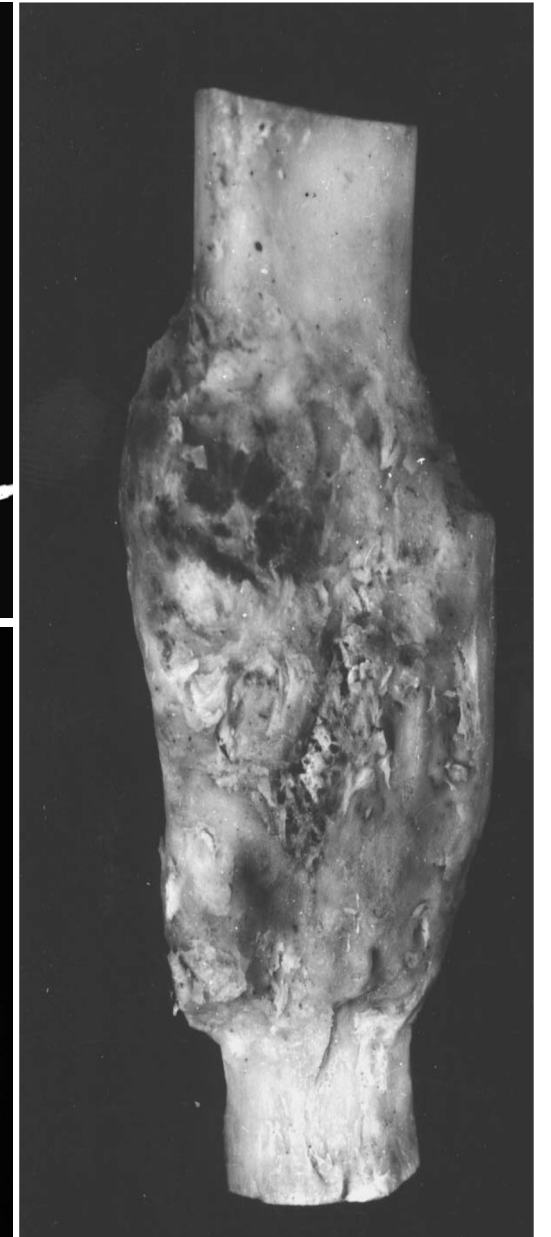
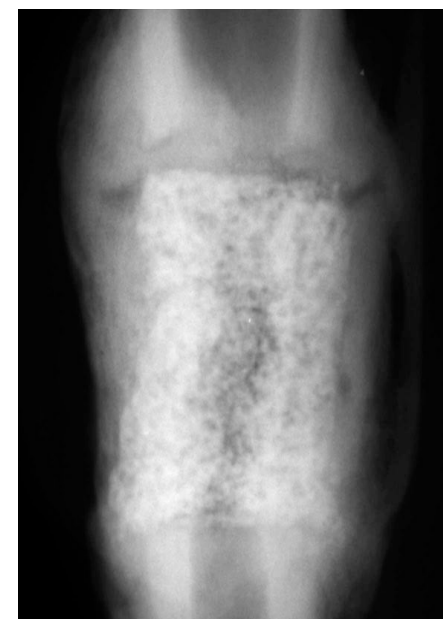
Preclinical models

Mice

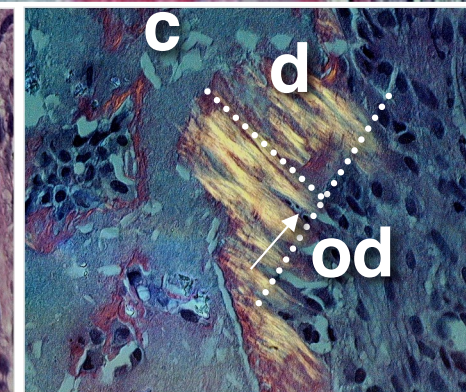
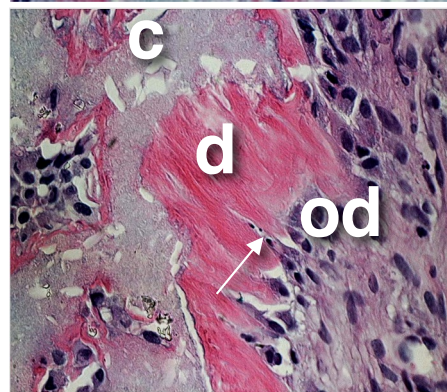
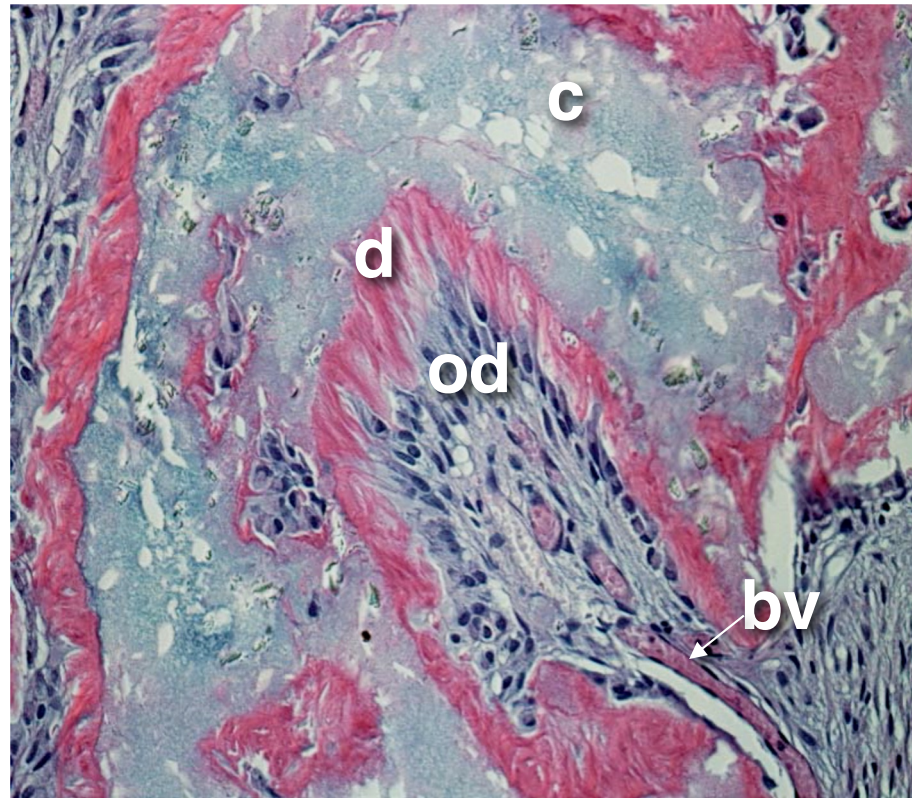
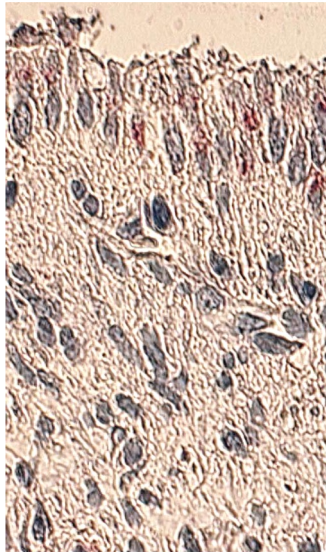
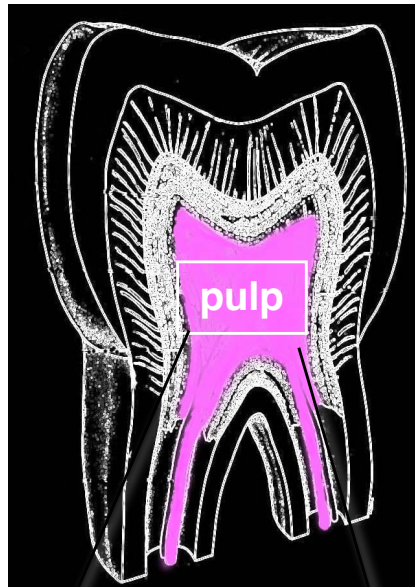
Dogs

Sheep

....



Post natal dental stem cells



MSC in therapy: problems

purification

CD146

expansion ex vivo

gmp/glp

culture medium quality

biomaterial

Way of injection

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Adult cell plasticity: an old concept - truth or not??

- Cloning experiments in amphibia (1962)
- Cloning experiments in sheep (1997)

provide evidence that the differentiated state in adult is not irreversible.

Adult genes in enucleated cell; fusions..

But more in detail? And without fusions or egg inductions?

“I have learned that the forest, humans and animals are interdependent, and if the forest is sick, then the animals will be sick, and animals will surely impact humans’ health too.”

Original idea on adult stem cells: **self renewal and differentiation potential**

hematopoietic stem cell

blood

Satellite cell

muscle

Skin stem cell

epithelium

Liver stem cell

liver

Plasticity of adult stem cells: self renewal, differentiation and transdifferentiation

Bone marrow derived cells:

Blood

Muscle

Brain

Liver

Heart

Vascular endothelium

Muscle cell

blood

CNS cell

Blood

muscle

BM stromal cells
(tissue injury...)

Adipocytes

Muscle

bone

General strategy for identifying cell fate transitions using BM-derived cells - same tissue

(transgenic mouse)

Genetically marked mouse



Harvest marrow



Isogeneic wildtype mouse



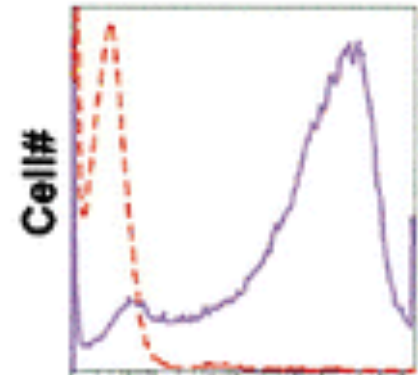
Lethal irradiation

Bone marrow transplant

"Labeled" mouse



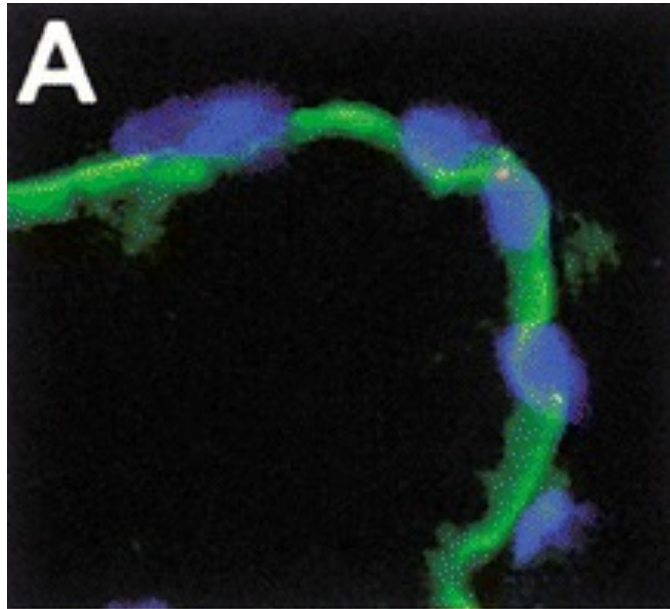
Blood assay



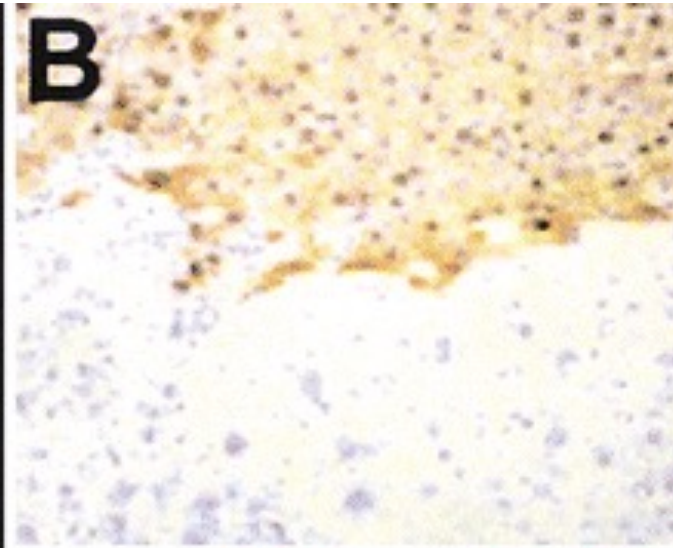
Wait ≥4 weeks
Confirm engraftment

Derivation of diverse tissue-specific cell types from BM-derived stem cells - different tissue

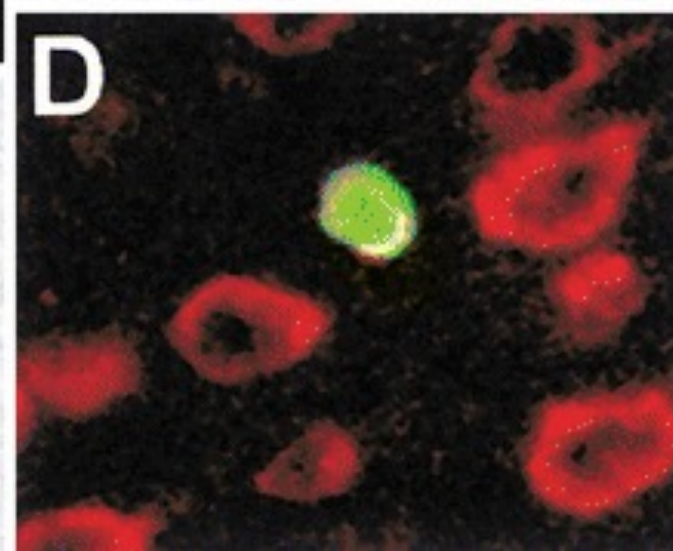
Dystrophin (green) and Y chromosome (blue) in BM-transplanted female mdx mice



Beta gal positive myocardium in a murine model of infarctum BM-transplanted (SP fraction intravascularly delivered)



FAH staining hepatocytes in FAH^{-/-} BM-transplanted mice
30-50% of liver mass 7 months posttransplant



Neurons (red) GFP positive (green) in the cortex of a mouse intravascularly delivered with GFP +BM.

Criteria for trans-differentiation

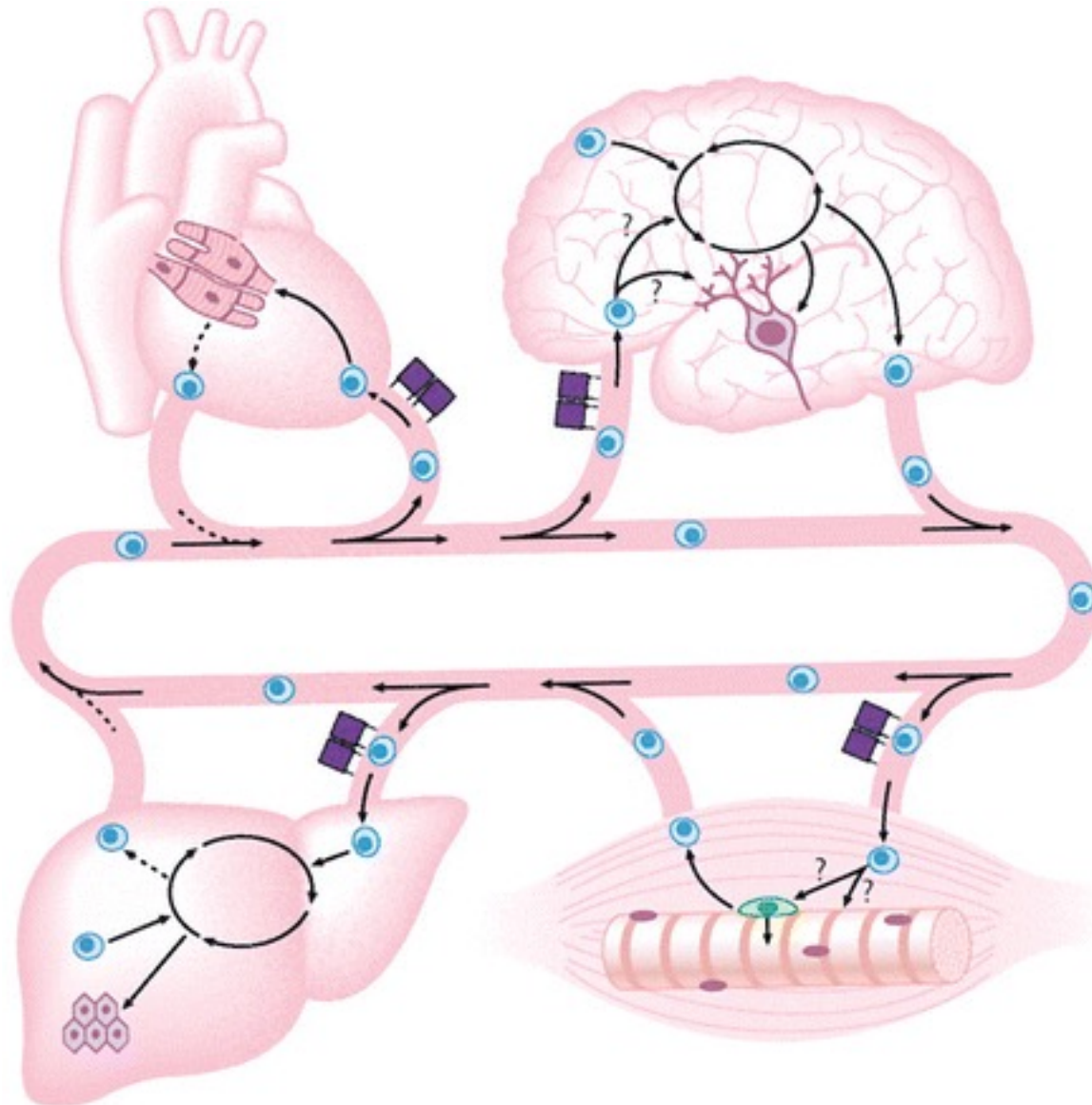
- New specific gene expression, in vitro and in vivo
- Marker of the stem cell (Y, GFP, lacZ..)
- Colocalization (confocal)
- Integration in the tissue
- Functional assay

Stem cells

Entity or function?

HM Blau Cell - 2001

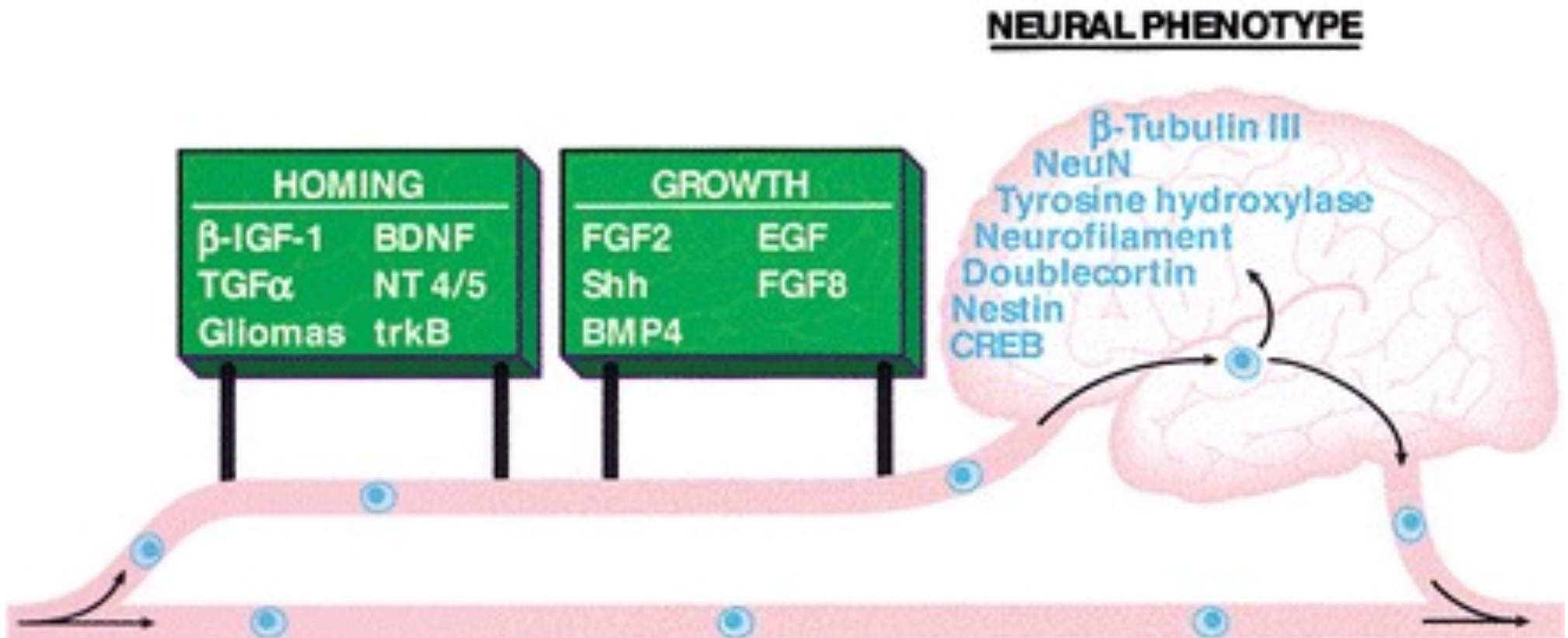
Circulation: the highway of stem cells



- contact with surrounding cells,
- Extra-cellular matrix,
- local milieu,
- growth and differentiation factors

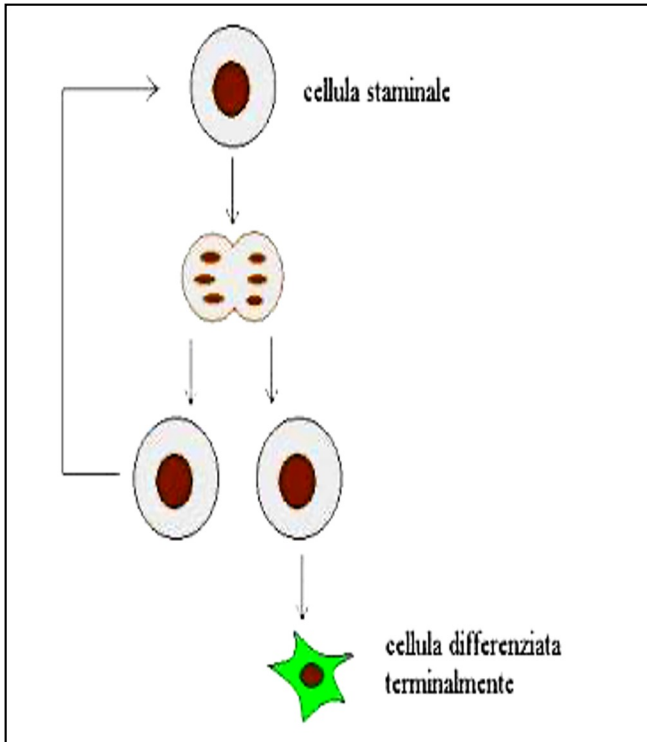
play a key role in determining stem cell function

Factors that control trans-differentiation

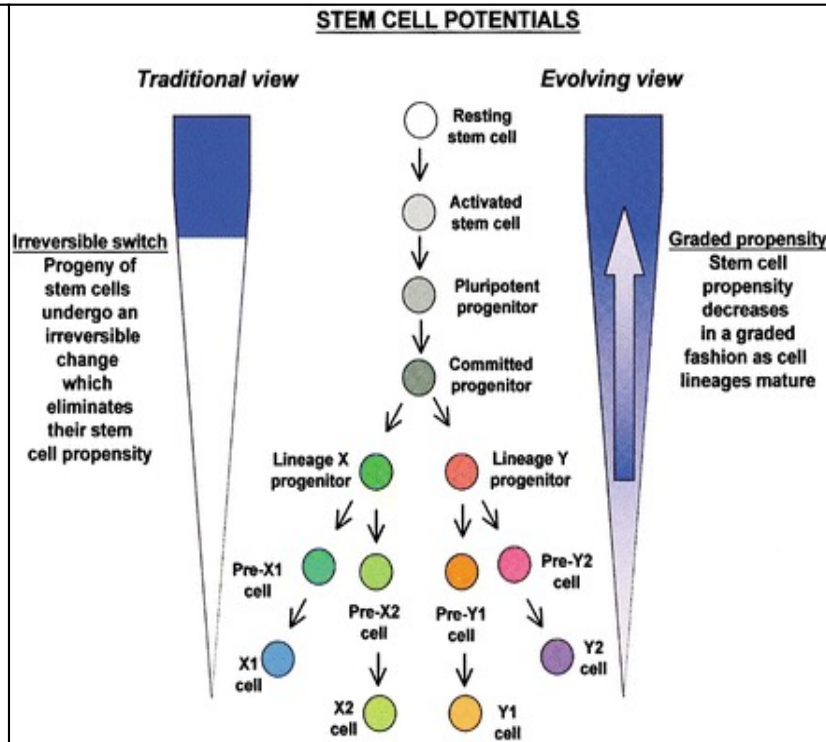


General concepts

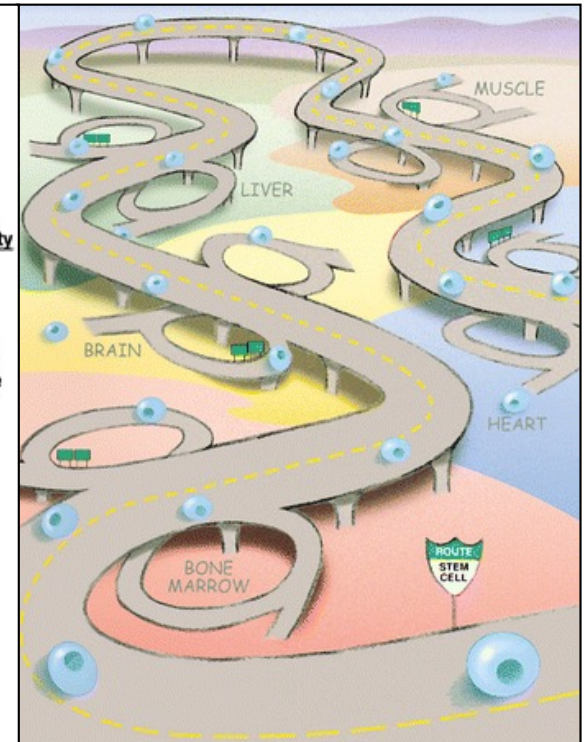
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QUESTIONS
REFERENCES