Hematopoietic stem cells and niche

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where is
the niche?

B. Lord, 1975

He showed that primitive cells tended to localize toward the endosteal margins, leading to the hypothesis that bone might regulate hematopoiesis

M. Dexter, 1977

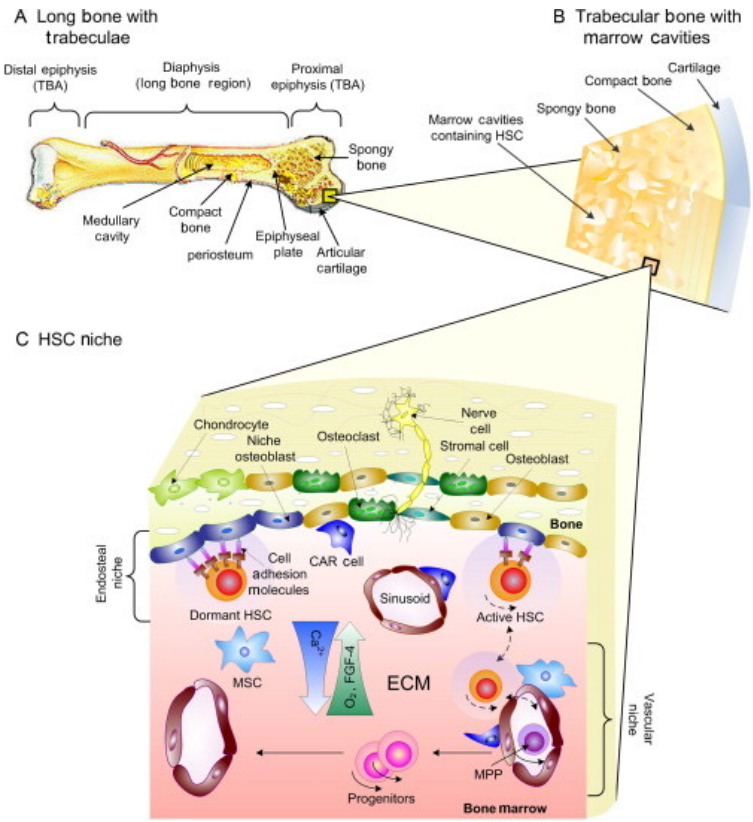
He showed that mesenchymal stromal cell cultures could maintain primitive haematopoietic cells ex vivo.

R. Schofield, 1978

A hypothesis is proposed in which the stem cell is seen in association with other cells which determine its behaviour.

Structural organization of the hematopoietic stem cell niche

- Controls on quiescence/ differentiation:
  - bone cell matrix
  - paracrine factors
  - systemic hormones
  - neuronal signals
  - O2 concentration
  - Ca^{2+} concentration



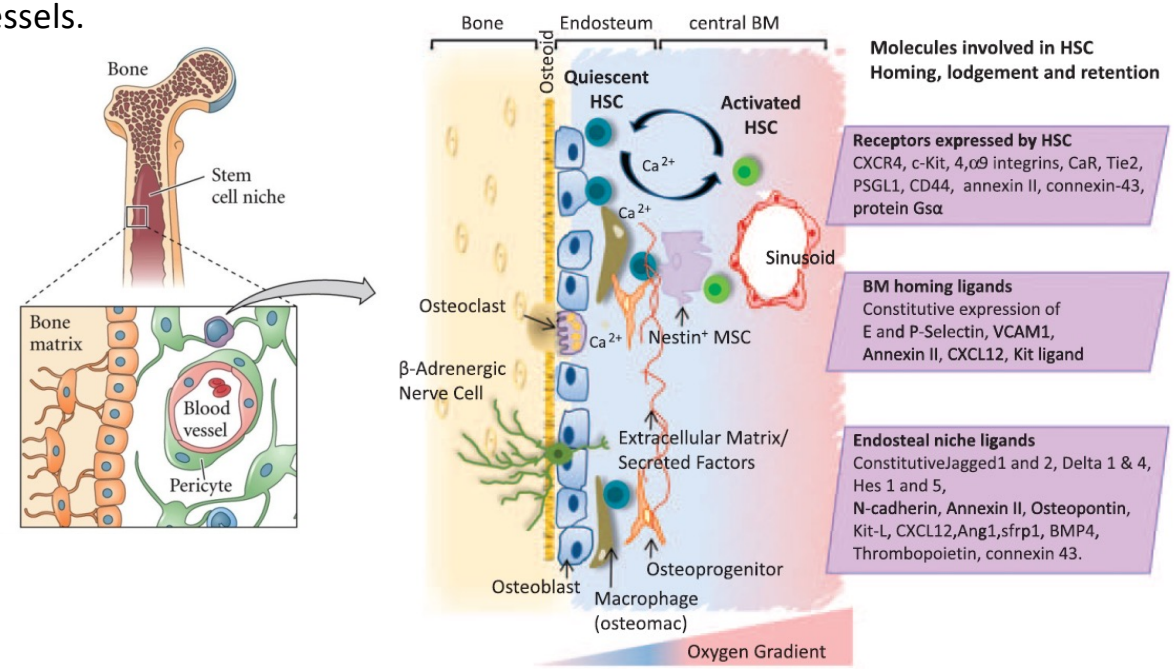
Wasnik et al, International Review of Cell and Molecular Biology 2012

Hematopoietic stem cell niche

It is the physiological microenvironment of Hematopoietic Stem Cells (HSCs) (Schofield 1978).

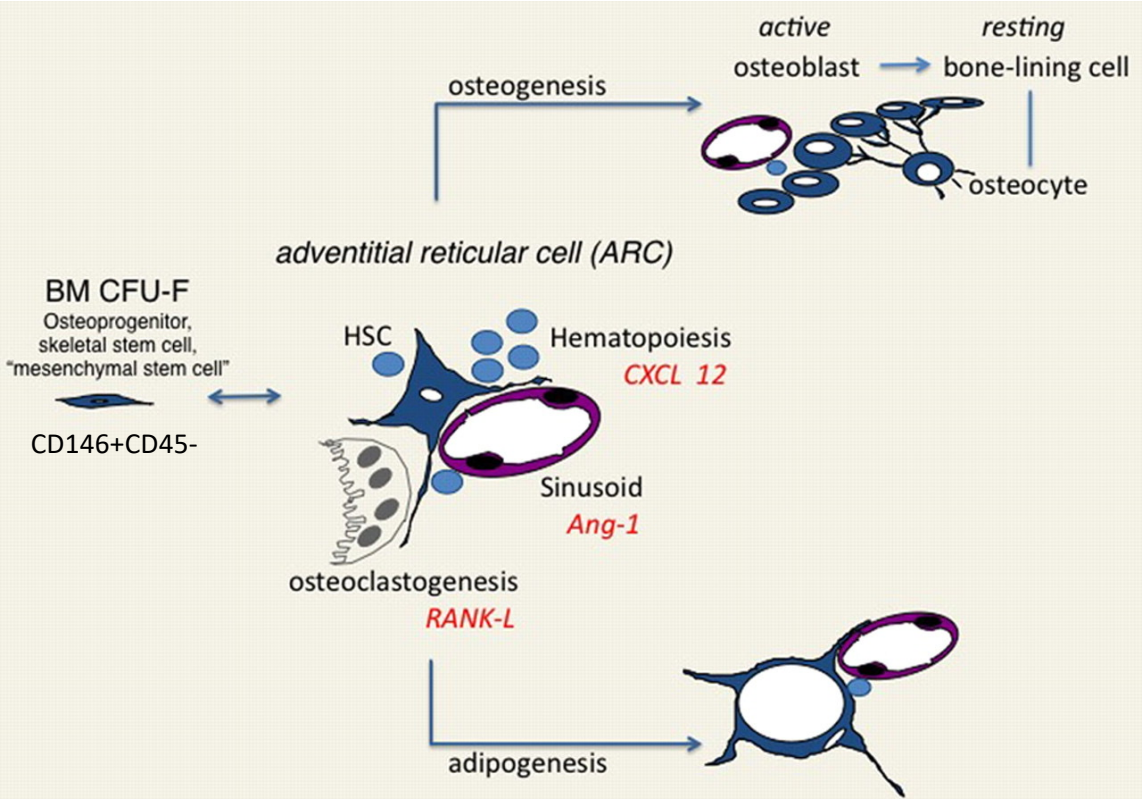
It is important for the balance between self-renewal and differentiation of HSCs.

It can be divided into endosteal niche, in which HSCs are maintained quiescent and into vascular niche, in which HSCs are activated, they proliferate, differentiate and go into blood vessels.



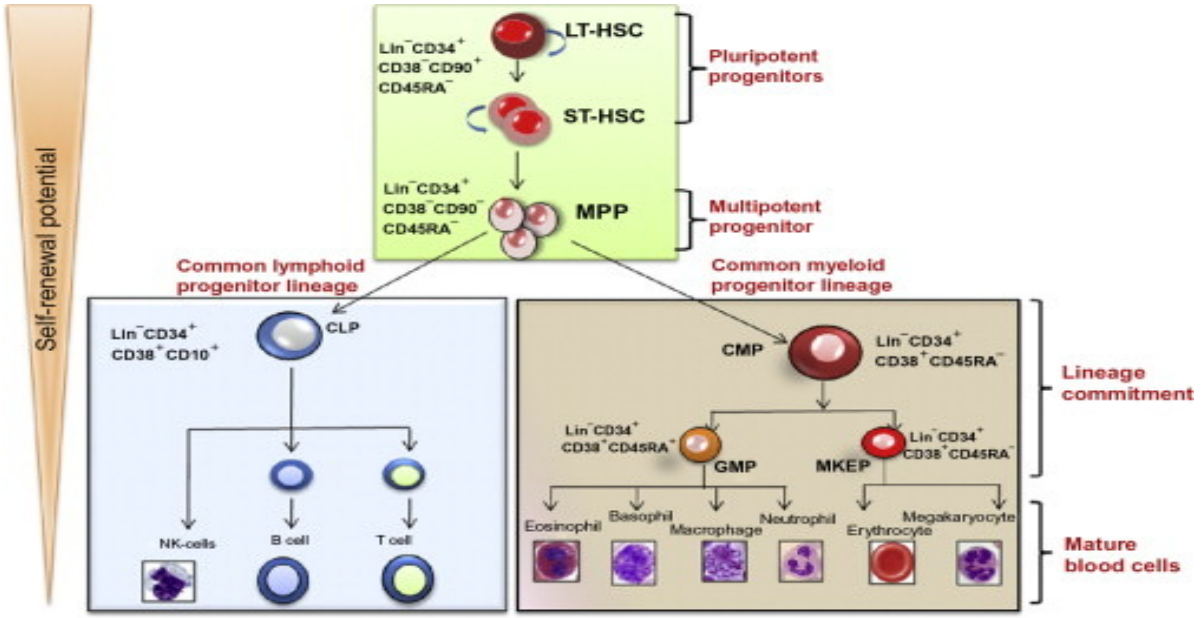
JP Lévesque et al. Leukemia (2010)

The central organizer of the hematopoietic niche

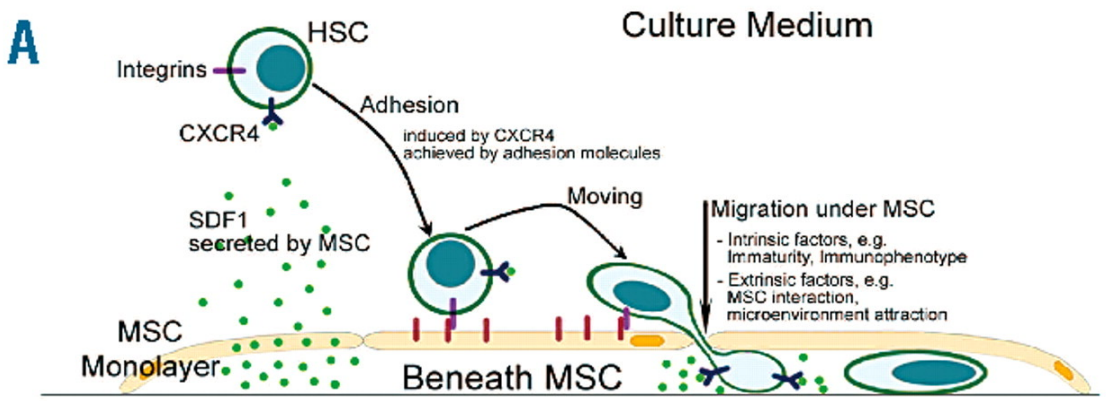


Bianco P., Blood 2011

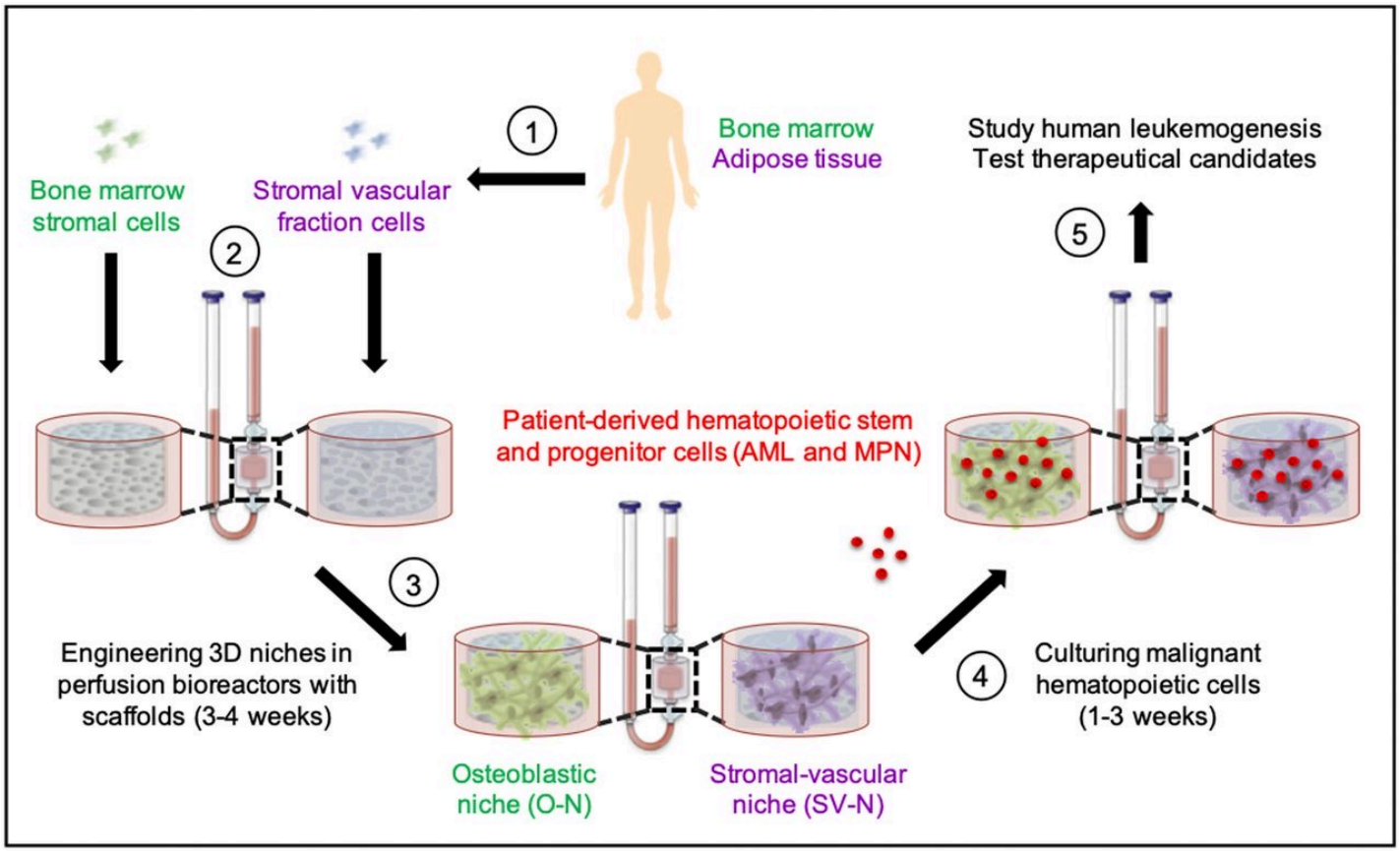
Hematopoietic cell hierarchy



Modeling the niche compartment in vitro

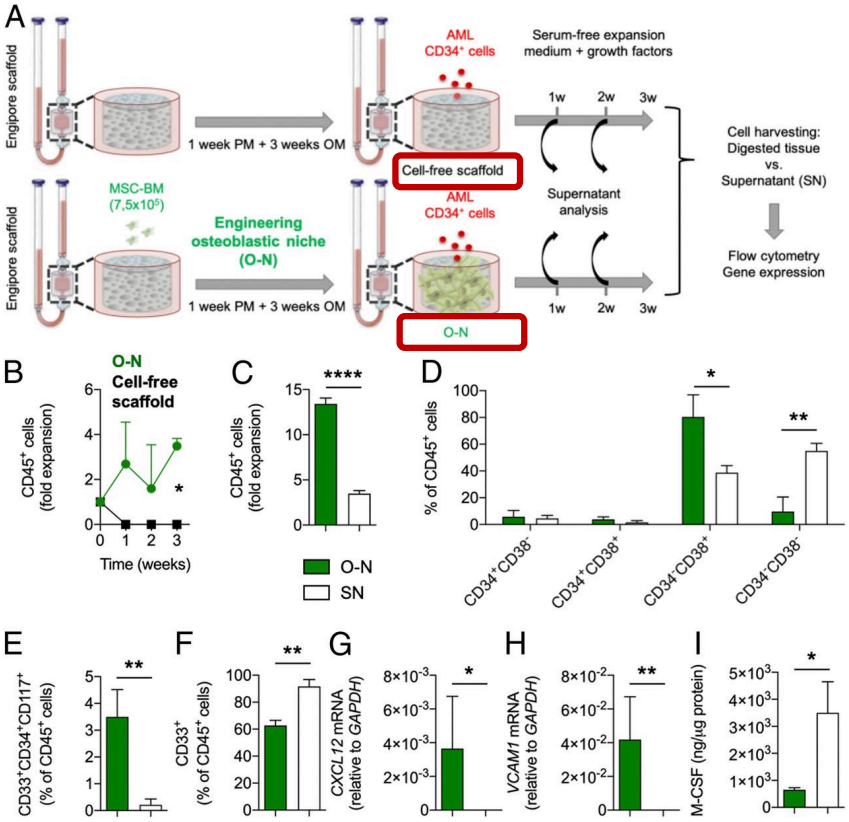


Bioengineering approach to generate patient-derived, biomimetic, and customizable 3D niches for malignant hematopoietic cells.



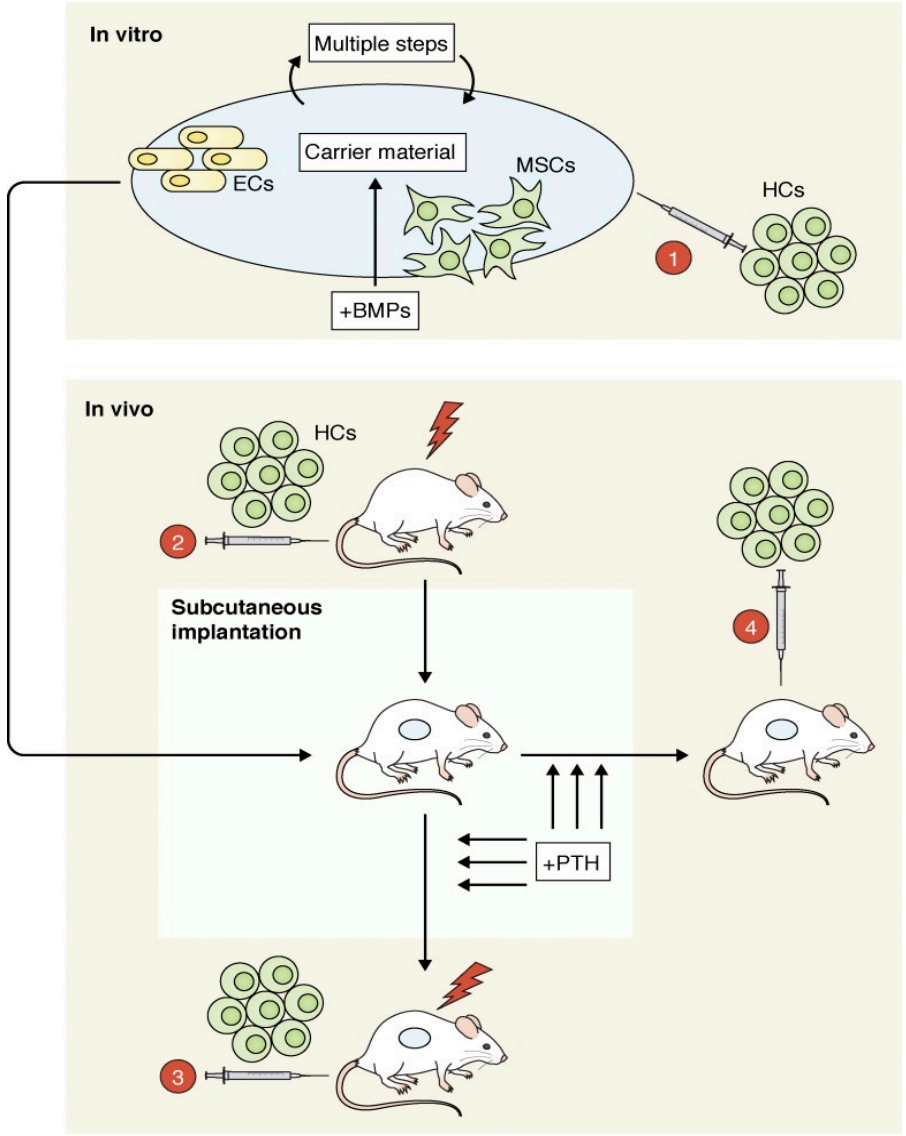
Andrés García-García et al. PNAS 2021;118:40:e2114227118

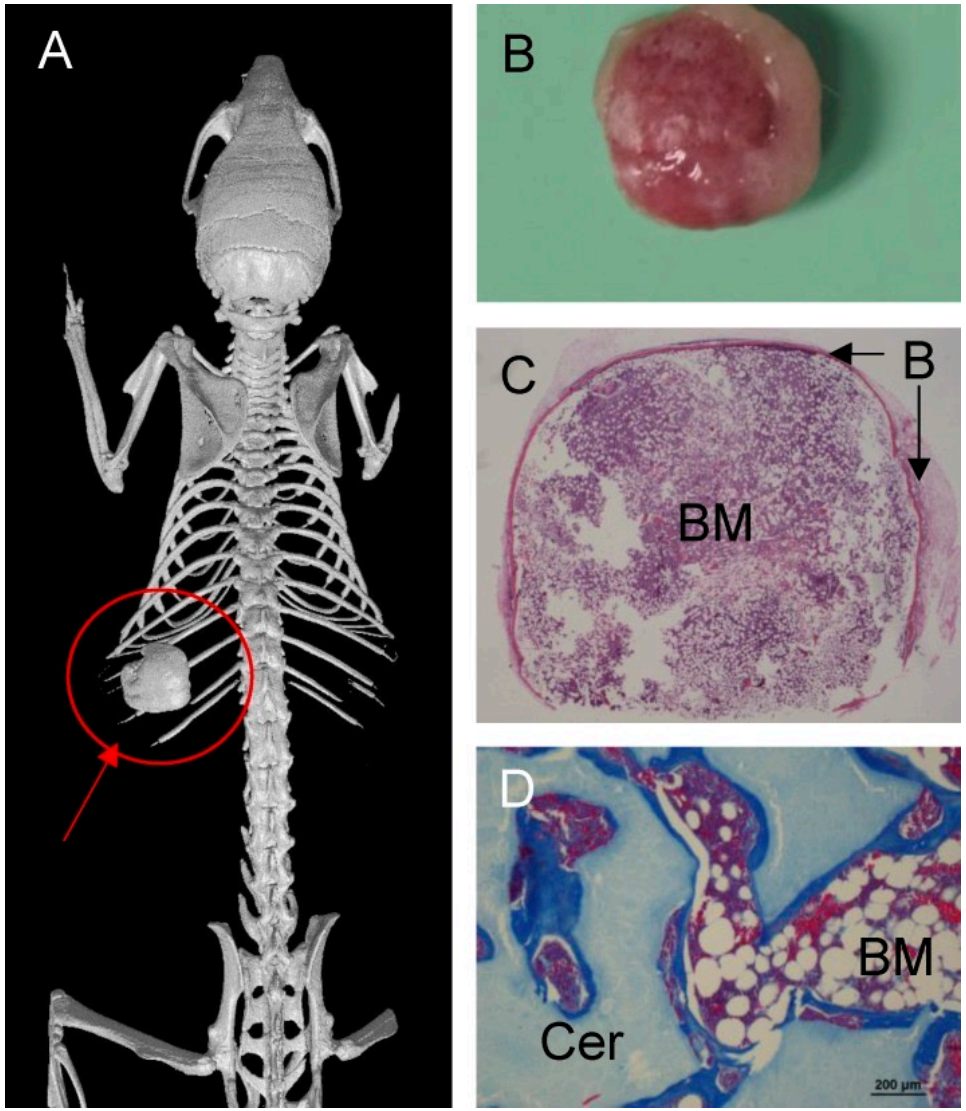
O-N can host patient-derived AML CD34+ cells preserving a fraction of leukemic progenitors and releasing differentiating cells to the supernatant (SN).



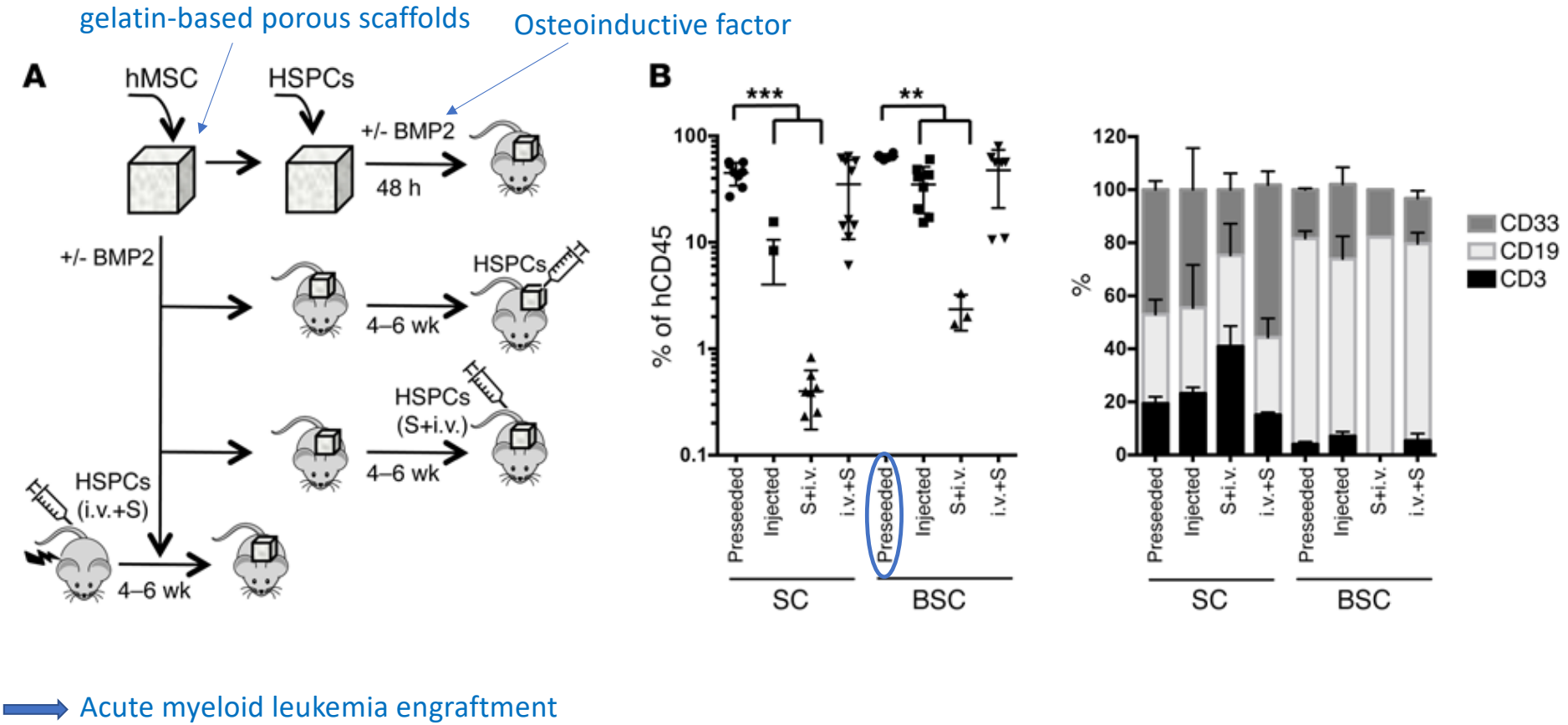
Andrés García-García et al. PNAS 2021;118:40:e2114227118

Modeling the Human Hematopoietic niche in vivo



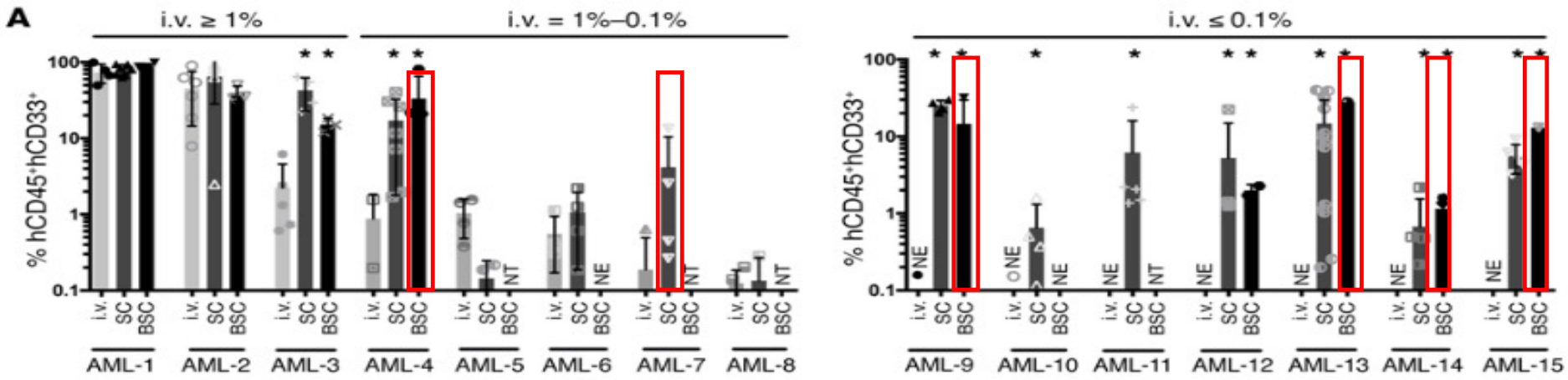

A
B
C
B
BM
D
BM
Cer
200 µm

Models of hematopoietic stem cell niche (1)



Acute myeloid leukemia engraftment

Abarrategi A, J Clin Invest 2017

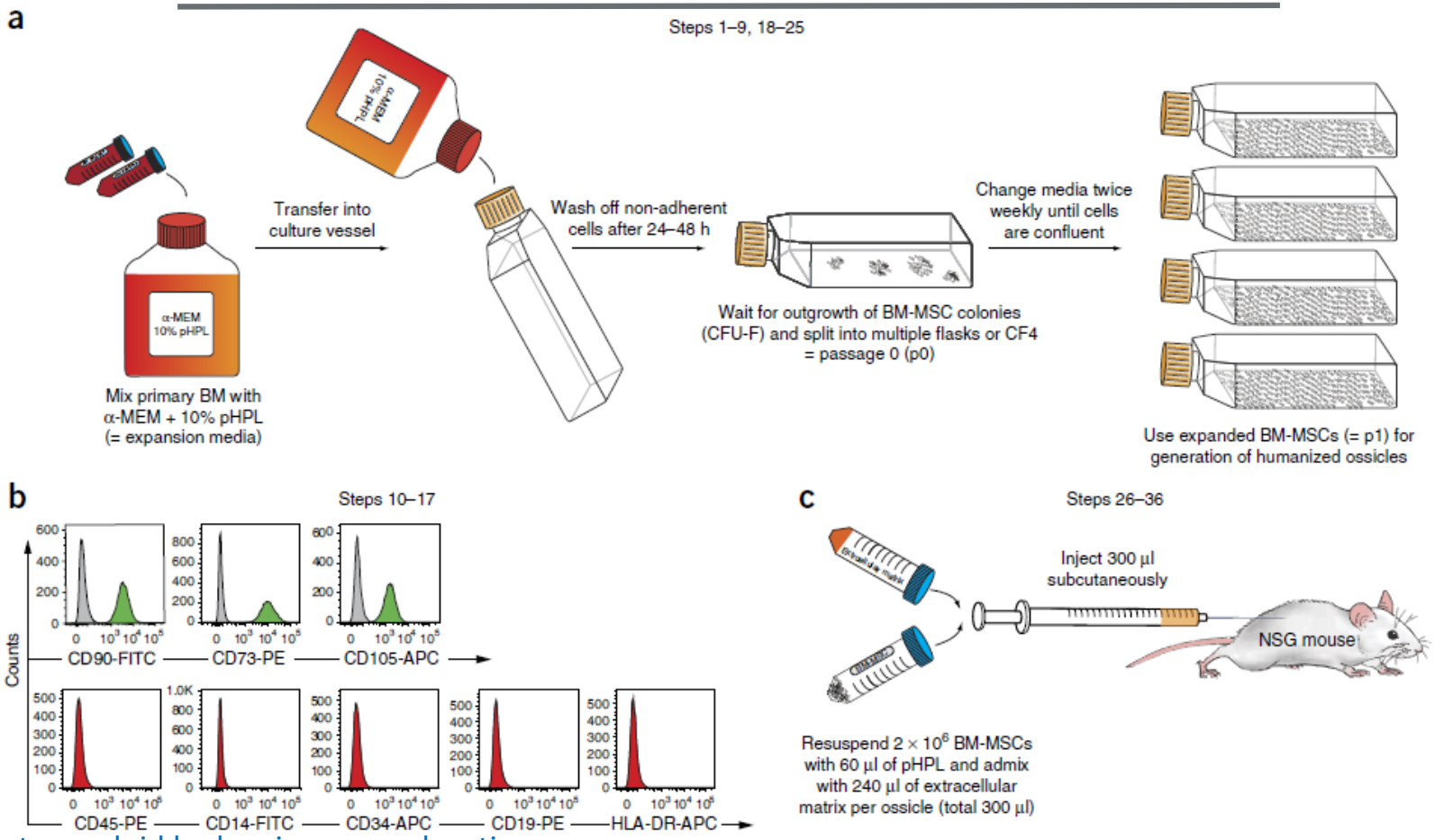


Low engrafters (between 1 to 0.1%) and non-engrafters (< 0.1%), that do not engraft with conventional i.v. transplantation, are able to engraft using the preseeding scaffold model

⟹ effective in vivo niche model for studying the human hematopoiesis

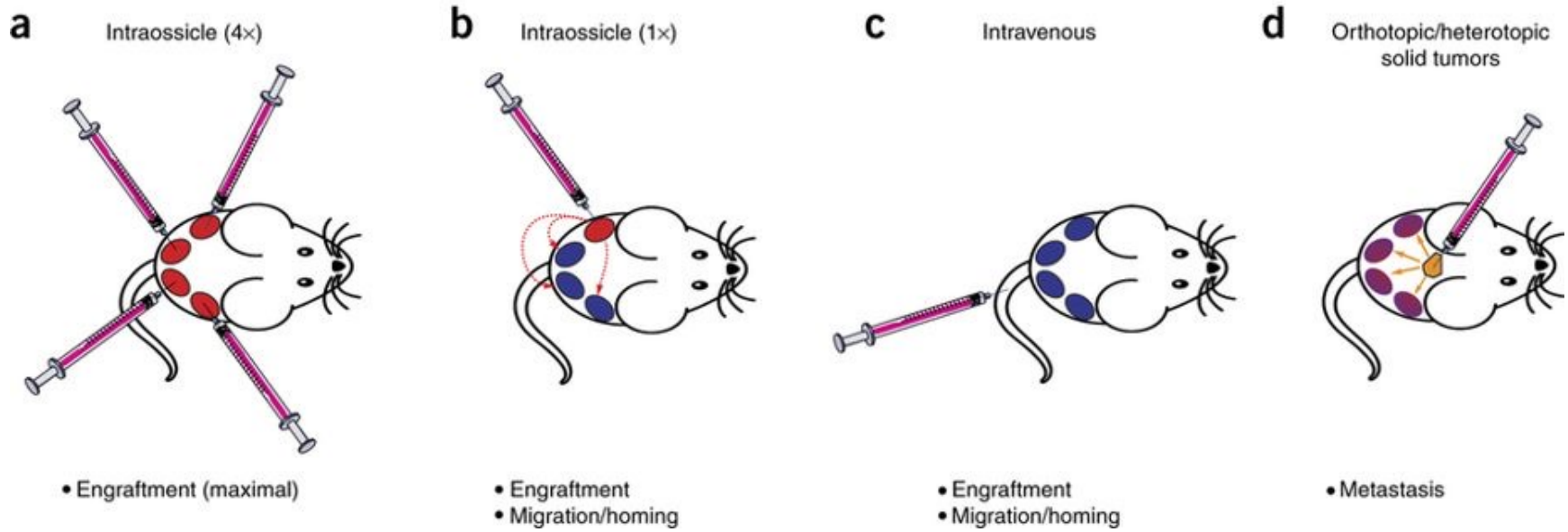
Abarrategi A, J Clin Invest 2017

Models of hematopoietic stem cell niche (2)

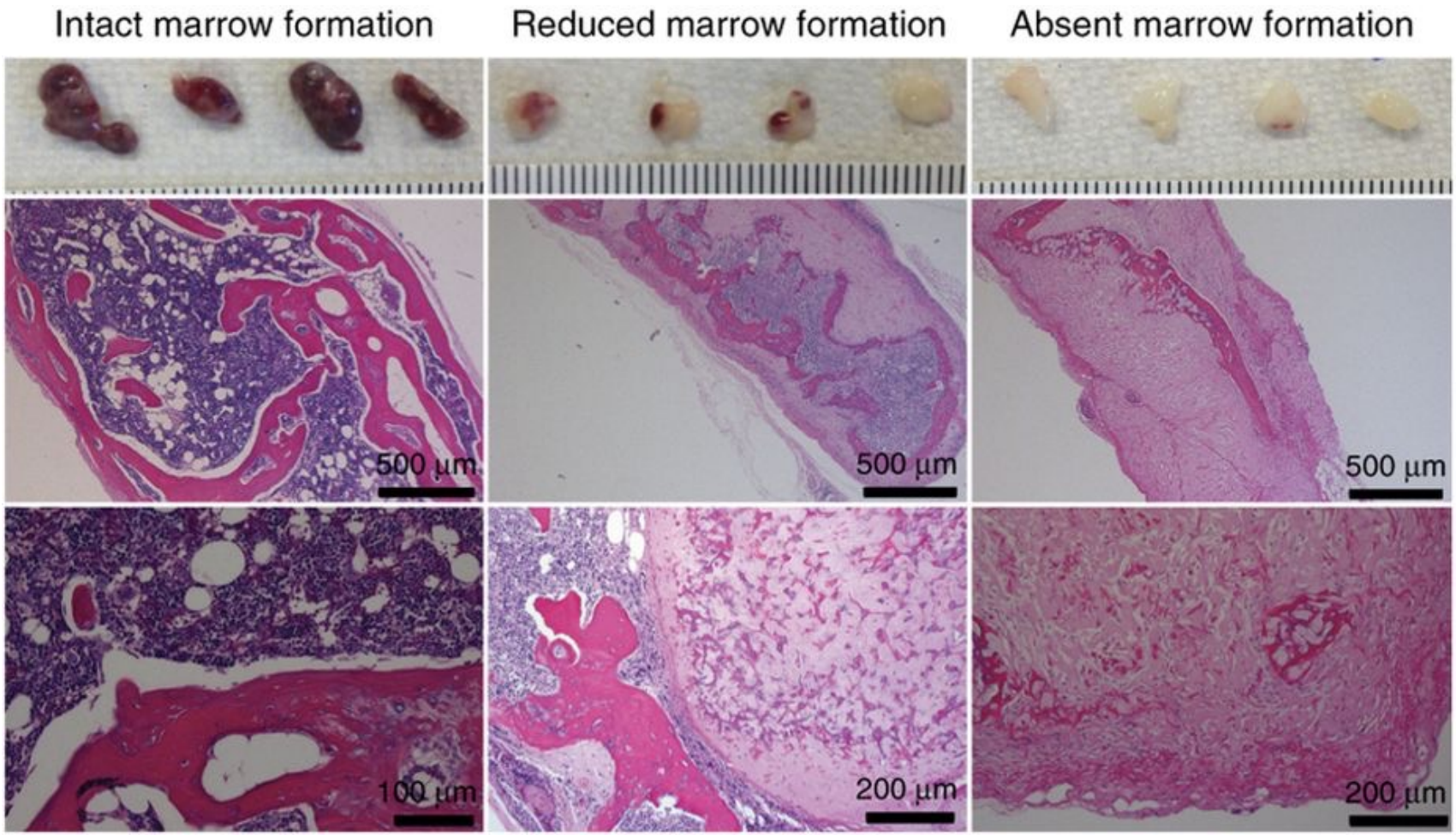


Acute myeloid leukemia, promyelocytic leukemia, myelofibrosis engraftment

Reinisch A, Nat Prot 2017


a
Intraossicle (4×)
• Engraftment (maximal)
b
Intraossicle (1×)
• Engraftment
• Migration/homing
c
Intravenous
• Engraftment
• Migration/homing
d
Orthotopic/heterotopic
solid tumors
• Metastasis

In vivo ossicle formation



Advantages of the method

- Generation of humanized ossicles that accurately reproduce BM microenvironment both morfologically and functionally
- Direct injection of human cells into a humanized microenvironment
- Direct intraossicle transplantation of hMSCs avoid cell loss due to intravenous transplantation
- Highly reproducible in vivo methodology for the study of normal and malignant human hematopoiesis

Limitations of the method

- Bone, cartilage, and MSCs within the ossicle BM microenvironment are of human origin, **but** the vasculature and the developing BM sinusoidal structures are mouse-derived → endothelial niche questions cannot be addressed
- Conditioning using sublethal irradiation significantly damage the BM niche and will therefore influence studies of HSC regulation by the humanized microenvironment

Reinisch A, Nat Prot 2017

Limitations

IN VITRO

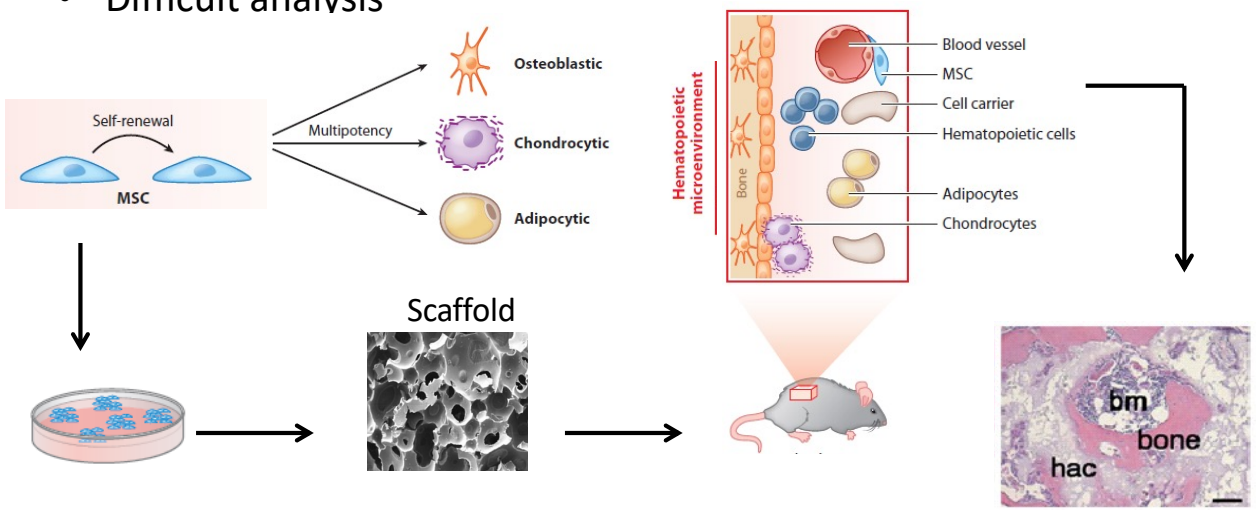
Based on co-colture of stromal cells with HSCs

- They do not recapitulate the complexity of the microenvironment

IN VIVO

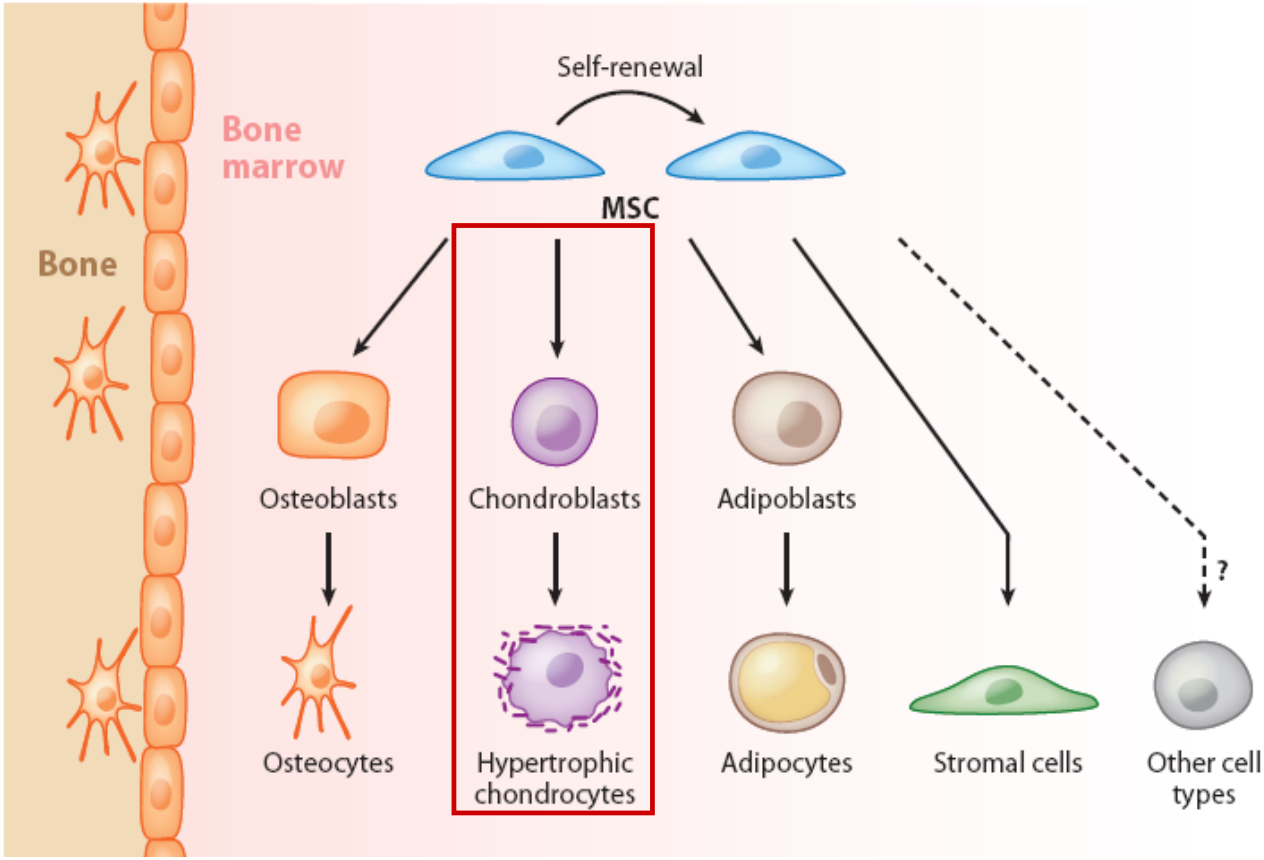
Based on the use of scaffolds

- They require lots of MSCs
- The exogenous scaffold cannot recapitulate the physiological process
- Difficult analysis



Adapted from PS Frenette et al. Annu. Rev. Immunol. (2013)

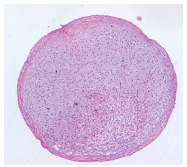
Bone marrow mesenchymal stromal cells

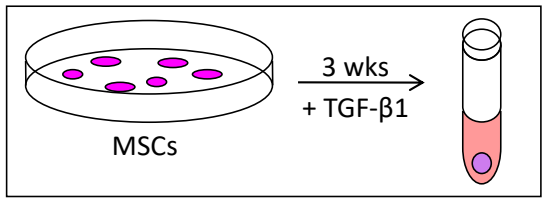


Frenette P., Annu. Rev. Immunol. 2013

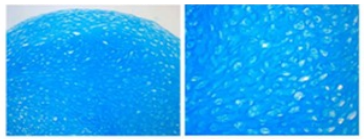
Chondrogenic differentiation of human mesenchymal stromal cells

H&E

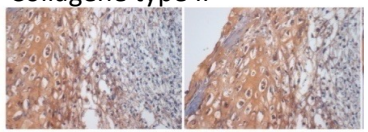




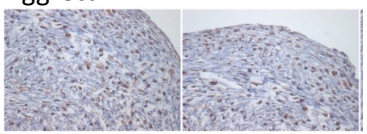
Alcian blue

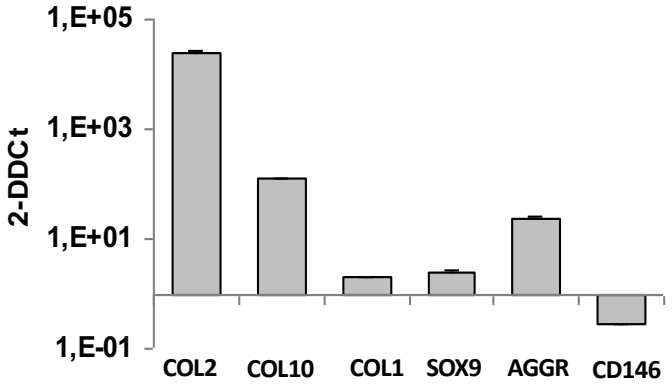


Collagene type II



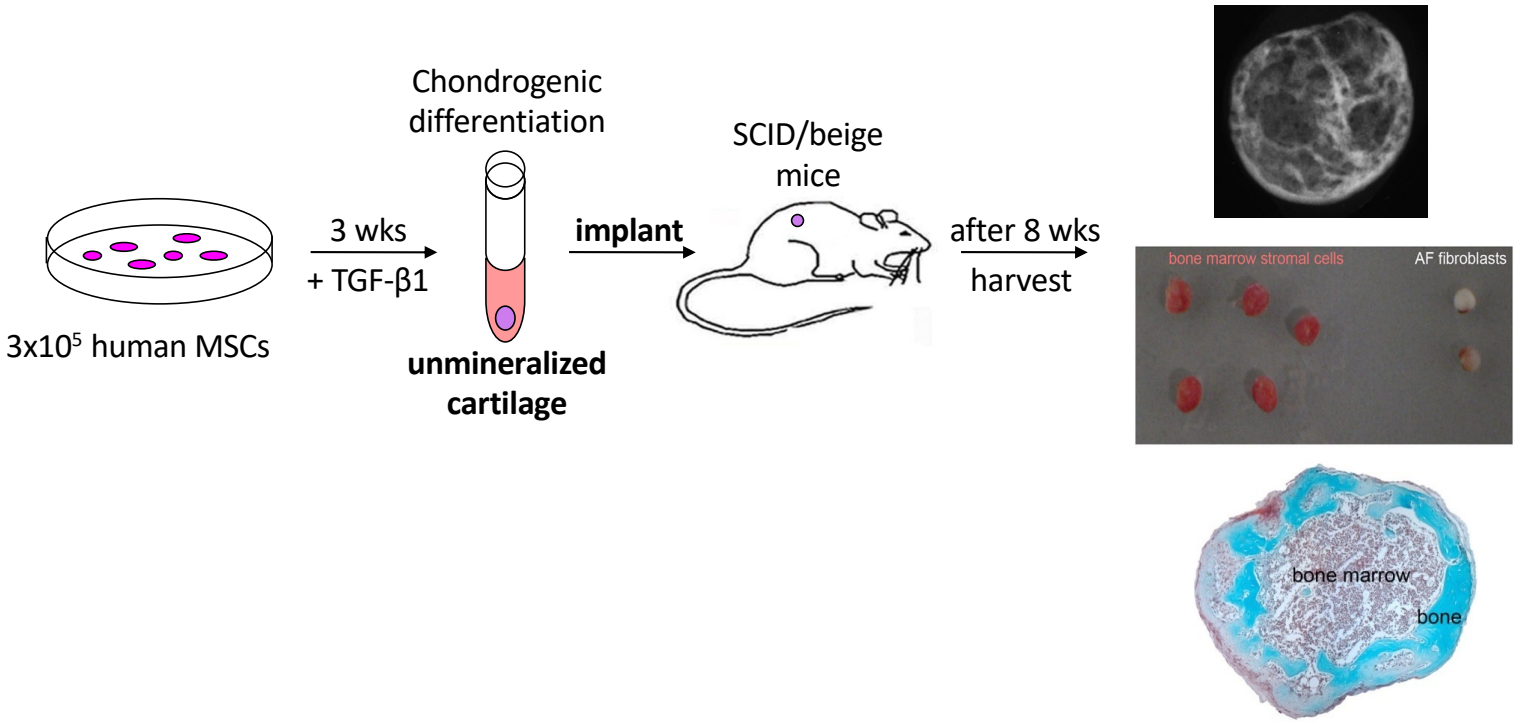
Aggrecan





Gatto F. et al, Stem Cells and Development 2012
Pievani A. et al, Cytotherapy 2014

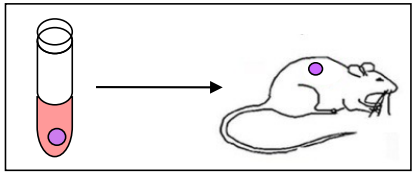
Generation of a Humanized Bone/Bone Marrow Organoid



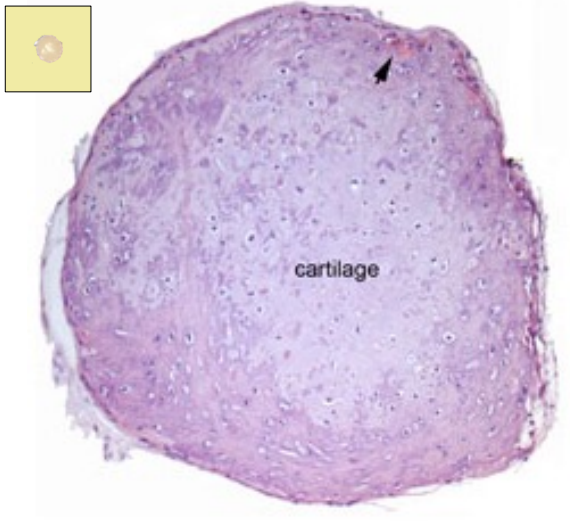
MSCs-derived chondroid pellets can generate heterotopic ossicles with no exogenous scaffold

Gatto F et al, Stem Cell and Dev 2012
Serafini M et al. Stem Cell Research 2014

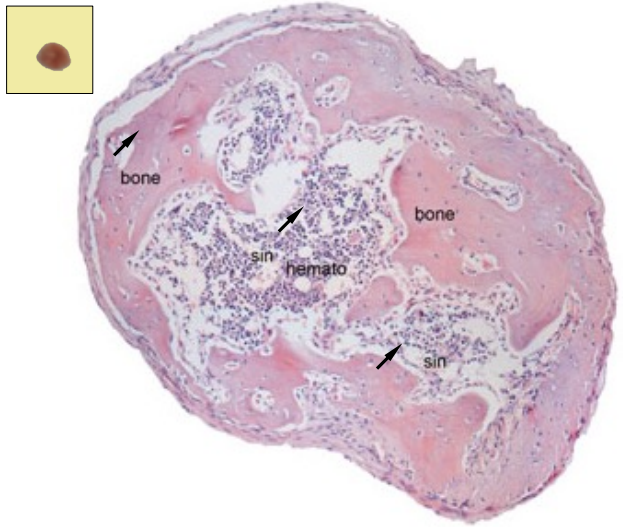
Formation of an Extramedullary Ossicle



Pre-implant

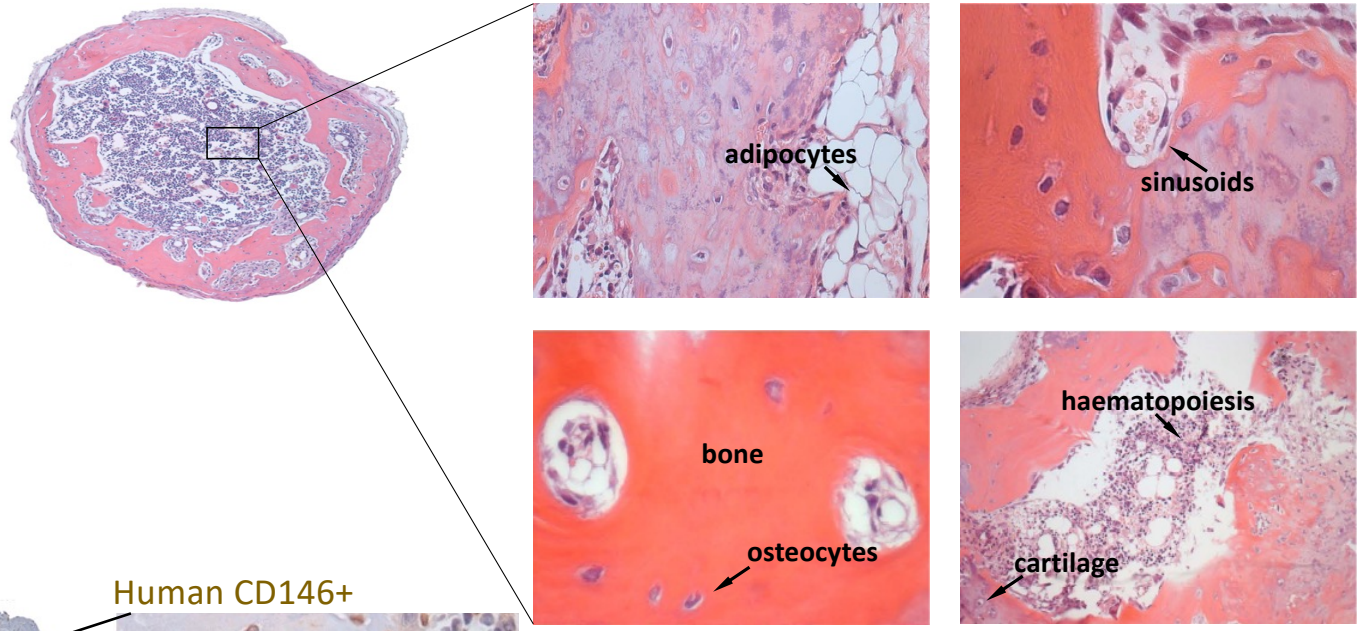


Post-implant (8 wks)

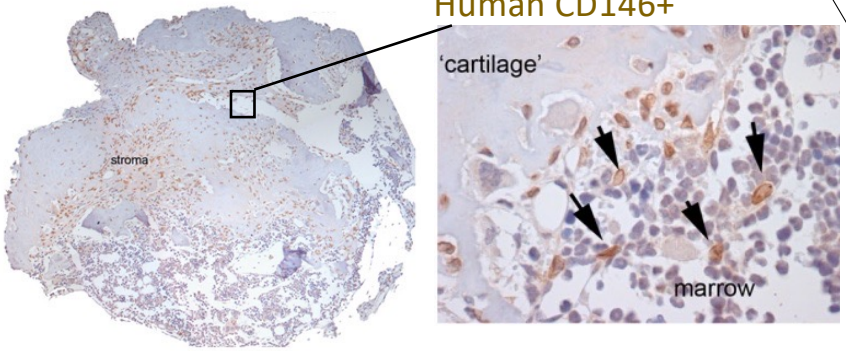


The replacement of cartilage by bone is known as endochondral ossification

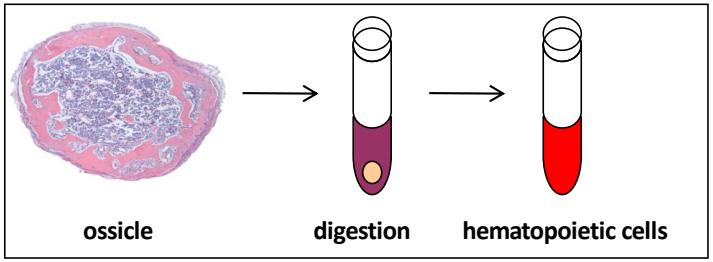
Bone marrow niche cellular players

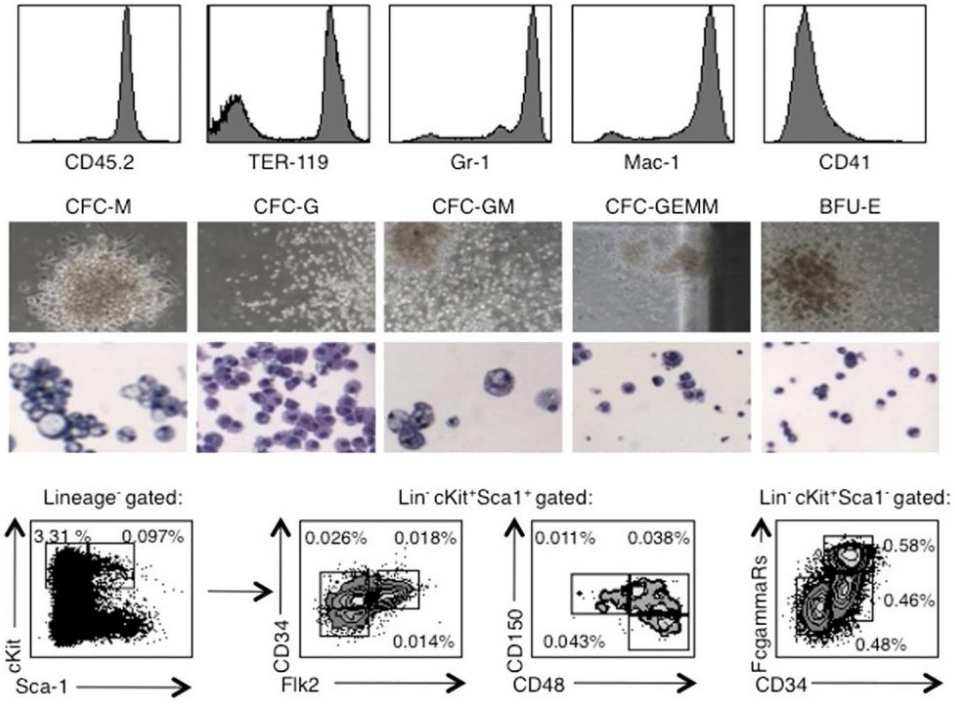


Human CD146+

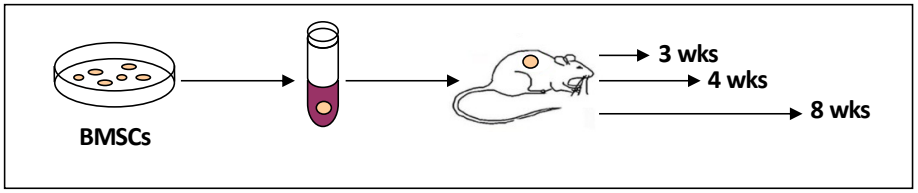


Hematopoietic lineages in heterotopic bone marrow

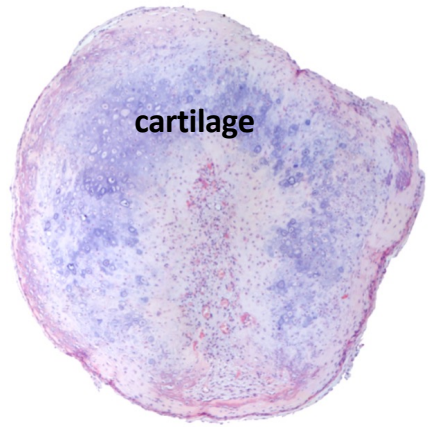




Ossicles formation at different time points

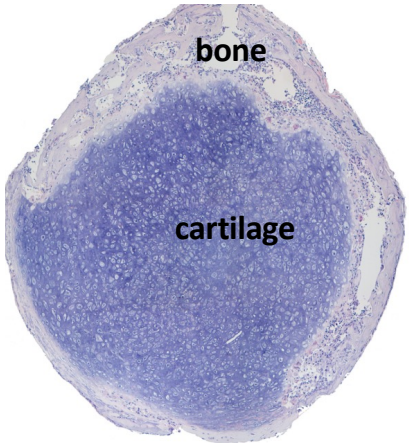


3 wks



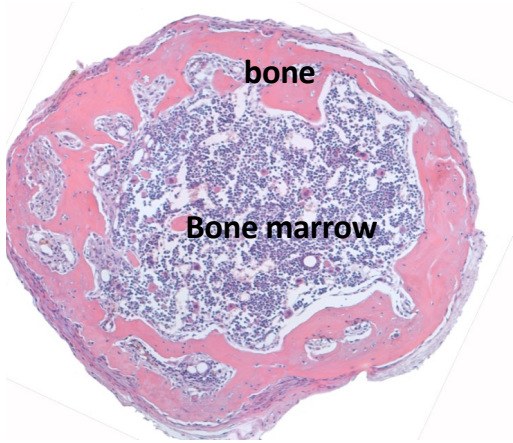
- Maturation of hypertrophic cartilaginous template

4 wks



- Development of a bony collar
- Vascularization
- Osteoclastic resorption of the cartilage template

8 wks



- Appearance of hematopoietic foci