«Regulation of Hematopoietic stem cells (HSC) by osteoblastic niches»

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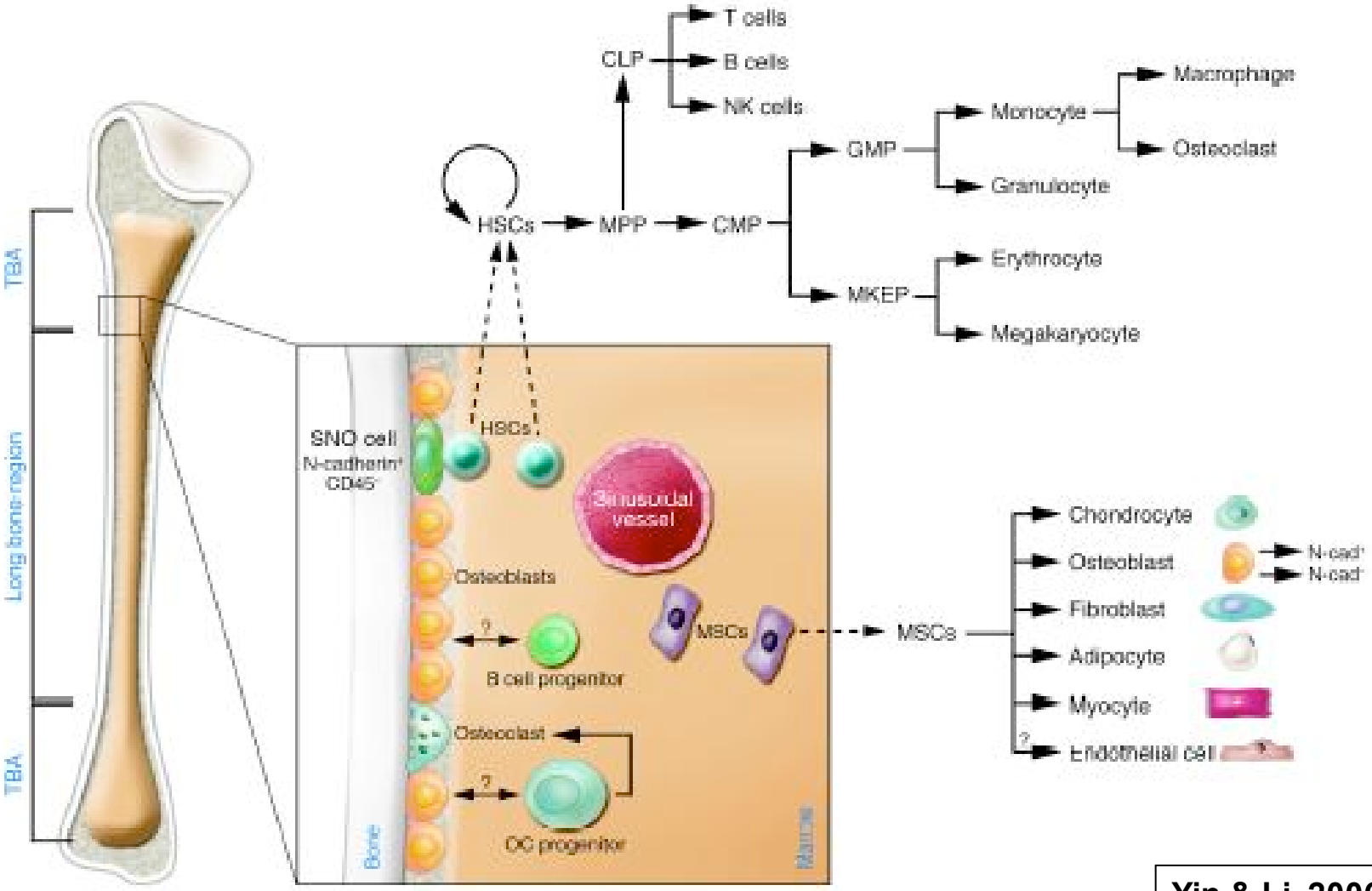
The opportunity to regulate HSC by “Niches”

HSC expansion is deeply desired for clinical hematology. The attempts to increase the number of long-term repopulating HSC ex vivo by cocktails of growth factors had failed.

«Niches» are composed of microenvironmental cells that nurture stem cells and enable them to maintain tissue homeostasis.

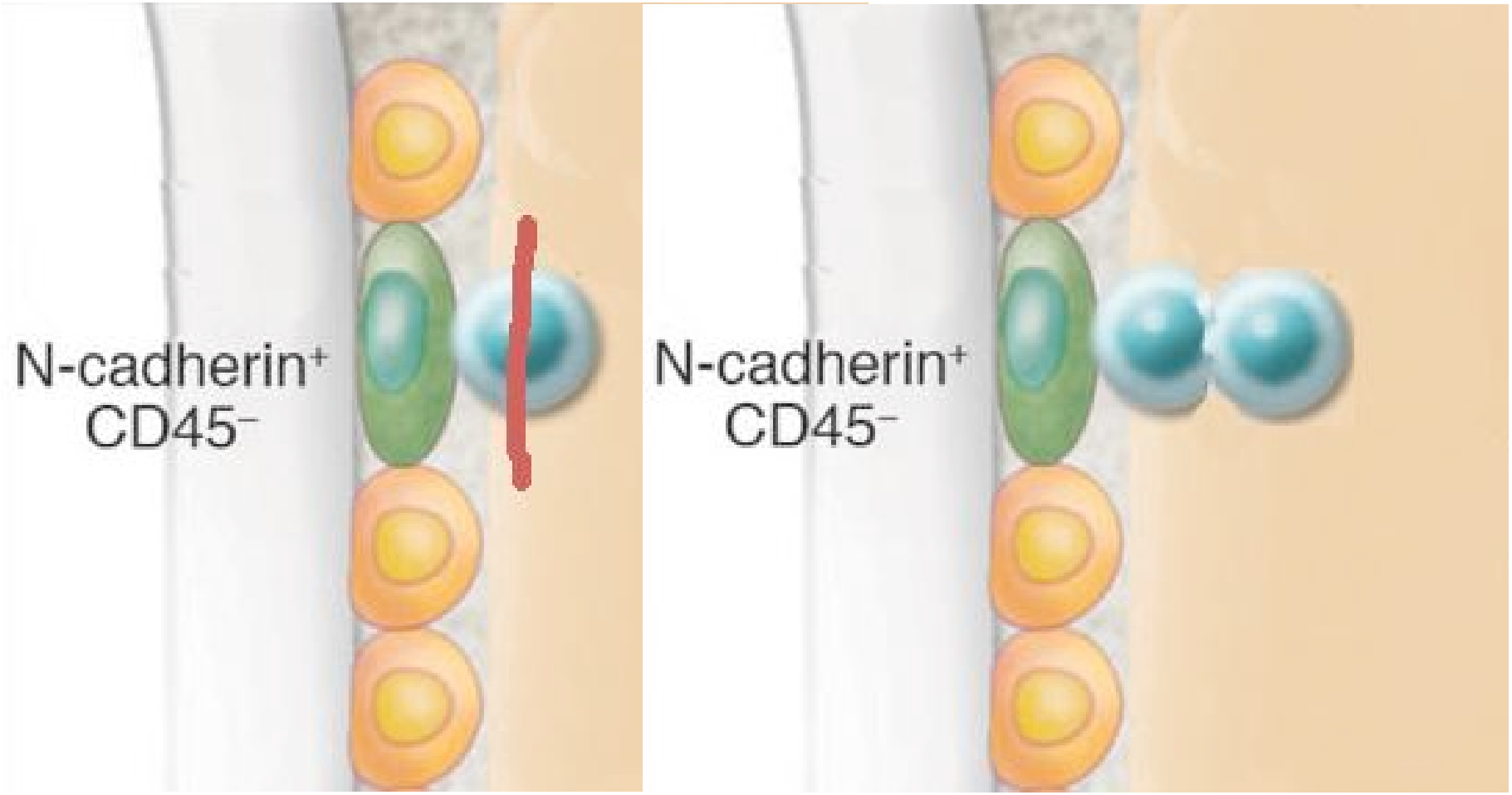
Expansion of HSC indirectly by influence upon niches, capable to maintain balance of stem cell quiescence and activity, could provide tools for achievement of clinically useful amount of these cells.

Model of support for hematopoiesis by osteoblasts

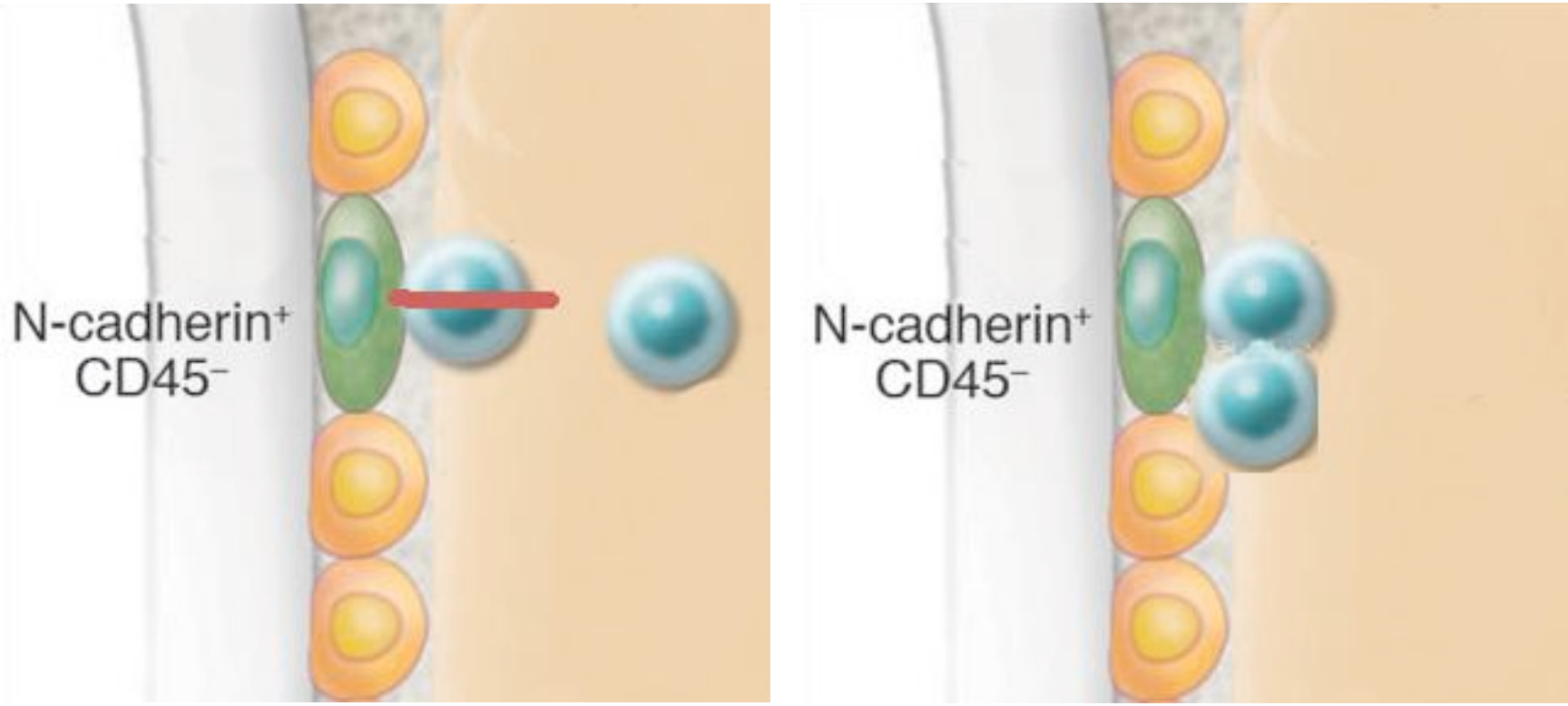


Yin & Li, 2006

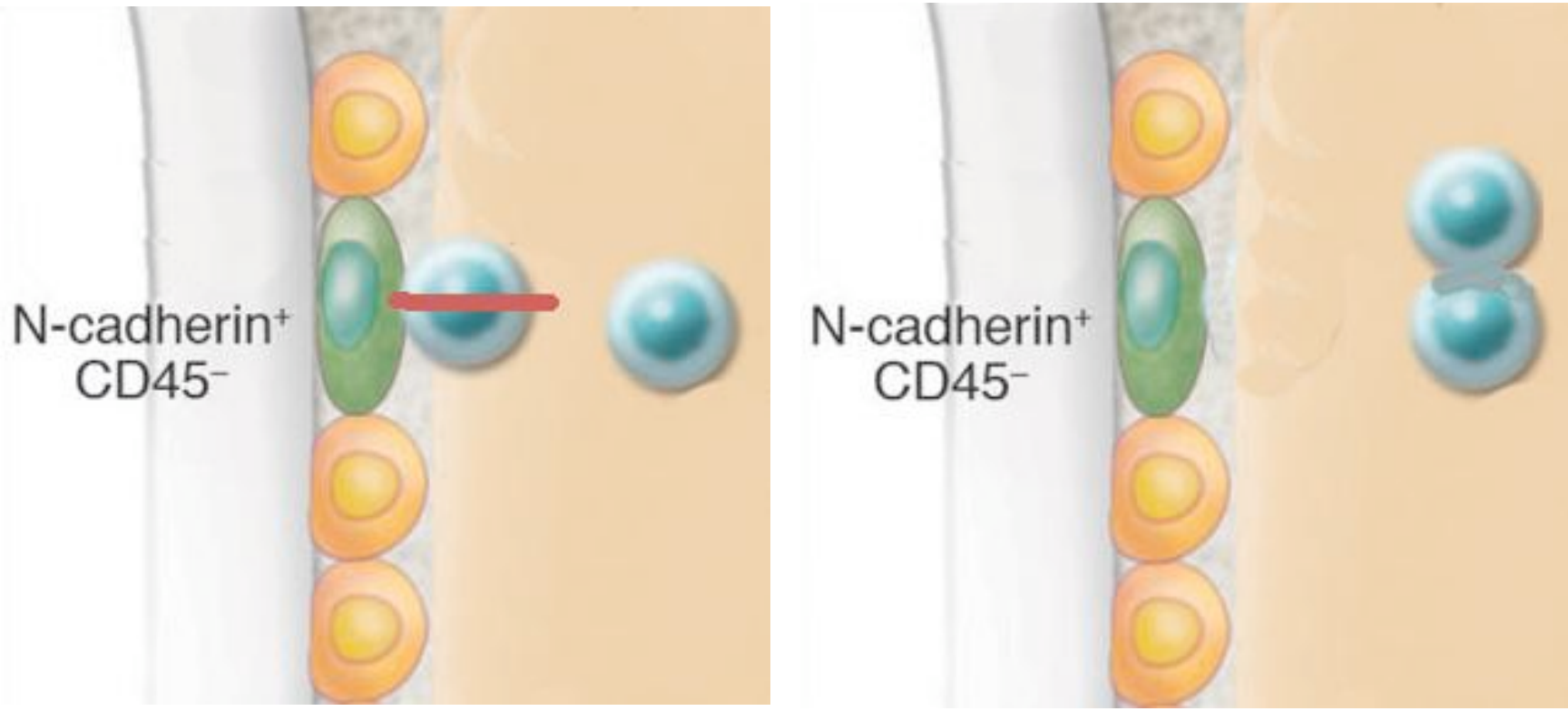
Model of maintenance of HSC by osteoblasts



Model of maintenance of HSC by osteoblasts



Model of maintenance of HSC by osteoblasts



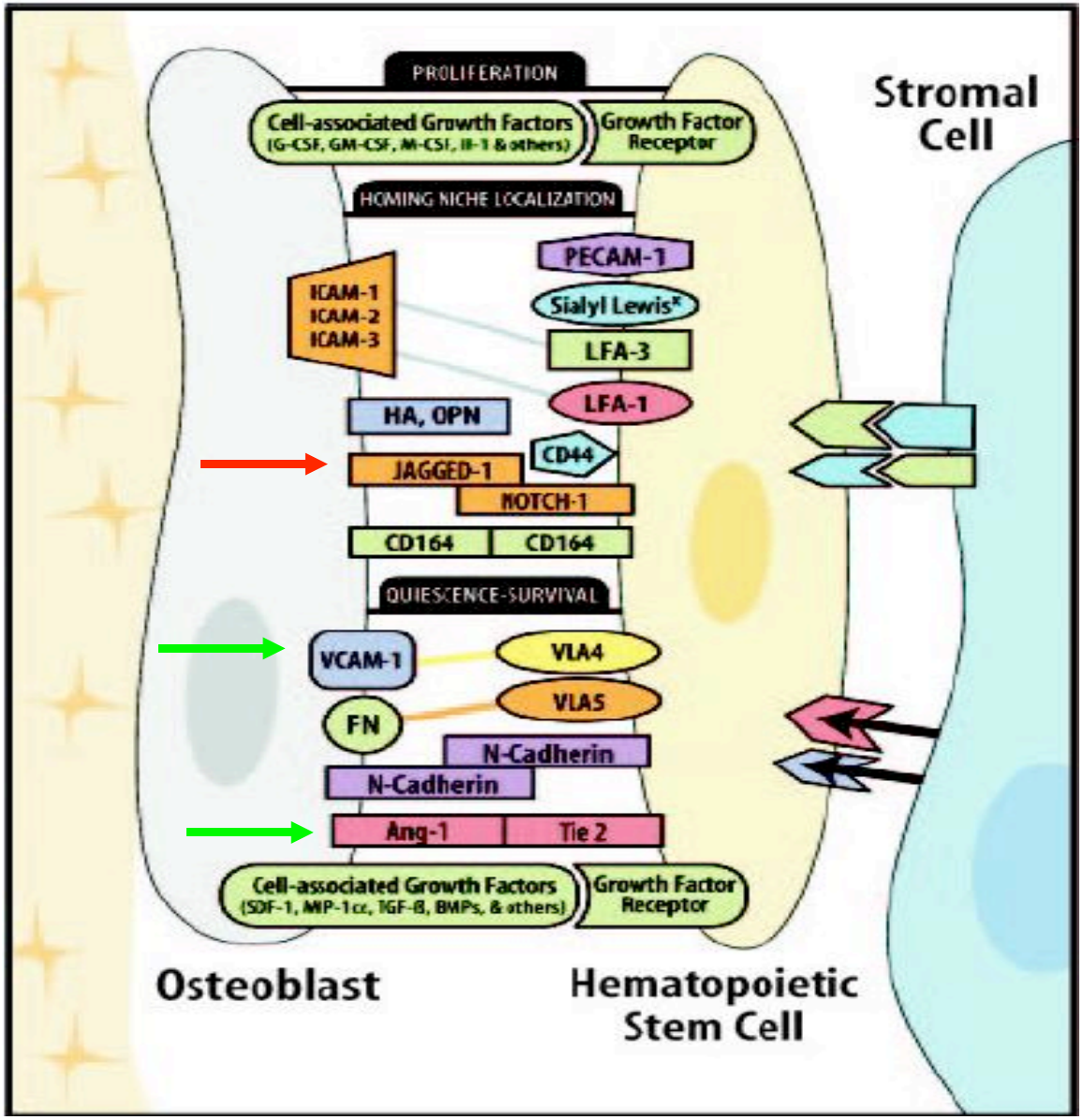
Model of support for hematopoiesis by osteoblasts

The system regulator of osteoblasts is **parathyroid hormone.**

Parathyroid hormone (PTH) activates osteoblasts

Parathyroid hormone (PTH) stimulates the division of osteoblasts

Osteoblast-HSC adhesion-ligand pairs



Taichman, 2005

Model of support for hematopoiesis by osteoblasts

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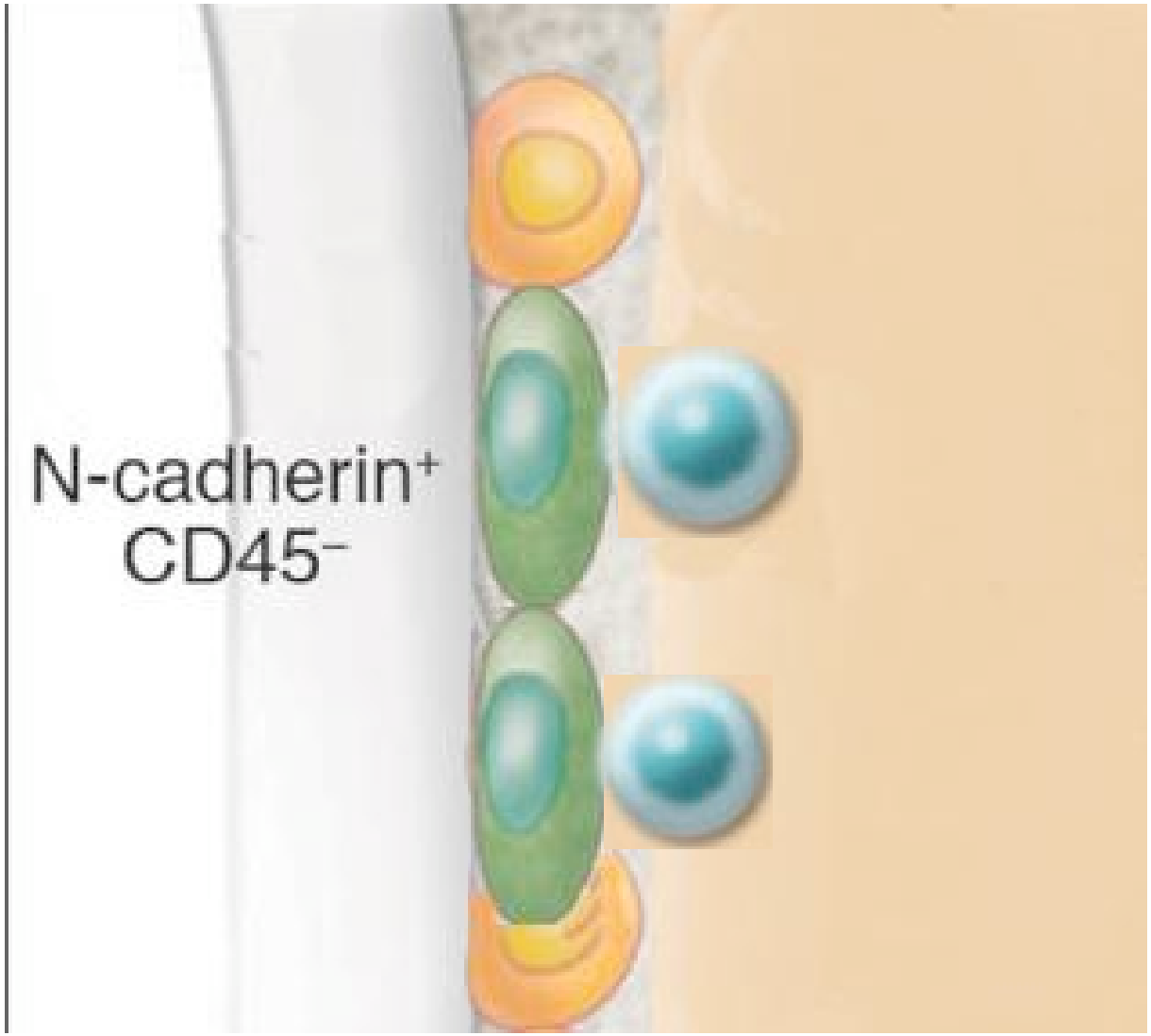
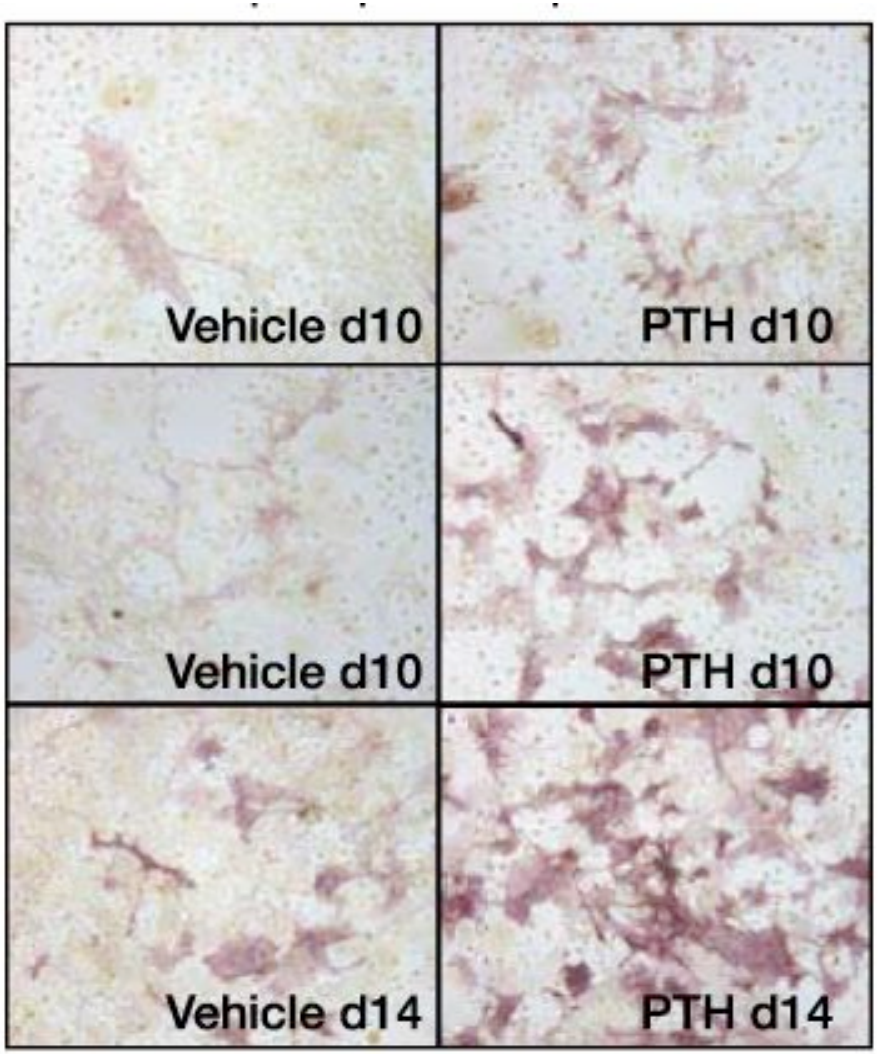
Model of increased number of osteoblasts

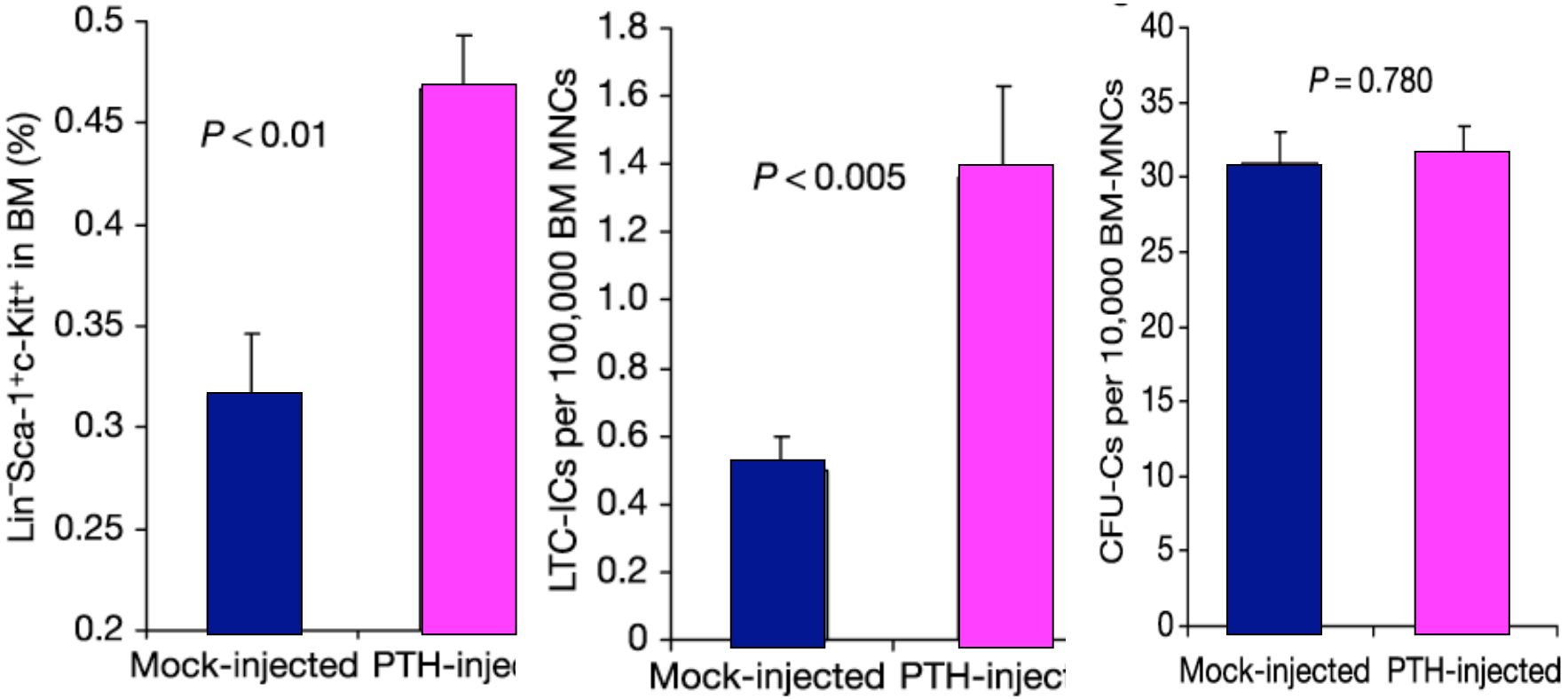


Model of increased number of osteoblasts – alkaline phosphatase expression.



Calvi et al, 2003

The increased number of hematopoietic precursor cells



Calvi et al, 2003

Competitive repopulation assay

- The only method for characterization of most primitive stem cells, capable for long-term reconstitution of irradiated recipients is competitive repopulation assay.
- This method allows to identify the selective advantage of stem cells maintaining hematopoiesis at the level of stem cells recruitment that serves to identify a more primitive class of stem cells with a greater capacity for competitive long-term repopulation.
- The stem cell defined by this assay is termed a competitive repopulating unit (CRU).
- In this assay, histocompatible but genetically distinguishable "test" stem cells are injected into lethally irradiated mice together with a large excess of marrow cells capable of reconstitution when transplanted alone.

Influence of PTH on HSC in vivo
Experimental design

Control groups

Experimental groups

PTH 80 g/kg
4 weeks

Bm

Bm

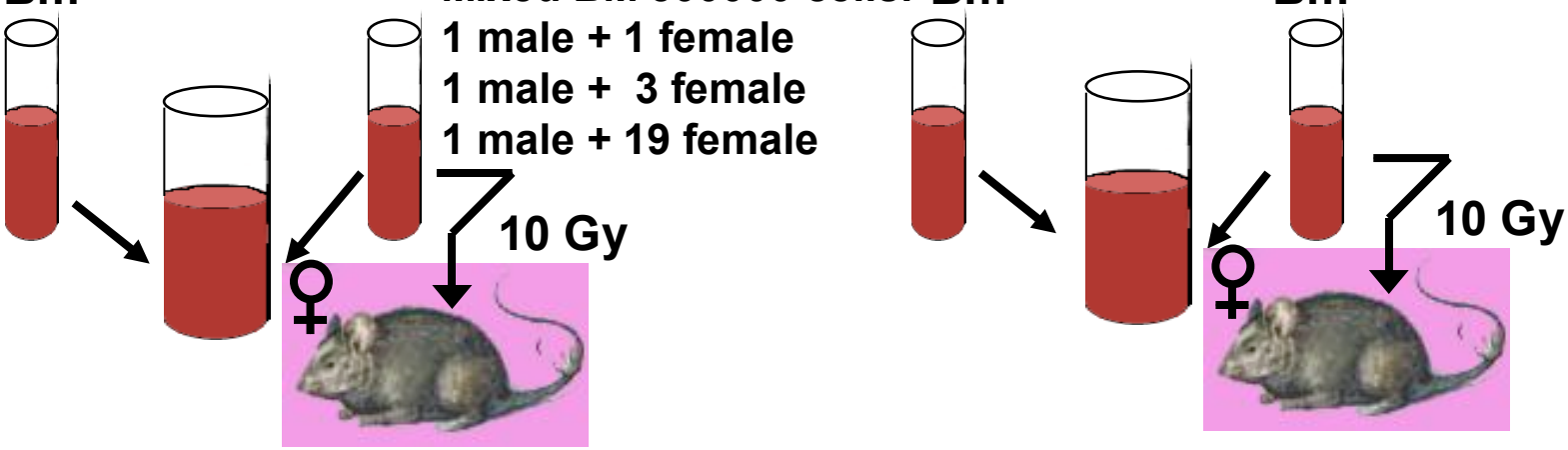
Mixed BM 500000 cells.
1 male + 1 female
1 male + 3 female
1 male + 19 female

10 Gy

Bm

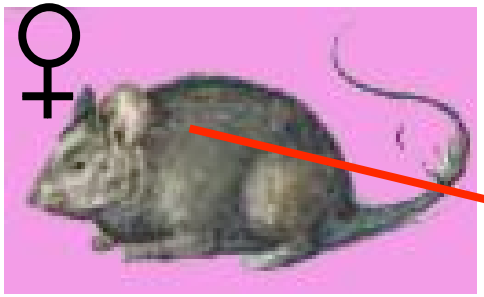
Bm

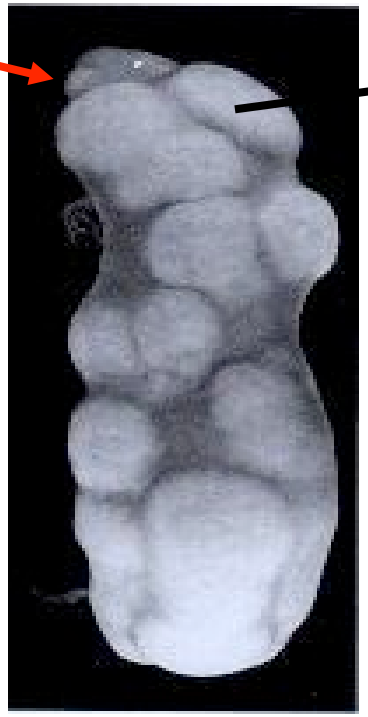
10 Gy



6 groups of mice reconstituted by limiting number of “test” HSC were prepared

Bone marrow cells from reconstituted mice 3, 10, 16 and 20 months later were aspirated repeatedly and transplanted into secondary irradiated recipients. The gender of CFU-S transplantation was studied 10 days later.

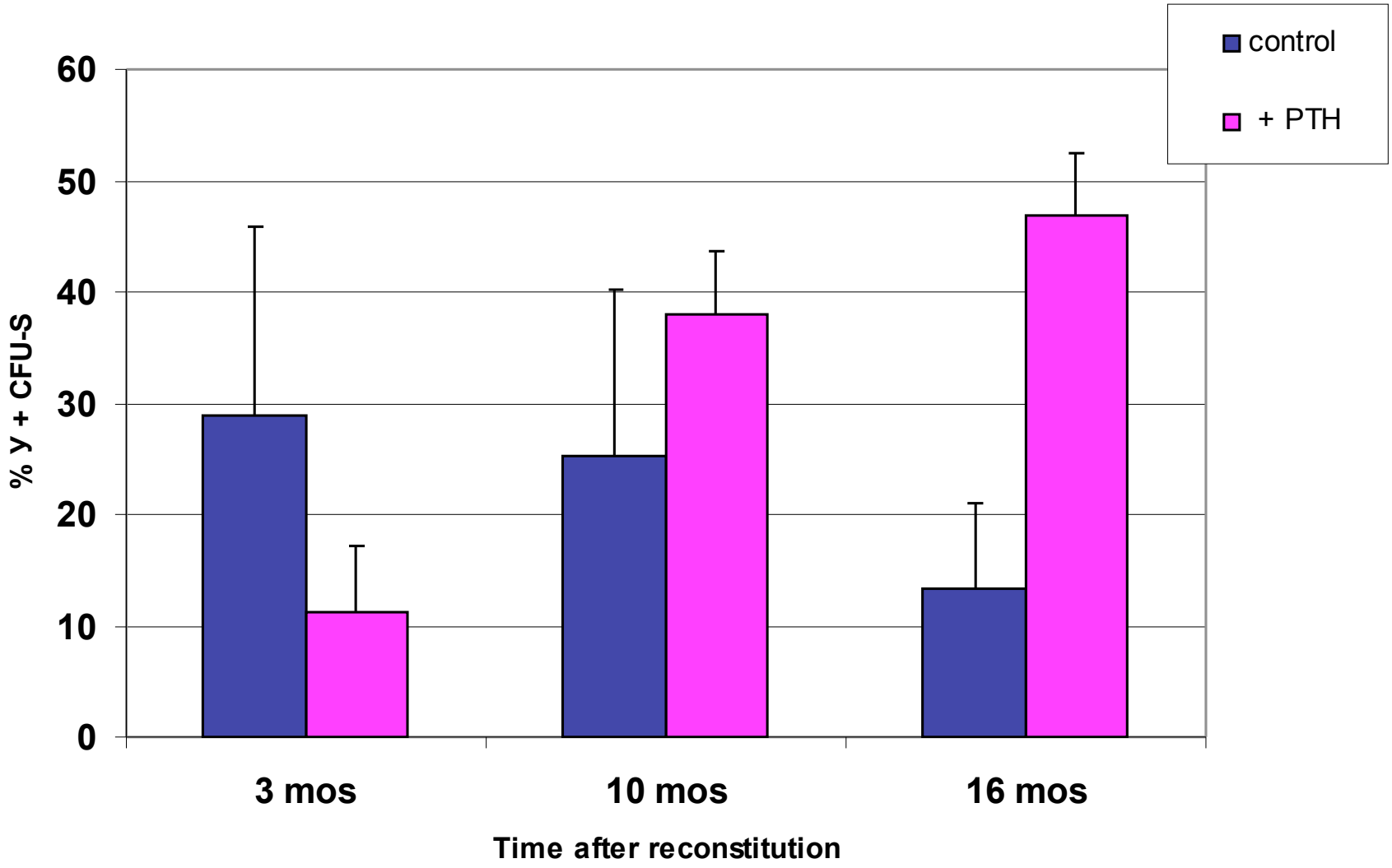




PCR analysis

For peripheral blood and bone marrow of reconstituted mice FISH method was used

Dynamics of У+ hematopoietic precursor cells in bone marrow of reconstituted mice

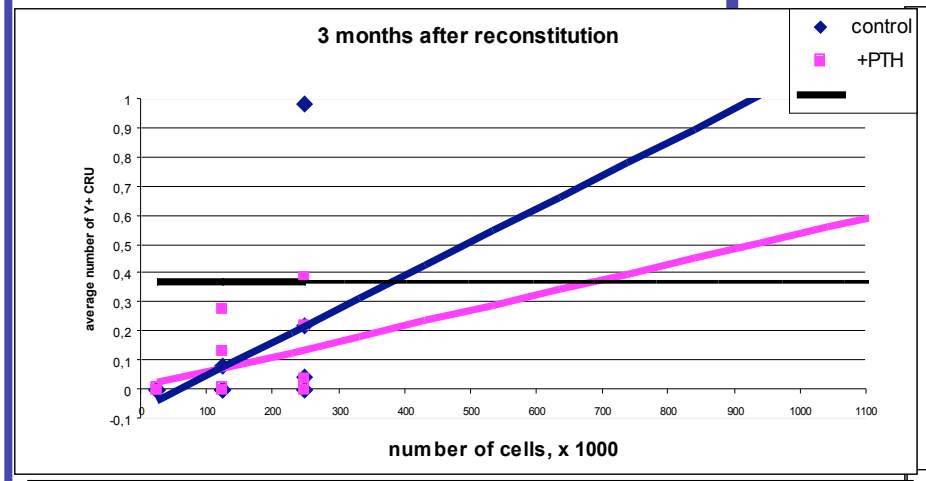


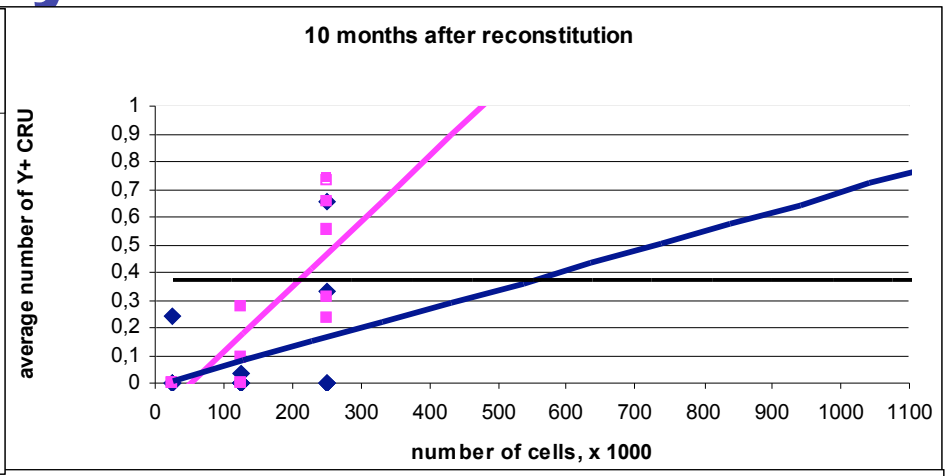
Male HSC worse than female repopulate recipient's bone marrow

Sex of donors and recipients	Number of mice transplanted with 1 one HSC	Number of reconstituted mice	% of reconstitution
male→ male female→ male female → female	**115**	**52**	**45**
male → female	**55**	**9**	**16**

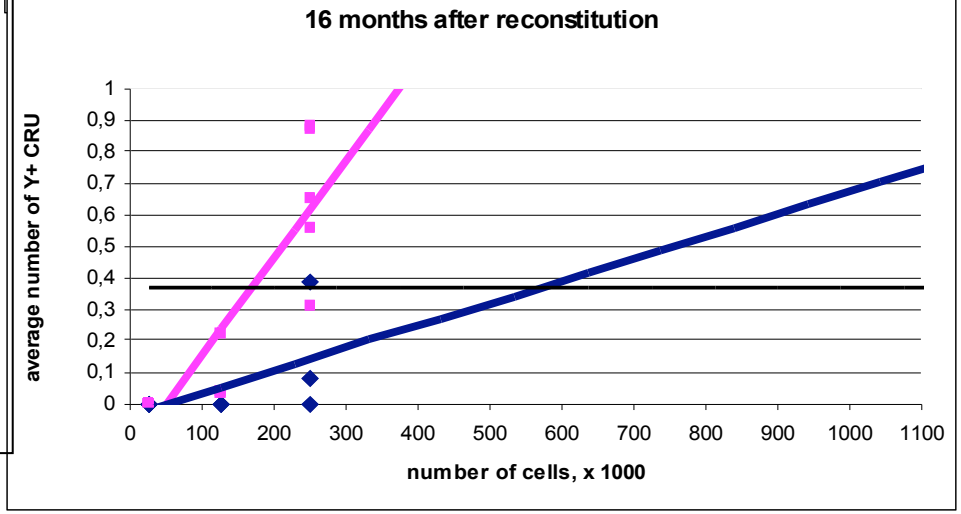
Uchida et al, 2003

Limiting dilution analysis of the frequency of CRU

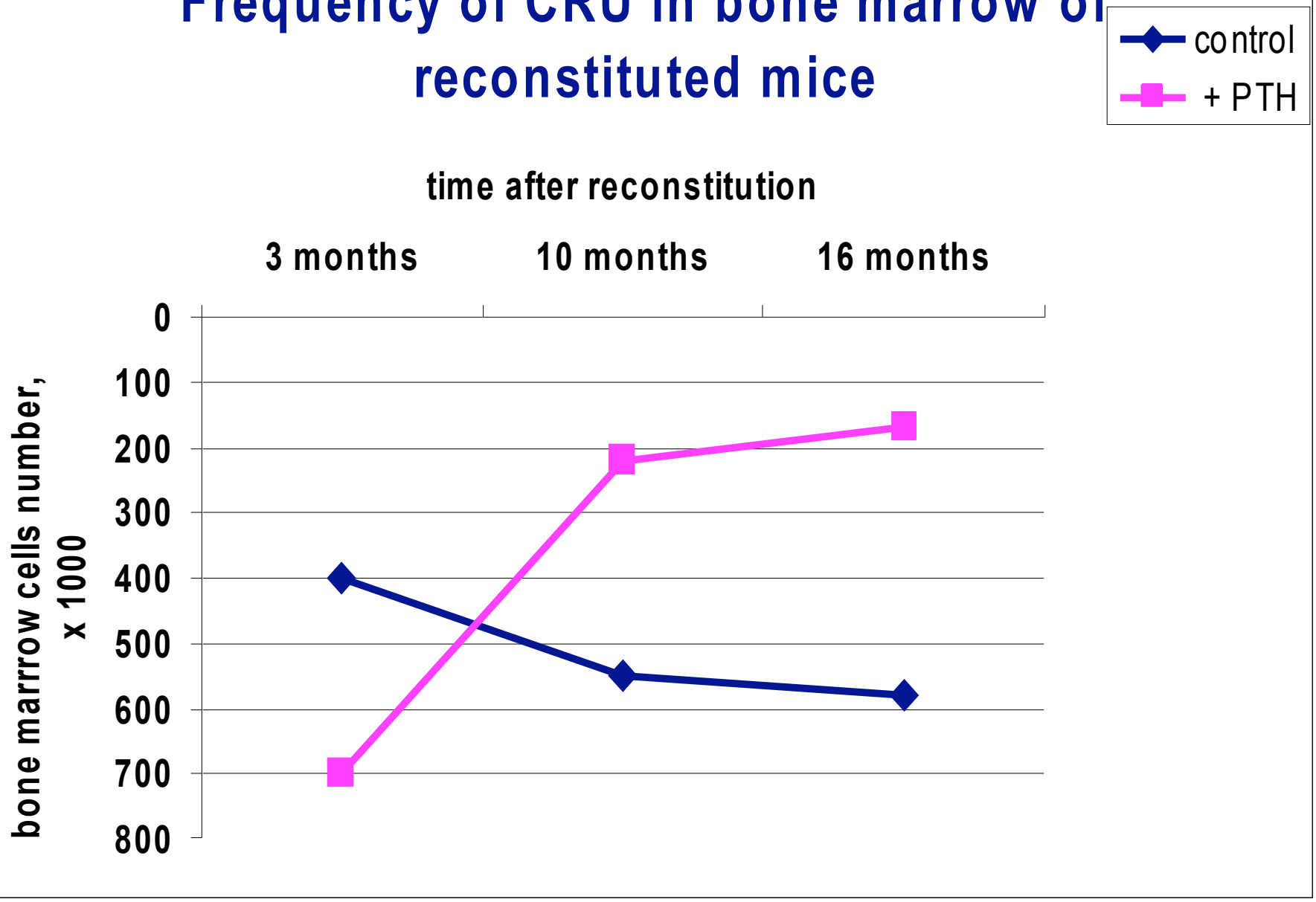




A line of best fit was generated using the maximum-likelihood method without forcing the data through the origin, and the frequency of CRU was then determined using standard statistical methods by interpolation of the number of test cells required a 37% negative response.



Frequency of CRU in bone marrow of reconstituted mice

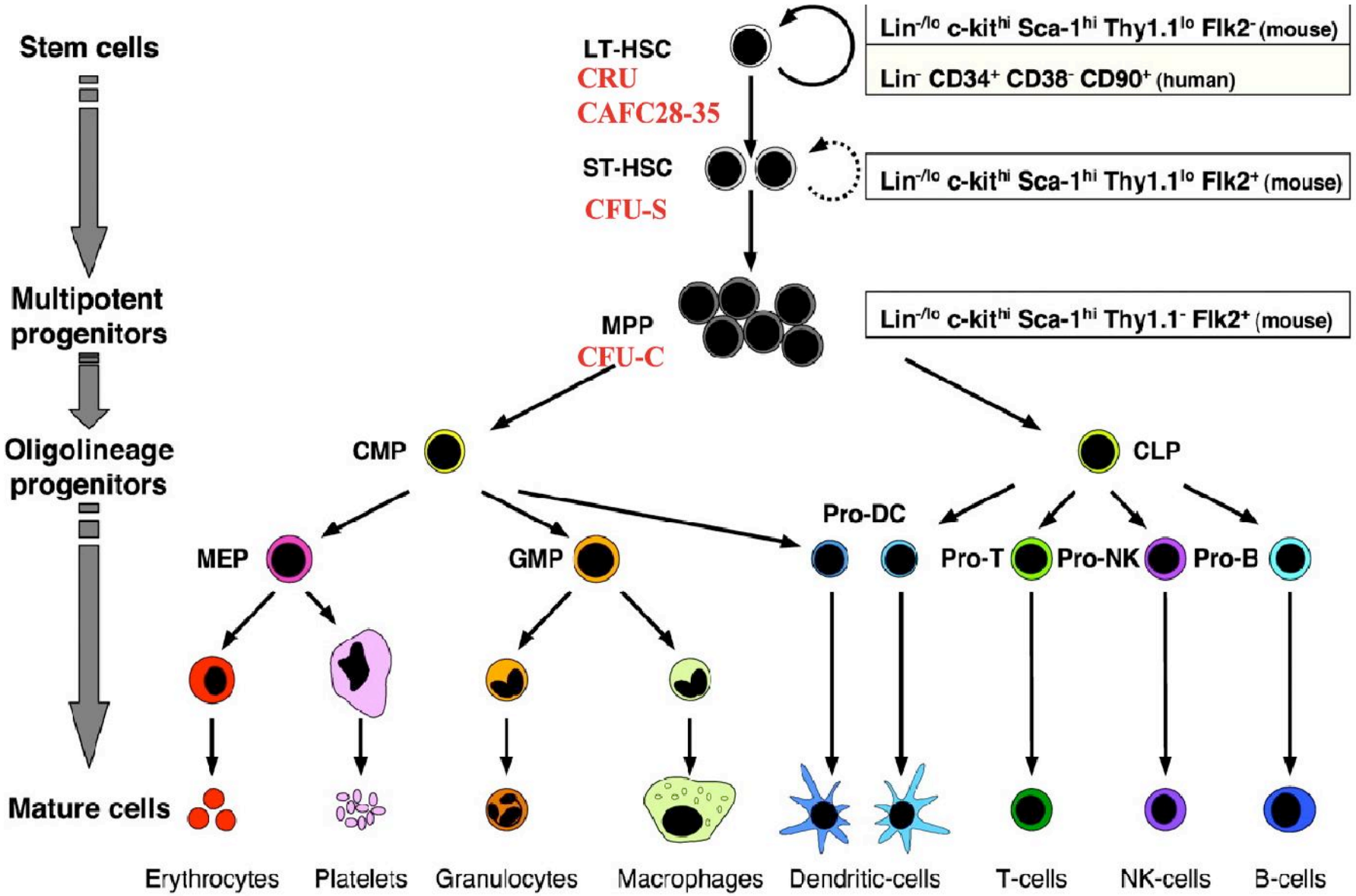


Conclusion

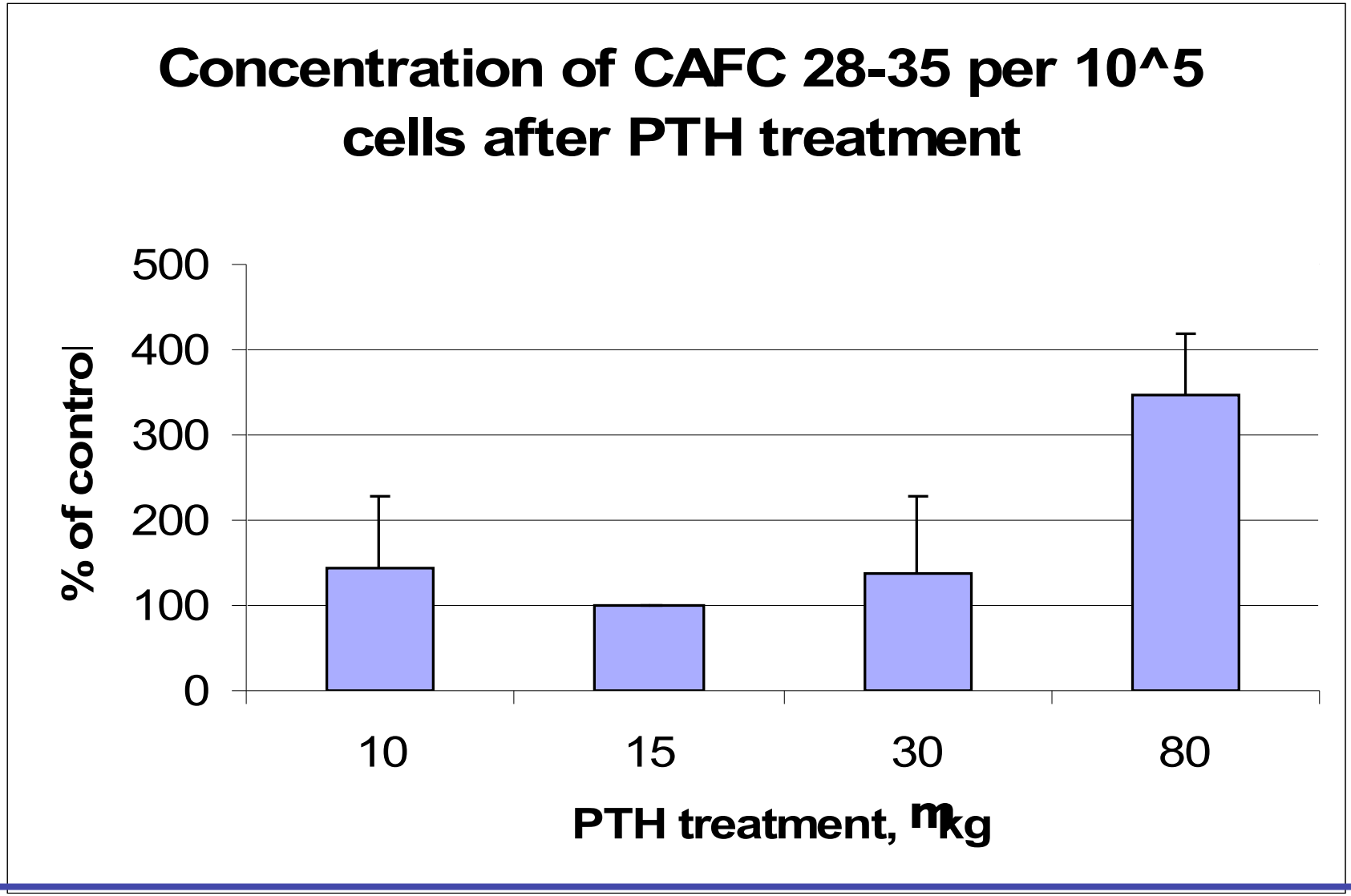
PTH treatment during 4 weeks leads to increase :

CRU about 4 fold

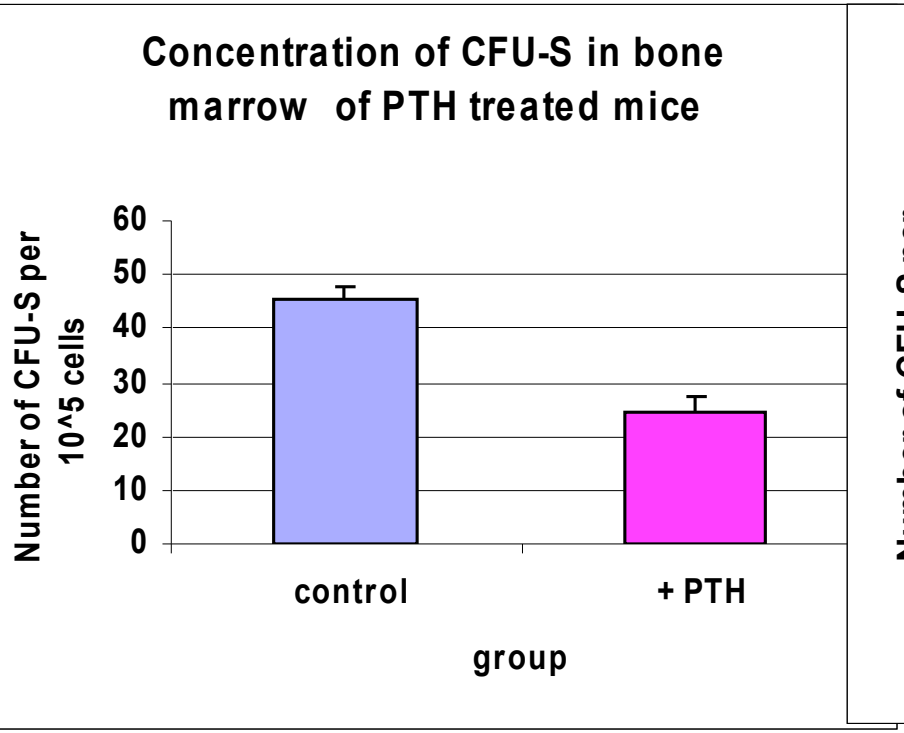
Scheme of hematopoiesis

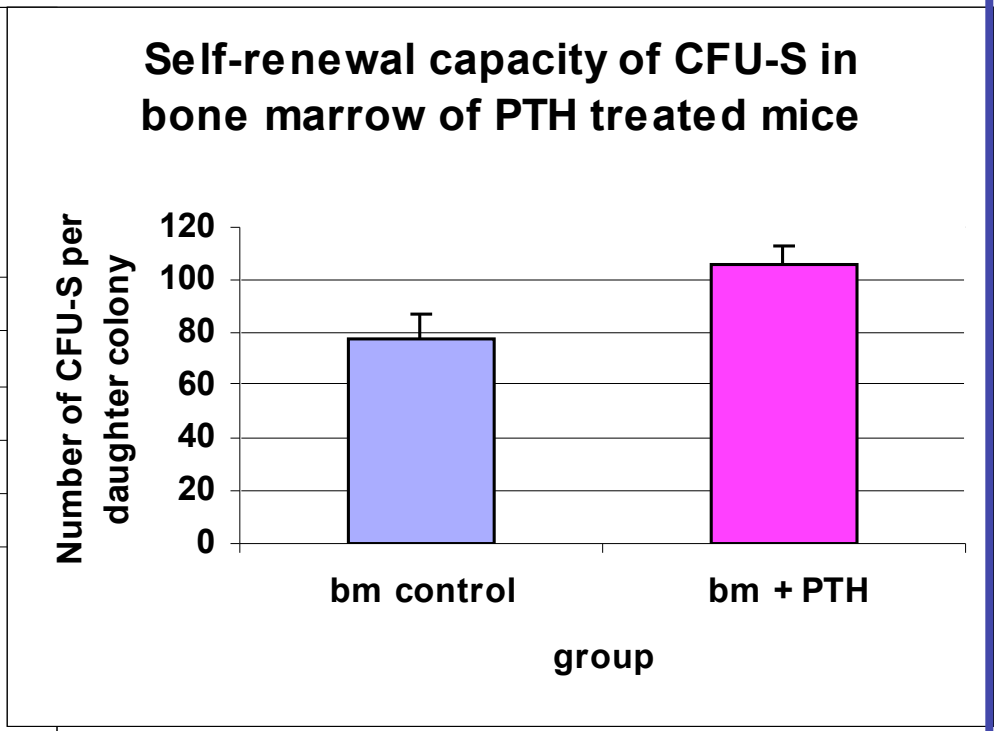


Frequency of early HSC in bone marrow after PTH treatment

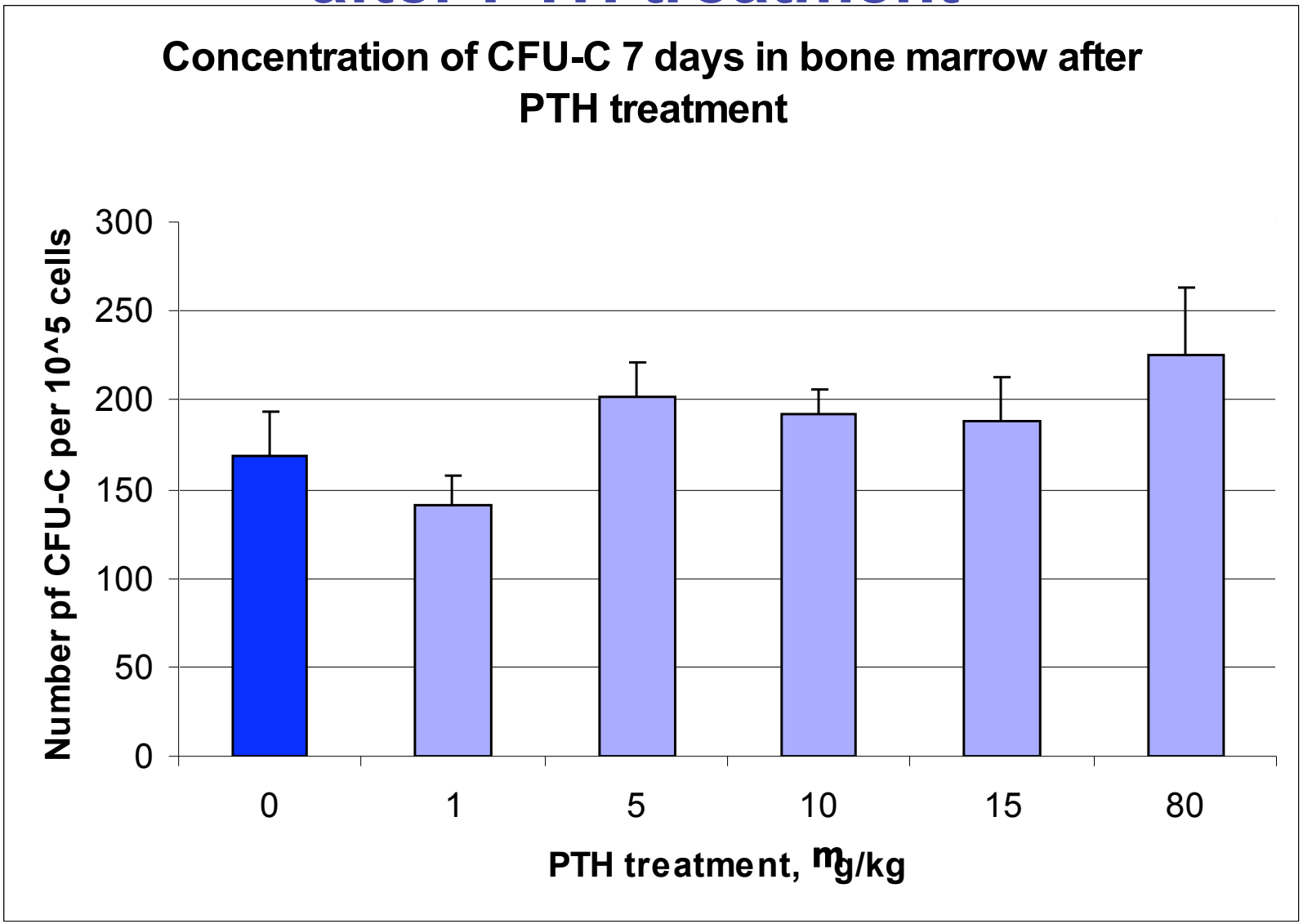


Concentration of CFU-S in bone marrow after PTH treatment





Concentration of CFU-C in bone marrow after PTH treatment



Conclusions

- PTH treatment during 4 weeks leads to increase :
- Lin-Sca1+c-Kit+ stem cells about 2 fold

(Calvi et al, 2003)

- CAFC28-35 in 3,5 fold
- CRU about 4 fold

- The concentration of more mature precursors did not change significantly

Questions

- Why the elevated number of early HSC does not lead to increase of more mature hematopoietic precursor cells and terminally differentiated cells?
- Do the properties of stromal microenvironment, apart from osteoblasts activation, change after PTH treatment ?

Experimental design of seeding efficiency analysis after PTH treatment

PTH 80 mg/kg
4 weeks

Both groups of mice were lethally irradiated and injected with $16x10^6$ bone marrow cells

24 hours later the number of CFU-S and CAFC 28 in bone marrow and spleen was analyzed

$$F\ 24 = \frac{a}{N} \times 100$$

Where a – the number of precursor cells seeded in organ,

N – the number of injected precursor cells

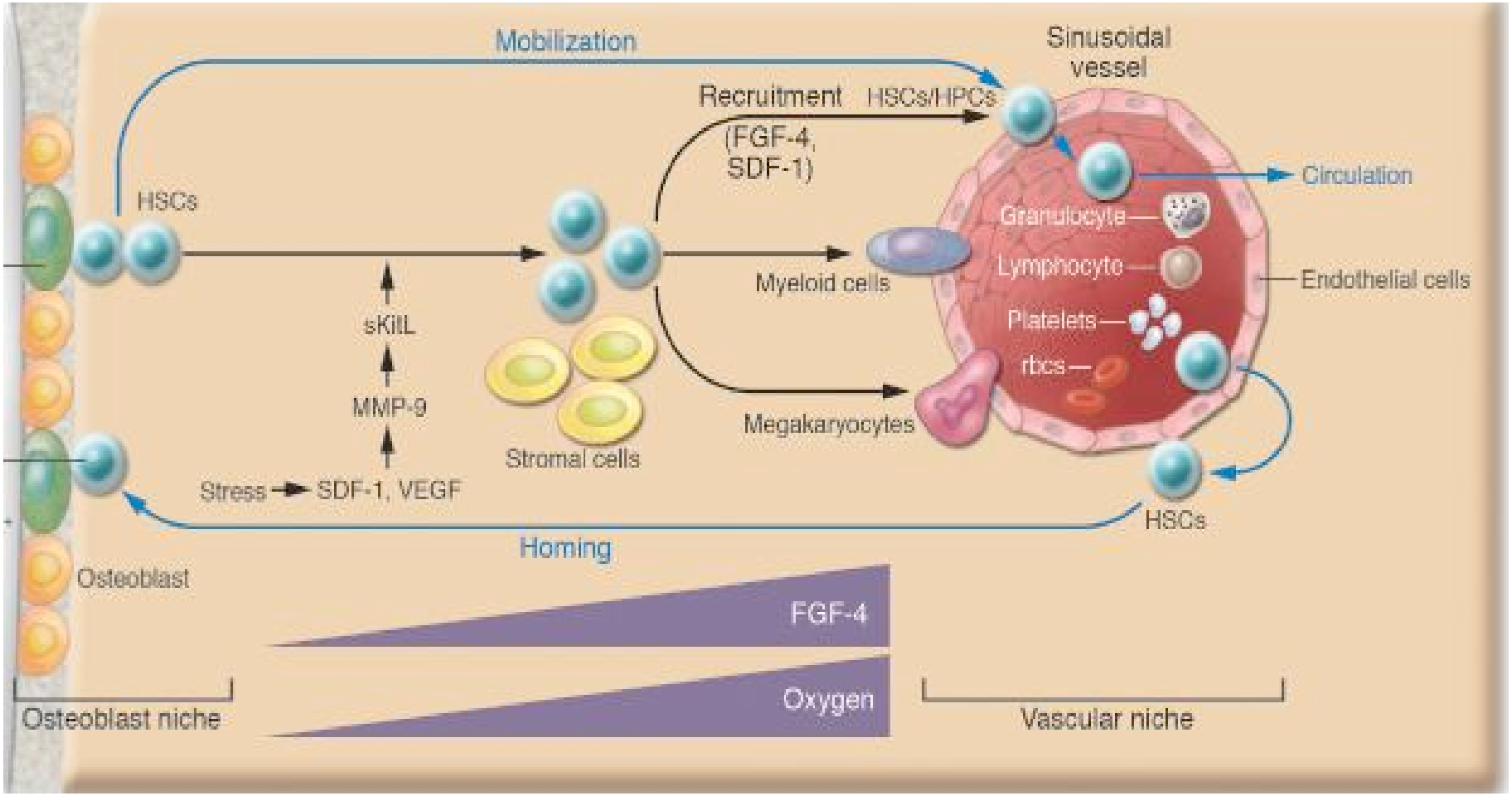
Distribution of CFU-S in hematopoietic organs of PTH-treated mice

Group	CFU-S
control	43% seeds in bone marrow
	5% seeds in spleen
	52% "lost"
PTH 80 g/kg	9% seeds in bone marrow
	6% seeds in spleen
	85% "lost"

Distribution of CAFC-28 in hematopoietic organs of PTH-treated mice

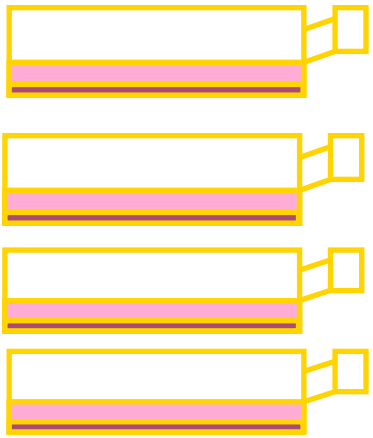
Group	CAFC 28
control	34% seeds in bone marrow
	7% seeds in spleen
	59% "lost"
PTH 80 g/kg	39% seeds in bone marrow
	1% seeds in spleen
	60% "lost"

Homing of CFU-S into bone marrow inhibited after PTH treatment



Yin & Li, 2006

Homing and adhesion of human CAFC after PTH treatment



control

+ PTH 10^{-8} M

+ PTH $5x10^{-8}$ M

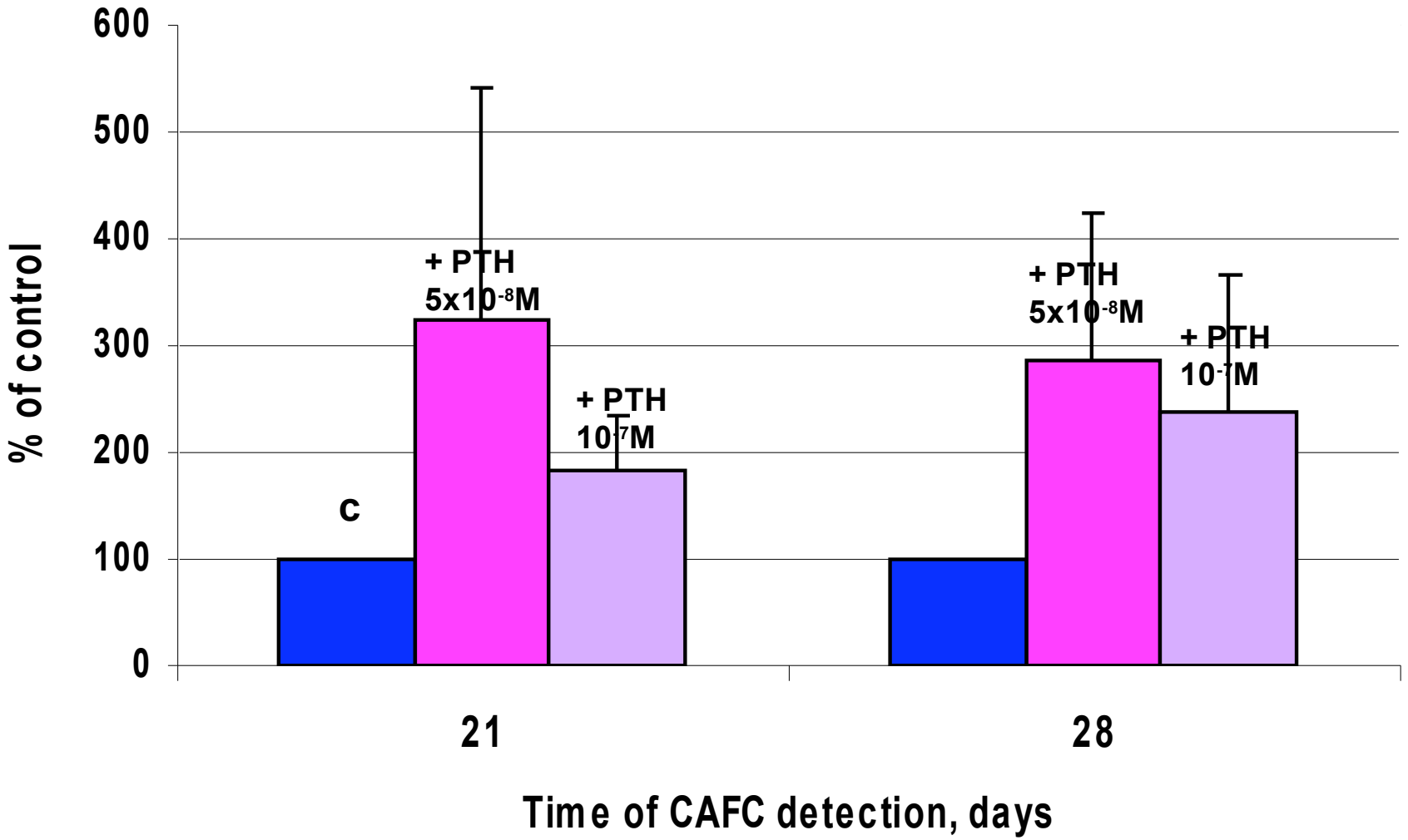
+ PTH 10^{-7} M

After 6 weeks in culture:

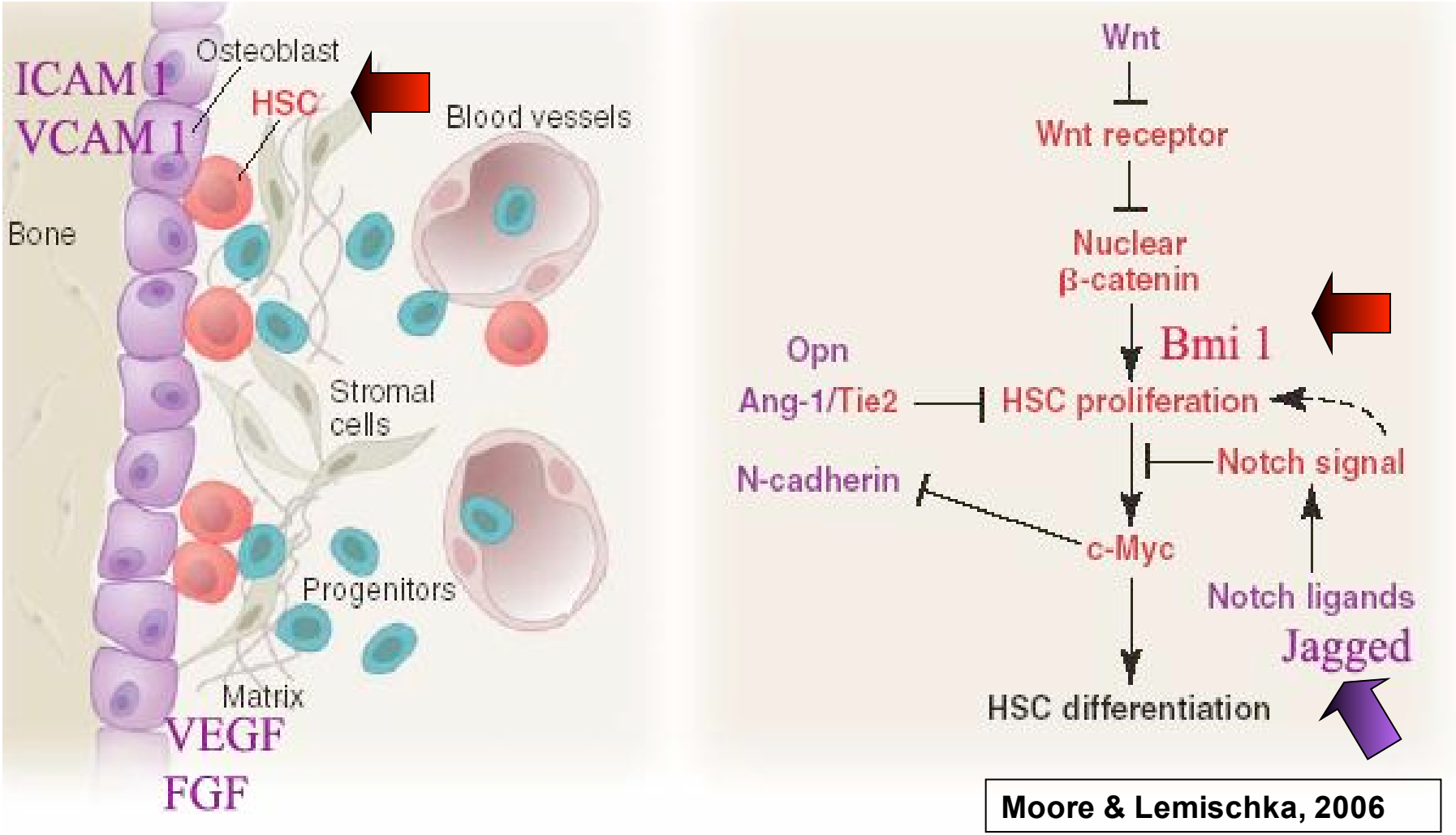
- The flasks were irradiated with 40 Gy;
- Donor bone marrow cells were implanted on adherent cell layers;

5 days later the frequency of CAFC survived on different cell layers were studied

Adhesion of CAFC28-35 after 5 days of cultivation

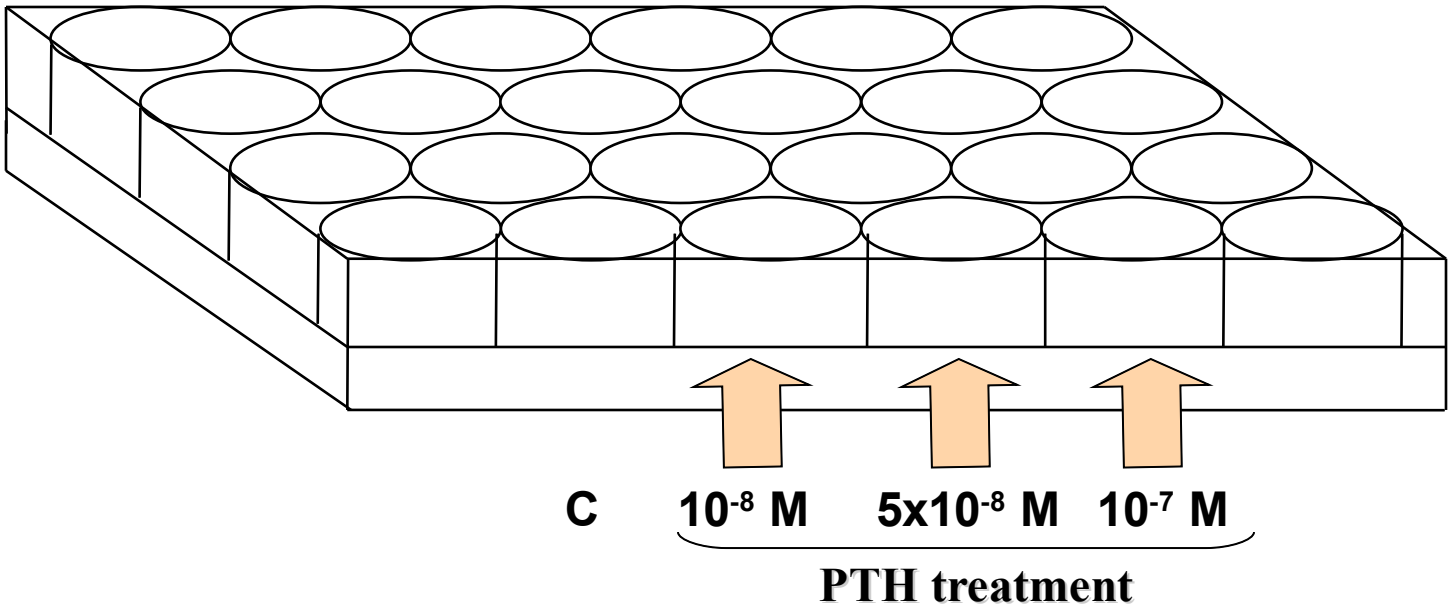


Interactions between osteoblasts, stromal cells and HSC



Moore & Lemischka, 2006

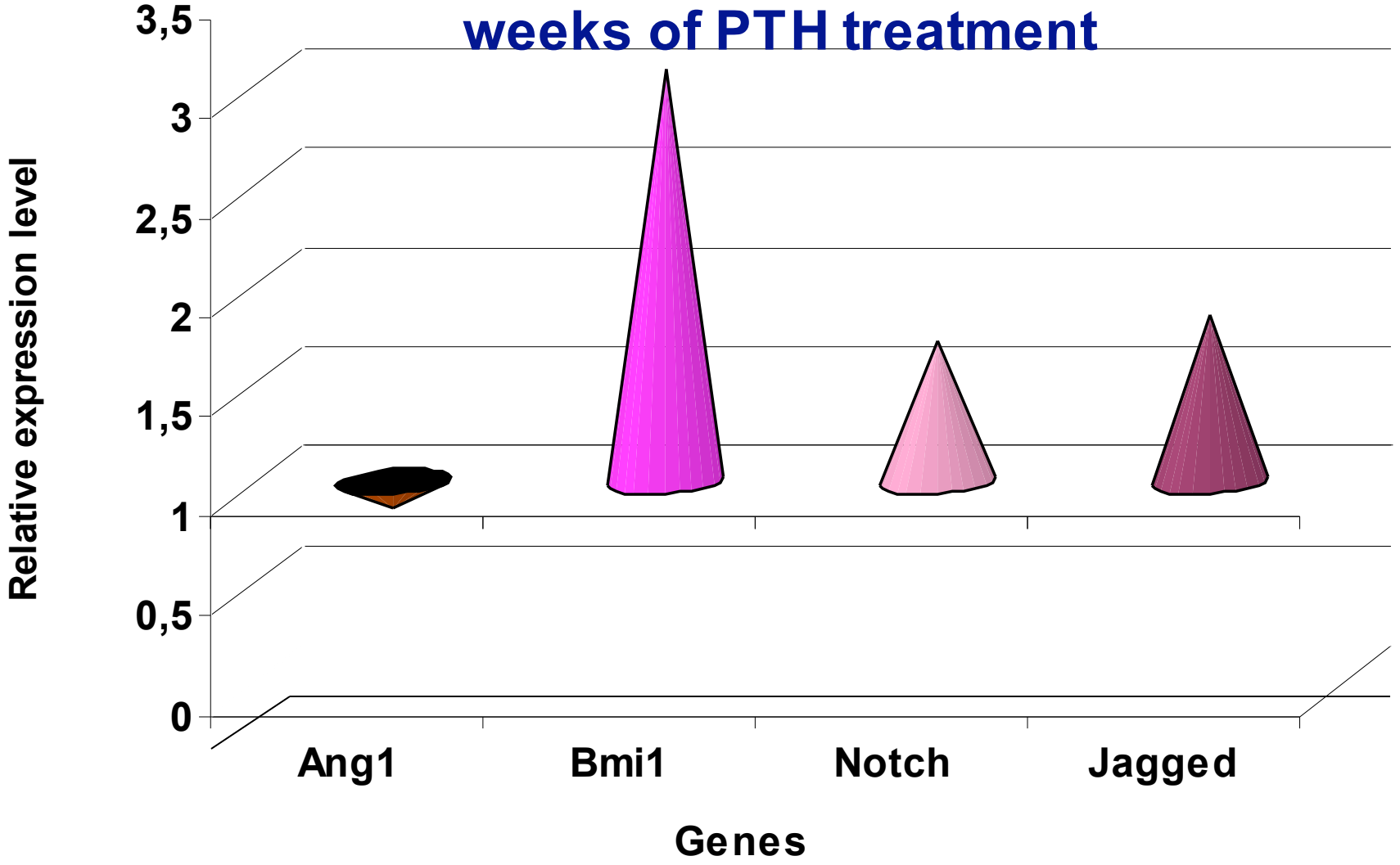
Analysis of gene expression in human long-term bone marrow culture



- 3 weeks in culture
- RNA extraction
- Semi-quantitive RT-PCR with subsequent Southern-blot hybridization (Phosphoimager analysis)

Ang 1, BMI 1, Notch 1, Jagged 1;
FGF, VEGF, Osteopontin, Aggrecan, ICAM 1, VCAM 1.

Alterations in genes expression after 3 weeks of PTH treatment



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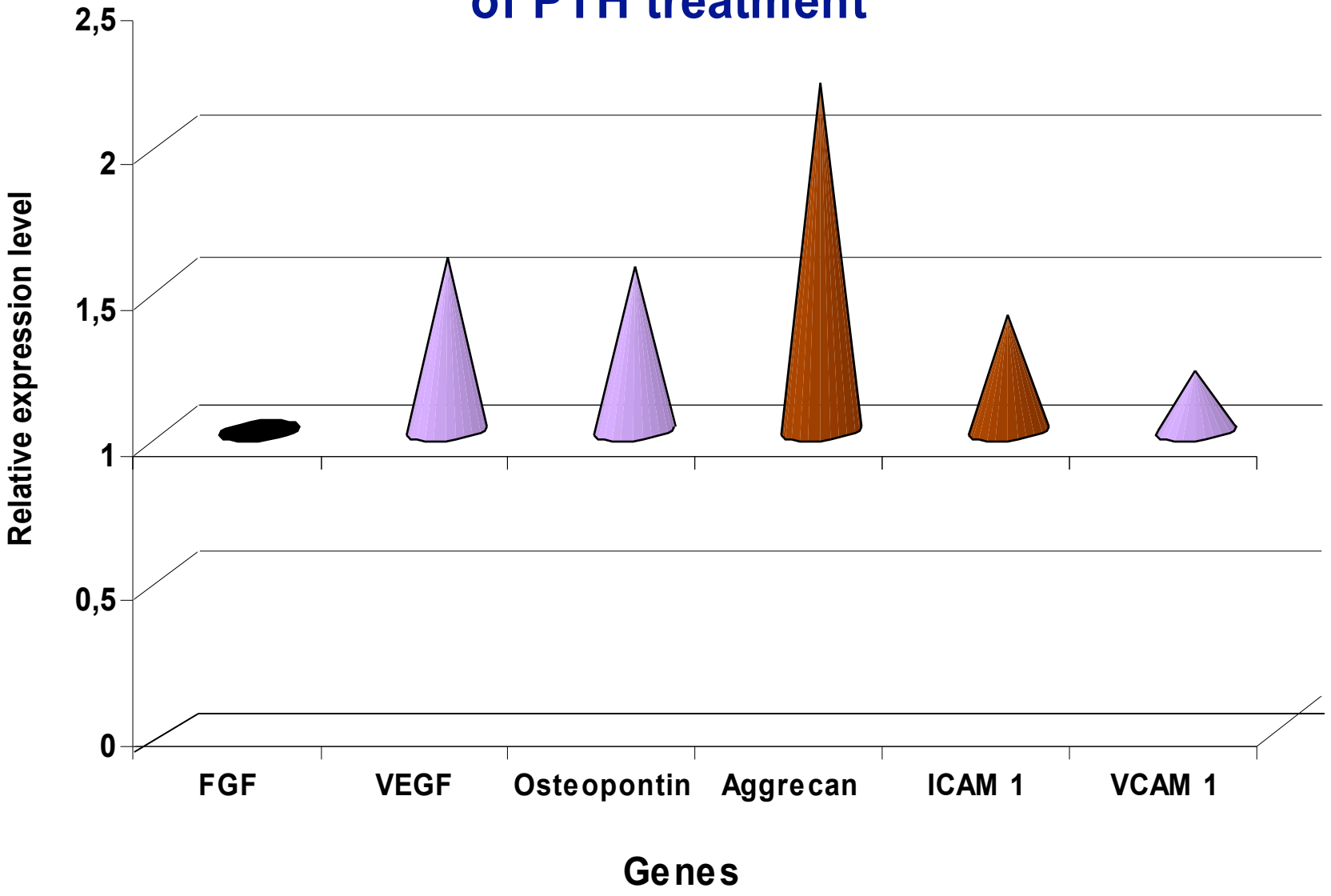
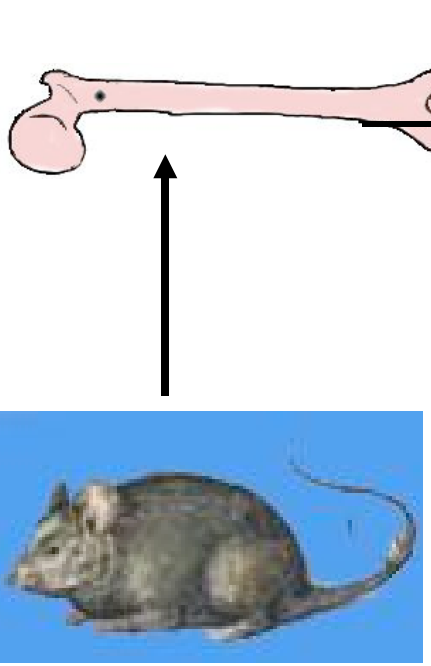


Question

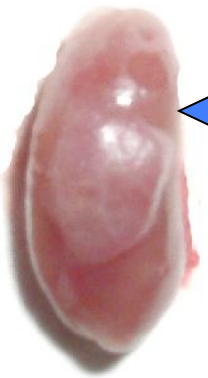
- **Could PTH treatment change the properties of mesenchymal stem cells, or alterations involve only osteoblasts and some other cells of hematopoietic stromal microenvironment?**

In vivo physiologic method of analysis of mesenchymal stem cells (MSC): ectopic foci formation



6 weeks

Ectopic foci



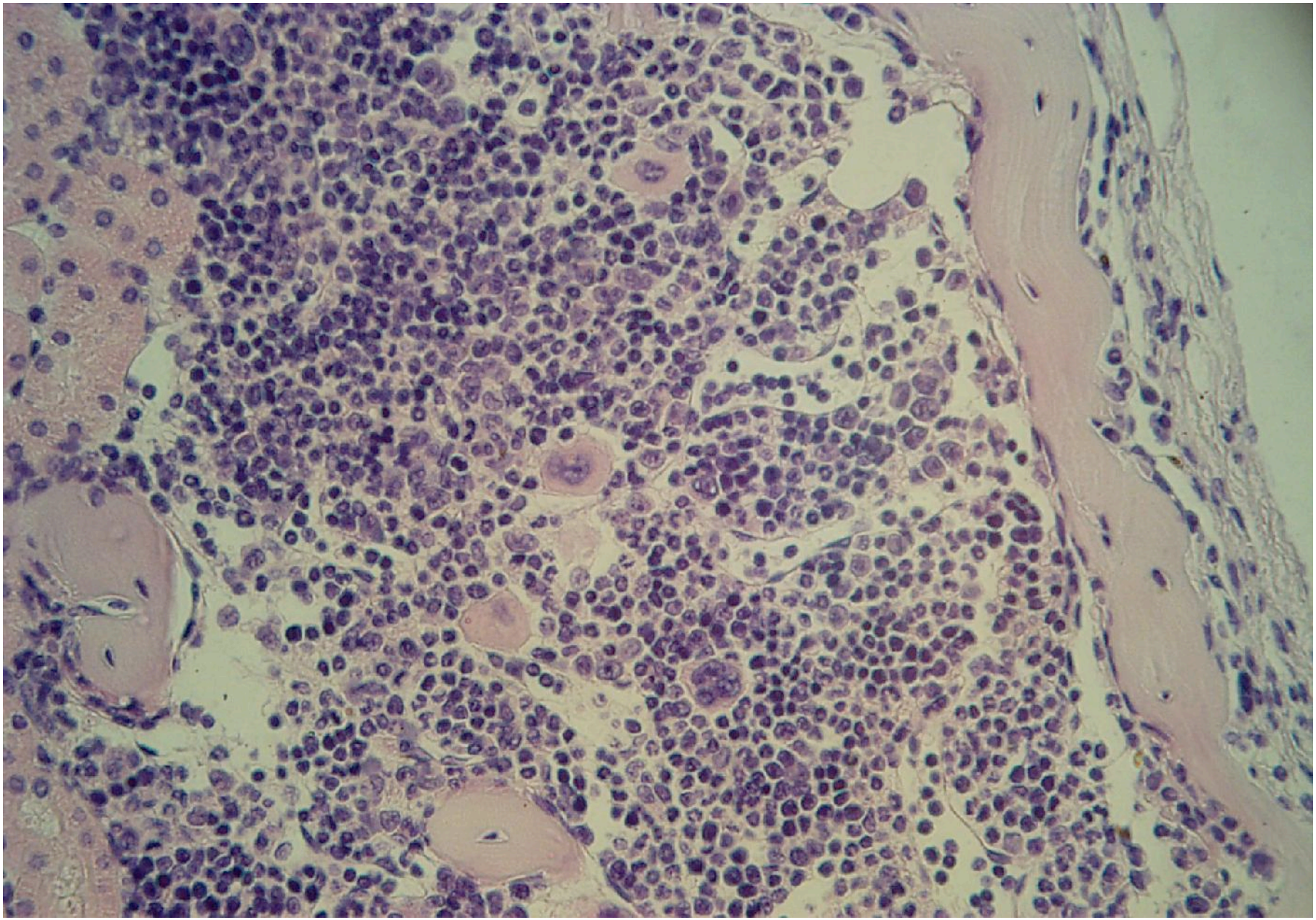
Ectopic foci:

=

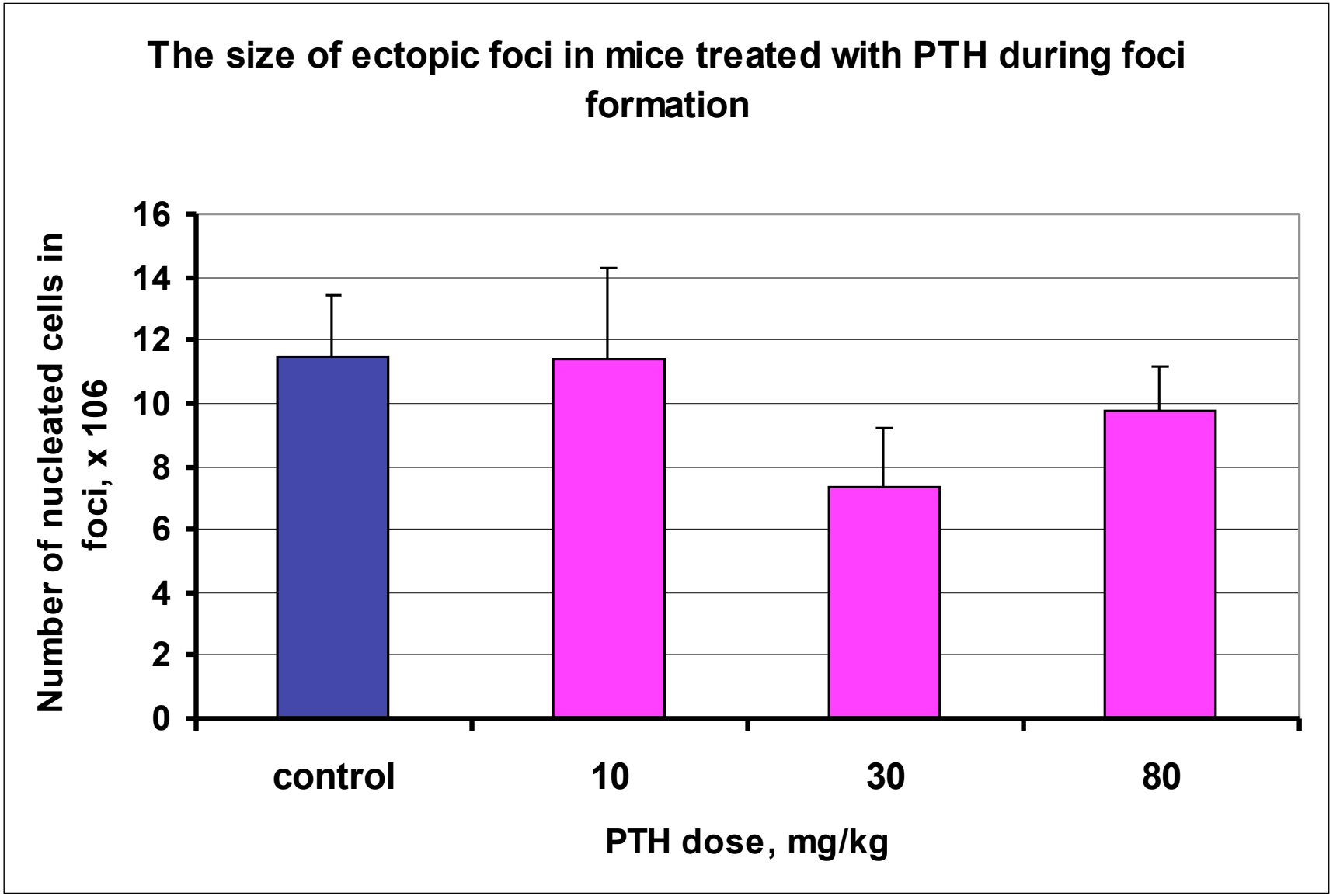
Hematopoietic cells

+

MSC → Hematopoietic stroma



MSC after PTH treatment



Conclusions

- **HSC are mobile – they get to niches and leave them**
- **PTH is important regulator of HSC and can influence their number and properties moving “steady state “ hematopoiesis due to multiple cell-to-cell interactions.**
- **The possibility to use PTH for HSC expansion in vivo is very attractive, but we should very carefully think about it.**