



Cord Blood Hematopoietic Stem Cells
*from biology to ex vivo expansion and
plasticity*

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Umbilical Cord Blood



HE BROXMEYER

UCB contains hematopoietic stem and progenitor cells at significant levels



E GLUCKMAN

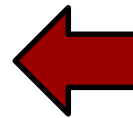
First UCB transplant



Clinical impact of UCB cells

- More than **30,000** HCT transplants have been performed using UCB cells
- **Cord blood banking** (public and private) throughout the world
- More than **700,000** UCB units stored in public banks, and more than **1 million** units in private banks worldwide

**Hematopoietic Cell
Transplantation**



Biological characterization and
in vitro manipulation of HSC

HSC-based Cellular Therapy

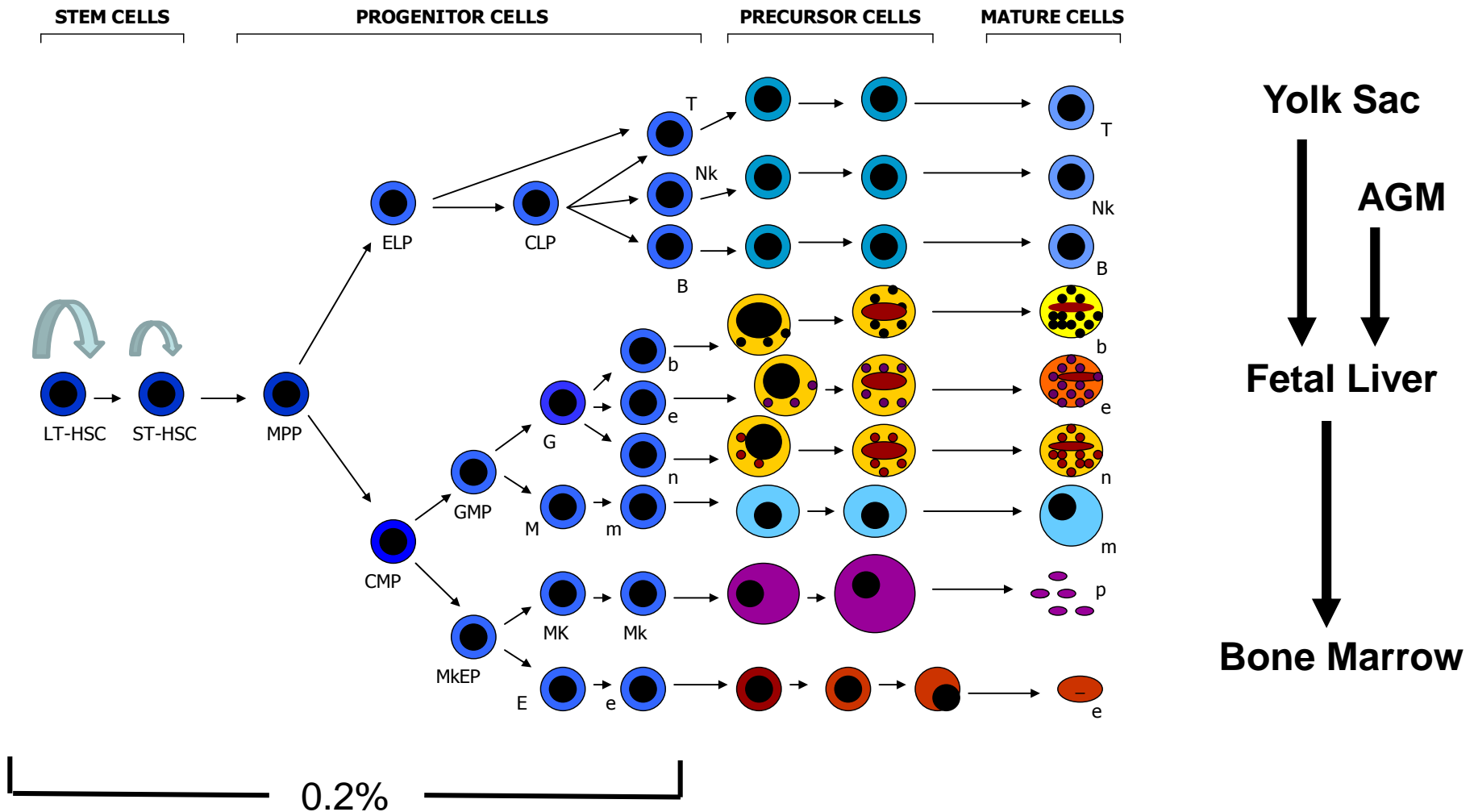


HSC Laboratory



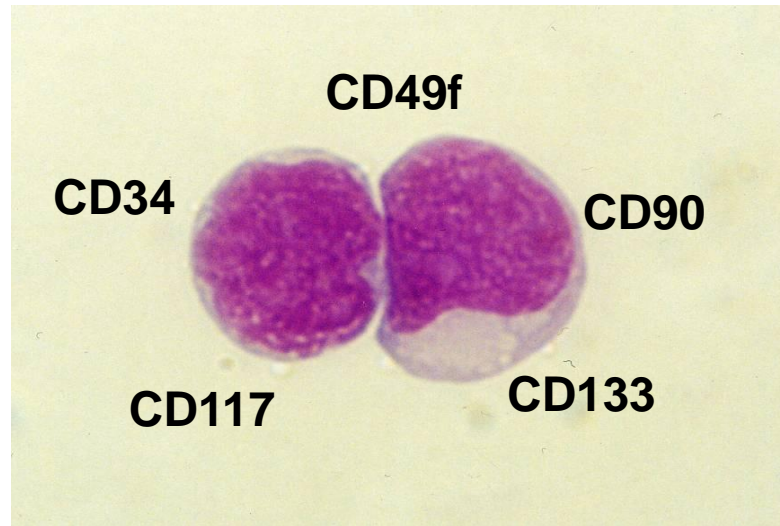
Funding:
IMSS-CIS (Mexico)
CONACYT (Mexico)
Terry Fox Fund (Canada)

The Hematopoietic System



Biological differences between HSC from UCB and adult BM

Hematopoietic Stem Cells



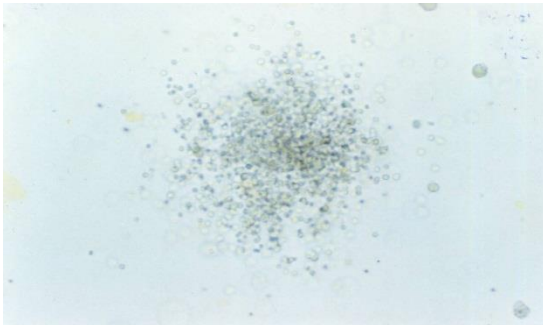
Adult Bone Marrow: 1 HSC per 60,000 nucleated cells

Umbilical Cord Blood: 1 HSC per **20,000** nucleated cells

Hematopoietic progenitor cell content in UCB and adult bone marrow

No. progenitors / 10^5 MNC

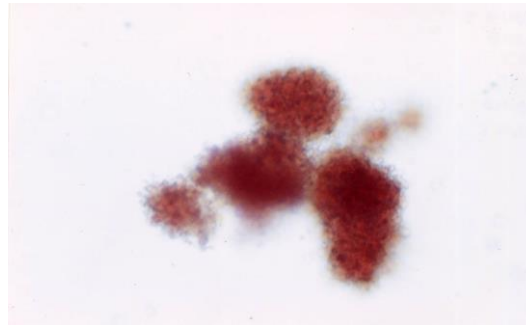
Myeloid



ABM = 164 ± 57

UCB = 160 ± 49

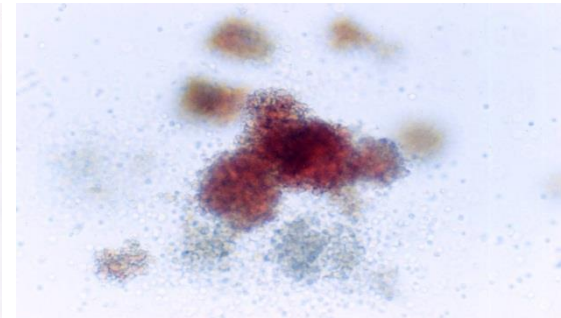
Erythroid



ABM = 137 ± 51

UCB = 179 ± 64

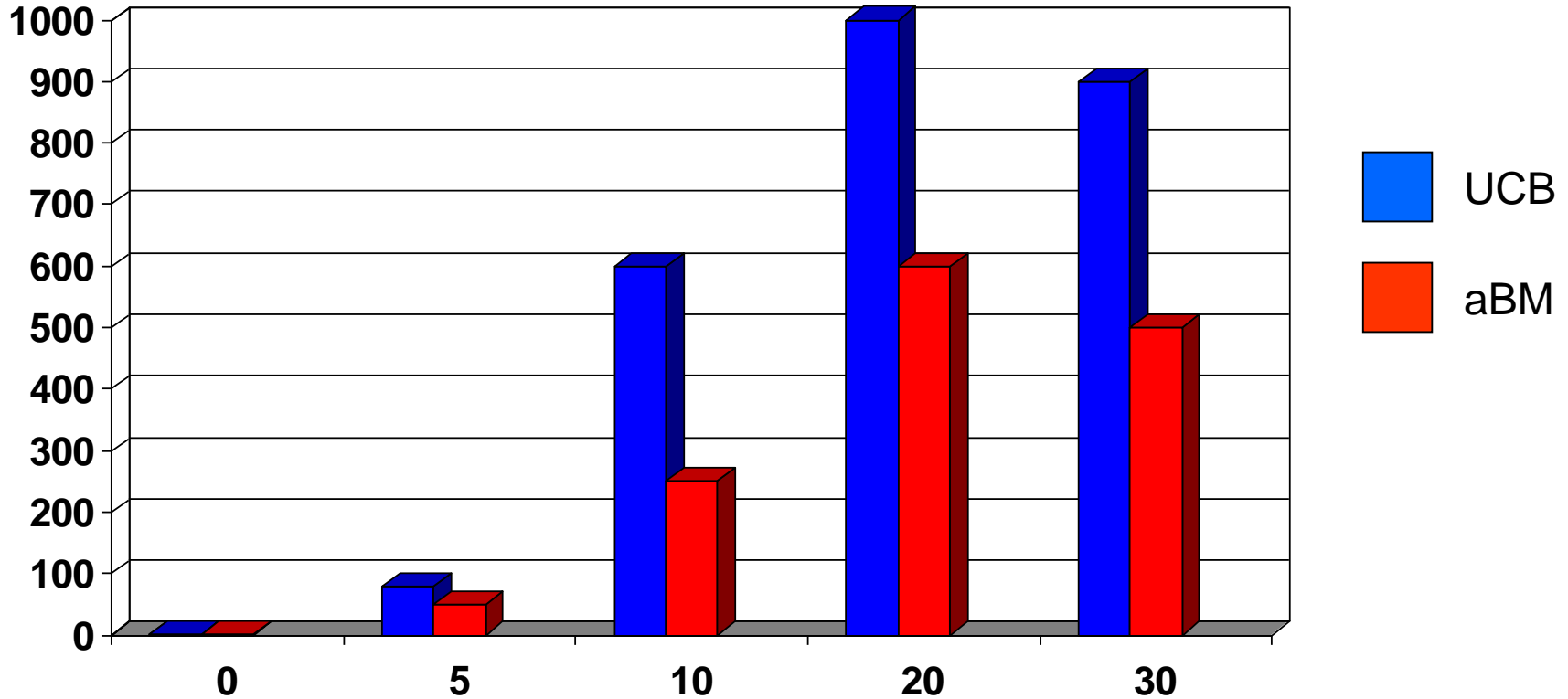
Multipotent



ABM = 4 ± 3

UCB = $21 \pm 10^*$

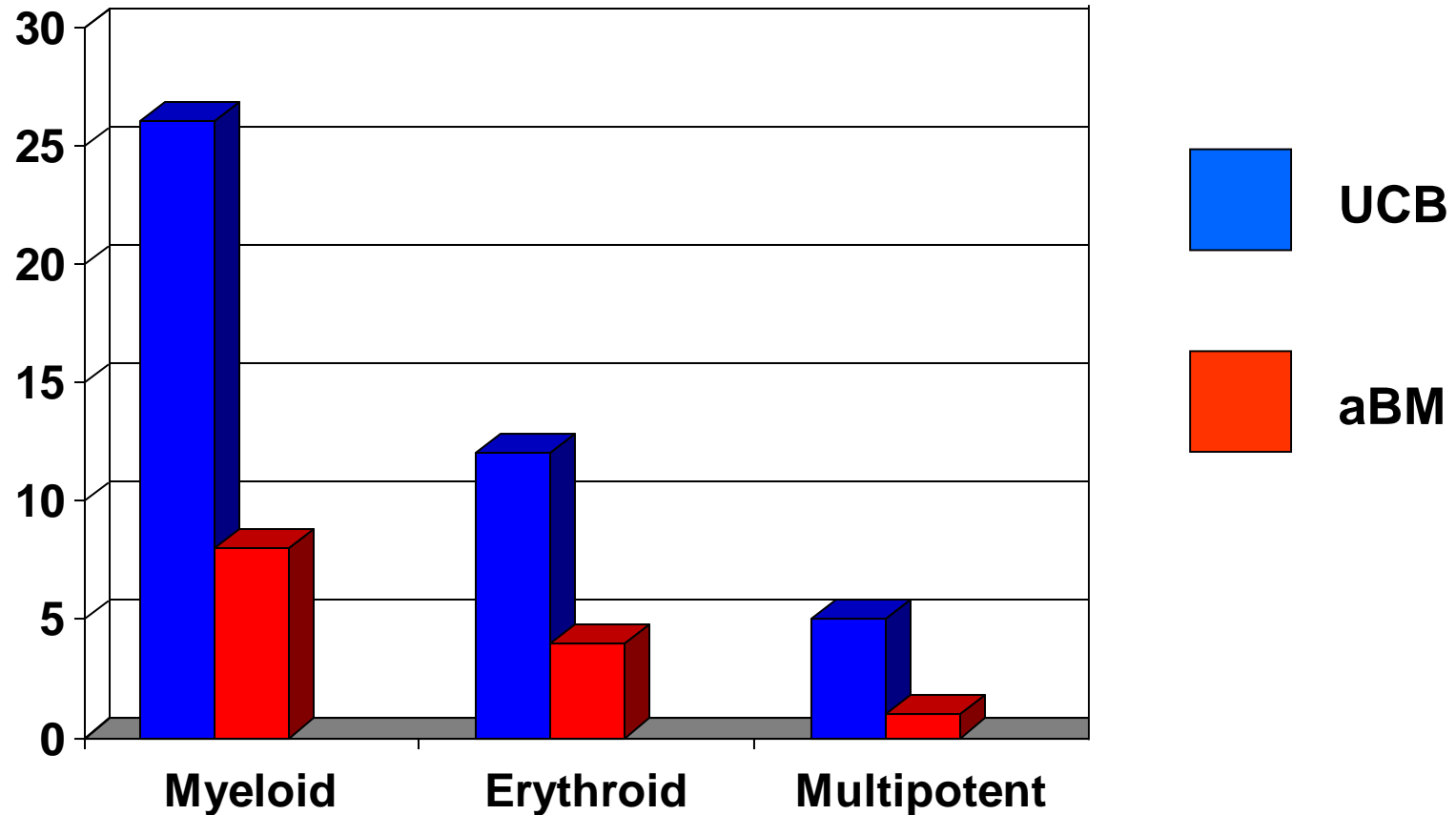
HSC/HPC from UCB possess higher proliferation* potentials than adult cells



* Fold-increase in total cell number

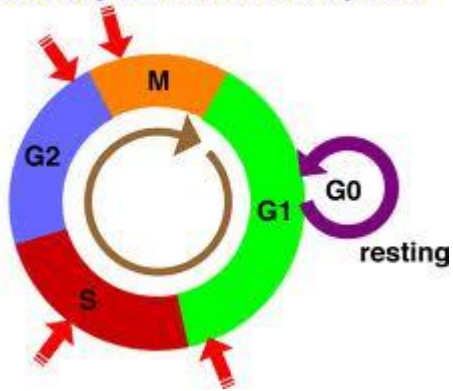
*Martínez-Jaramillo G, et al
Stem Cells Dev 2004*

HSC/HPC from UCB possess higher expansion* potentials than those from ABM



* fold-increase in HPC number

The Cell Cycle and the Checkpoints



Cell cycle kinetics in culture

Alvarado-Moreno A, et al
Stem Cells Dev 2007

UCB

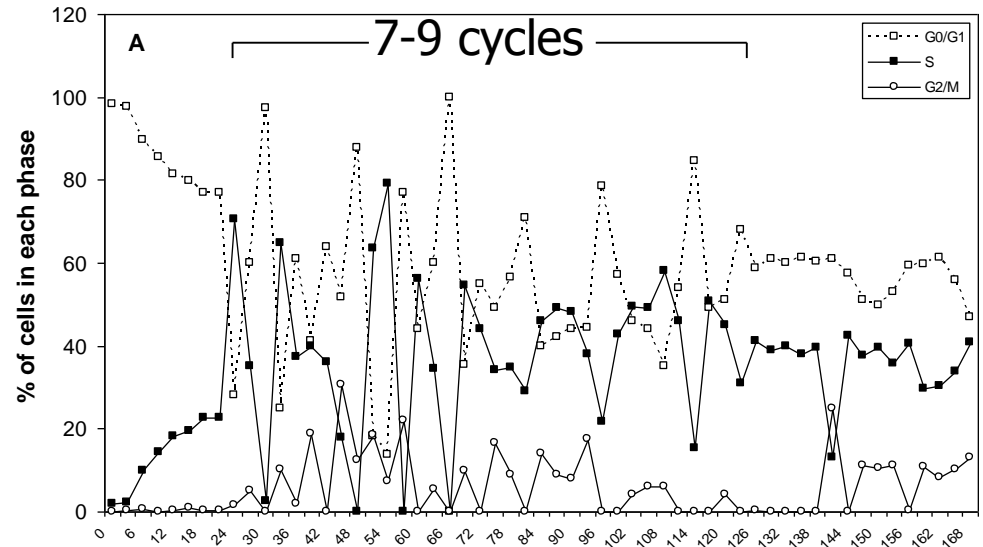


Figure 2

aBM

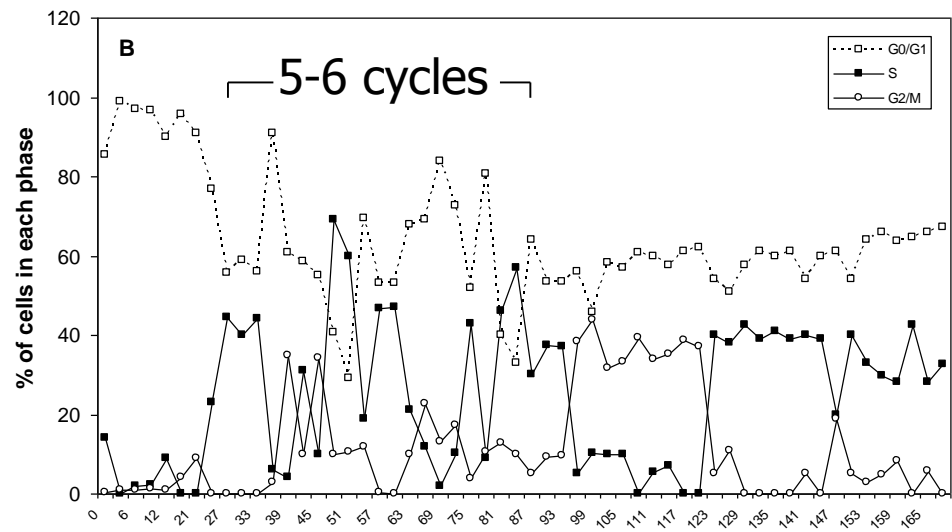


Figure 2

Expression levels of cell cycle regulators

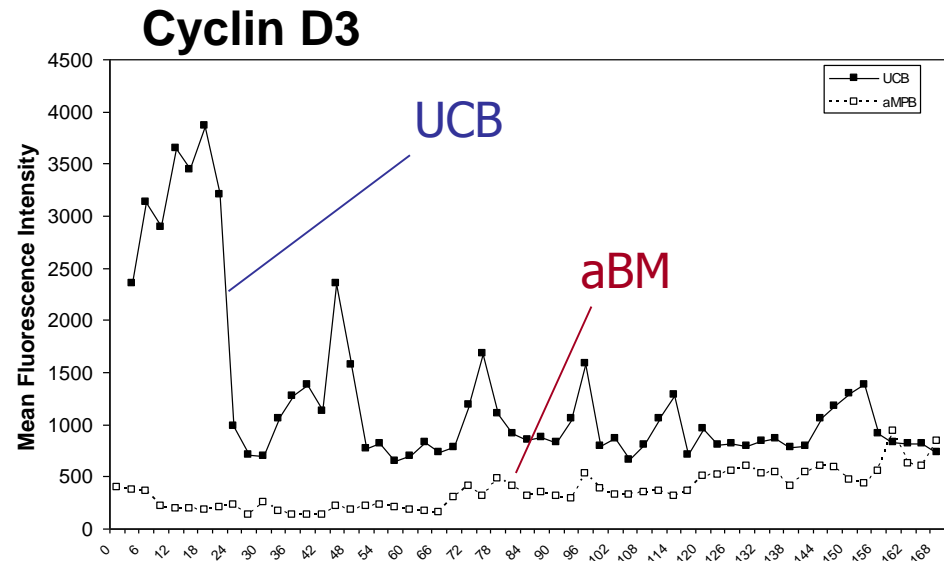


Figure 3

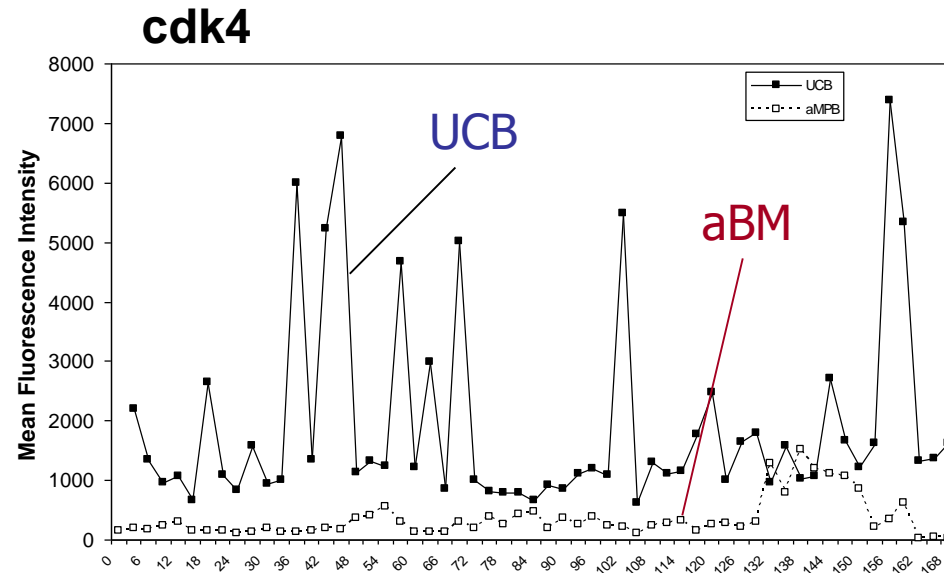
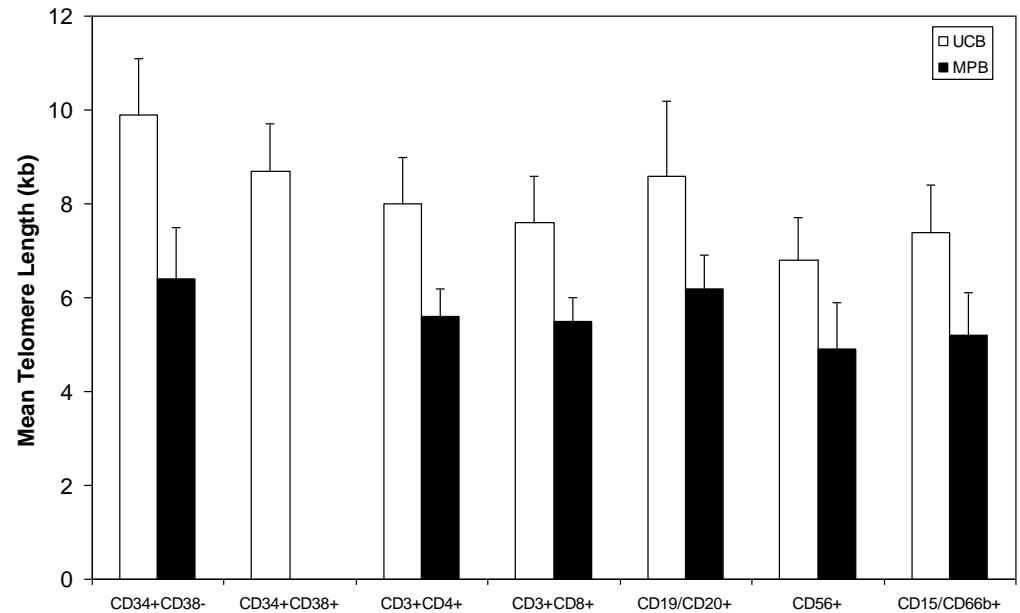
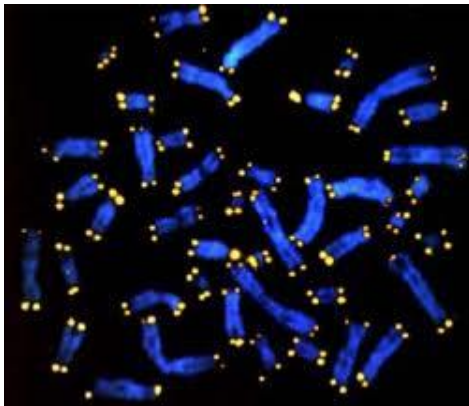


Figure 4

HSC from UCB possess longer Telomeres than those from adult subjects

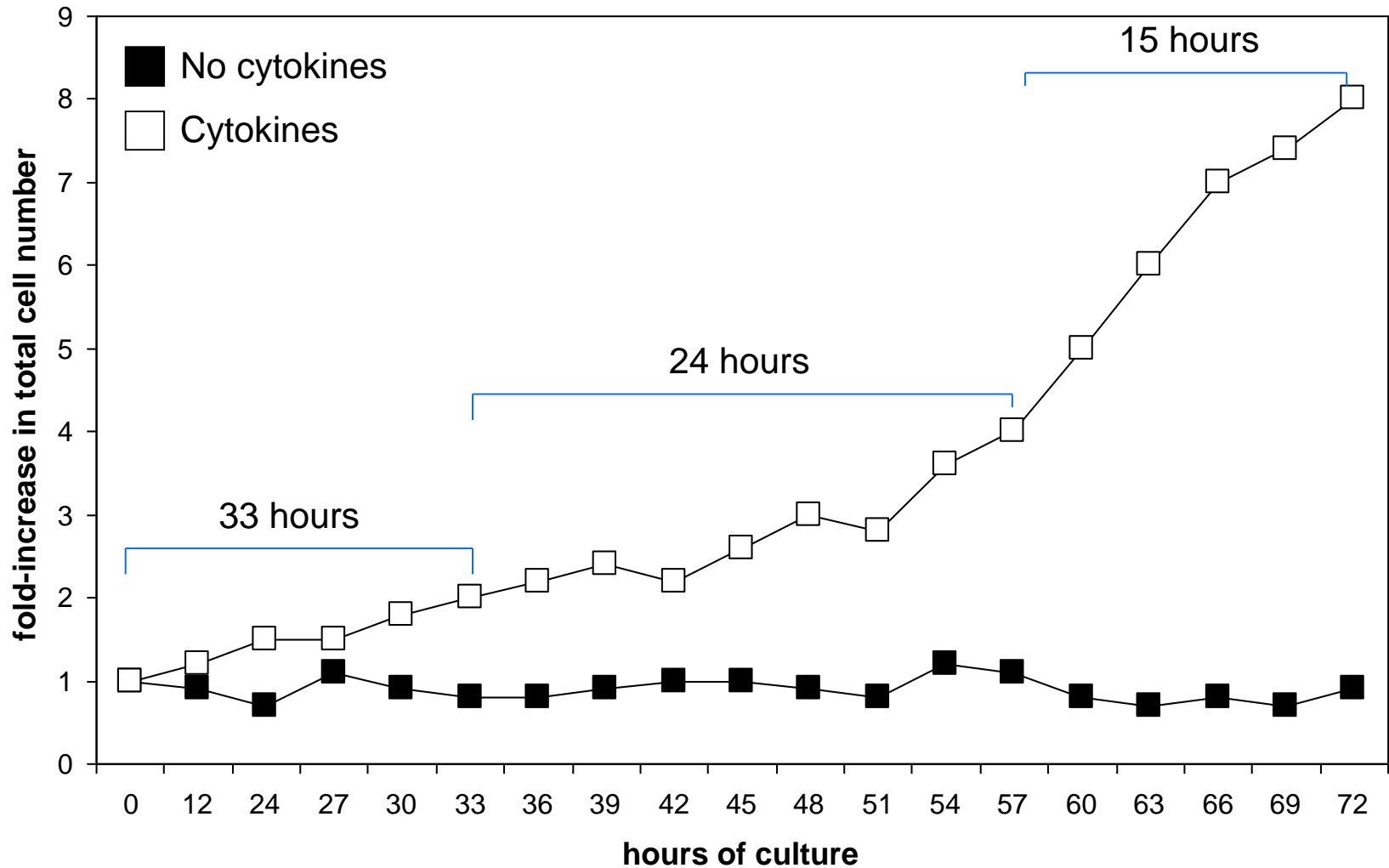


HSC: UCB vs aBM

- UCB contains a **higher frequency** of HSC and multipotent HPC than aBM
- HSC/HPC from UCB possess **higher proliferation and expansion potentials** in vitro than equivalent cells from aBM
- Such differences in proliferation and expansion potentials seem to be due to **differences in the expression of cell cycle regulators and telomere length**

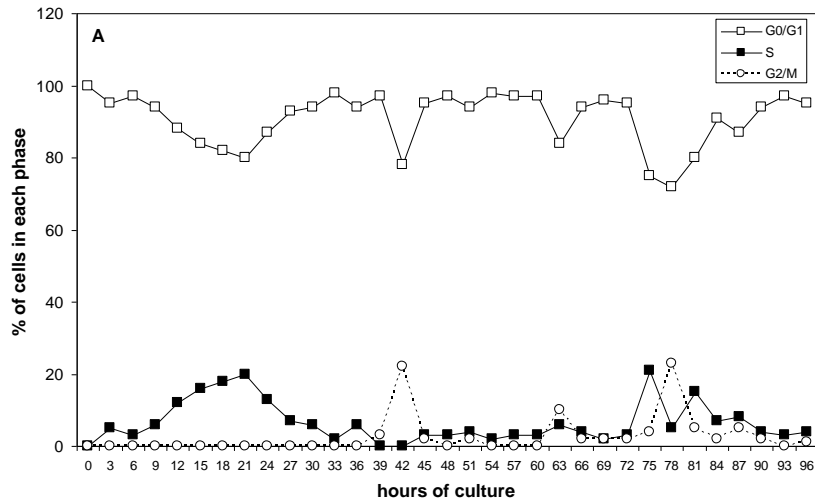
Role of hematopoietic cytokines on the in vitro biology of HSC

Doubling time in liquid cultures

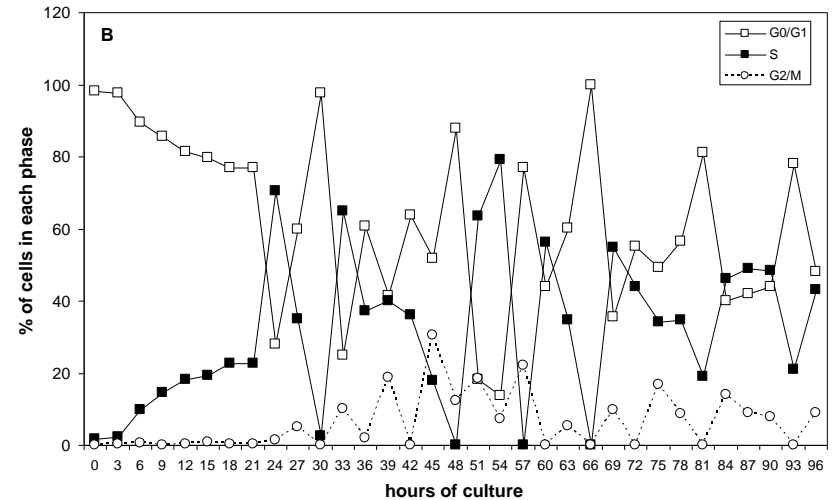


Cell cycle dynamics in culture

No cytokines



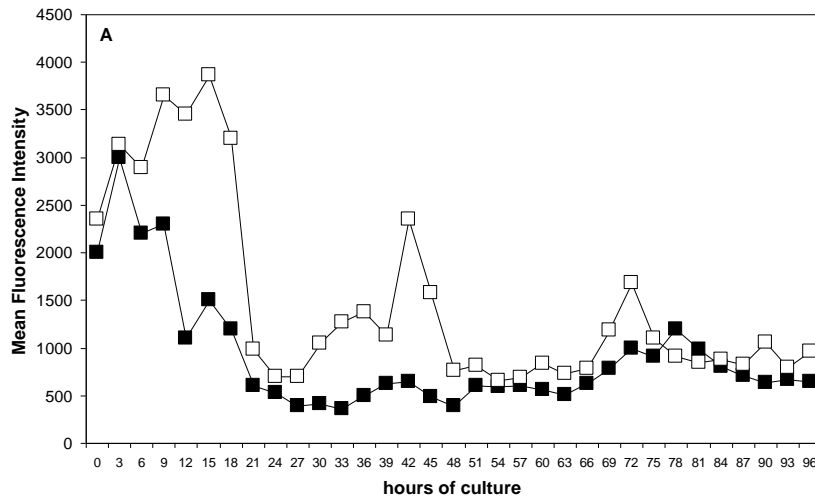
Cytokines



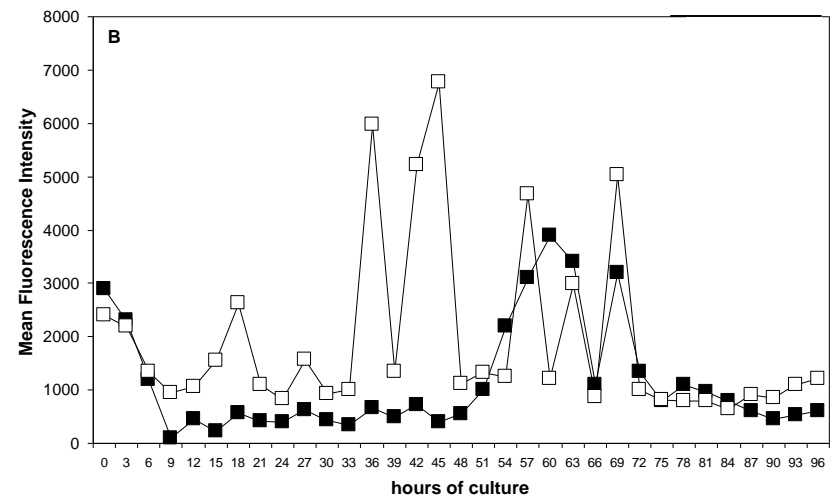
Cytokines = SCF, FL, TPO, IL3, IL6, GM, G, EPO

Expression levels of cell cycle stimulators

Cyclin D3



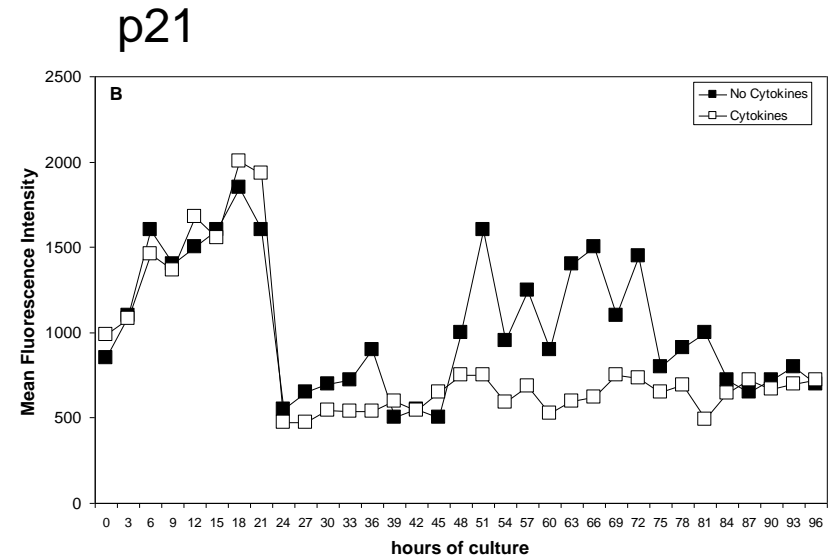
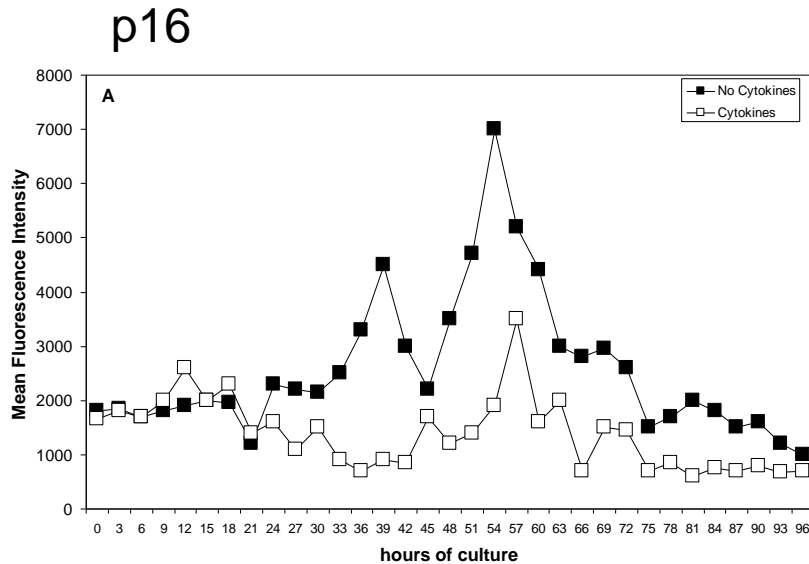
cdk4



■ No cytokines
□ Cytokines

Cytokines = SCF, FL, TPO, IL3, IL6, GM, G, EPO

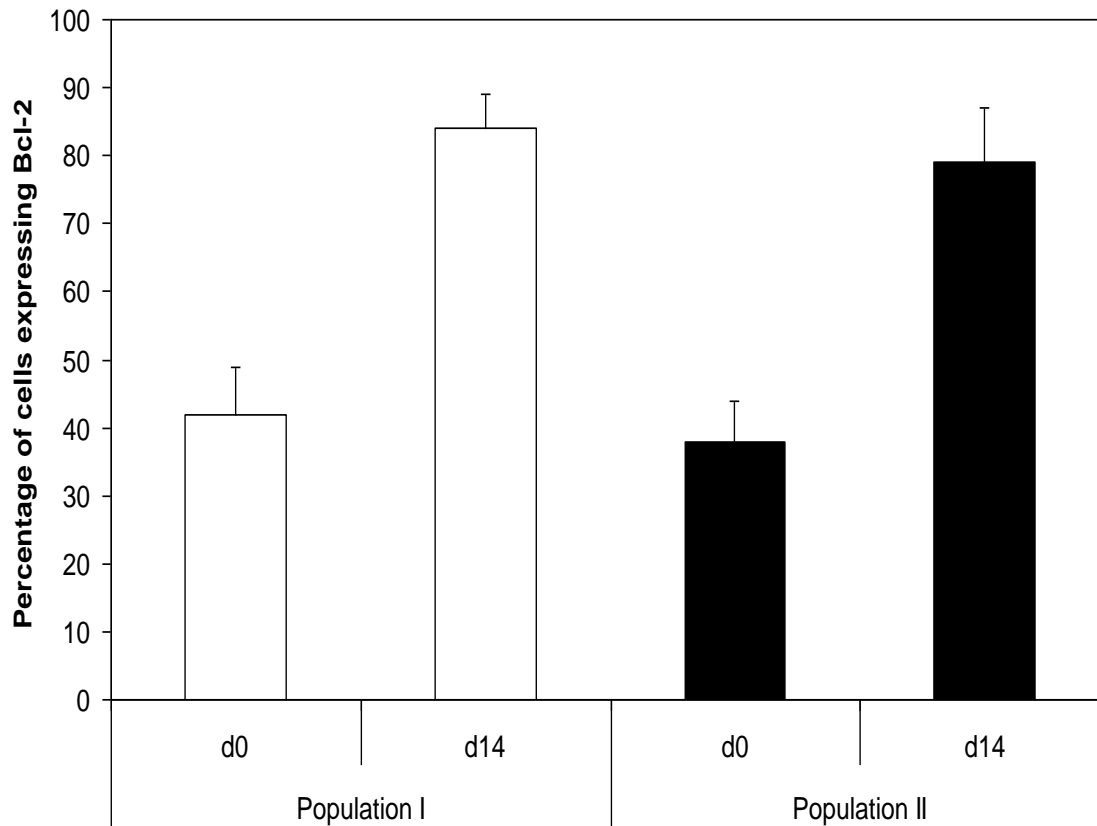
Expression levels of cell cycle inhibitors



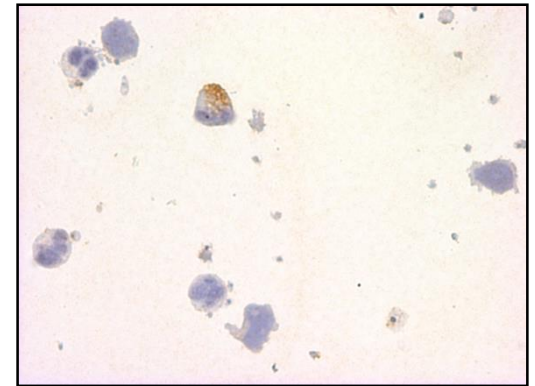
■ No Cytokines
□ Cytokines

Cytokines = SCF, FL, TPO, IL3, IL6, GM, G, EPO

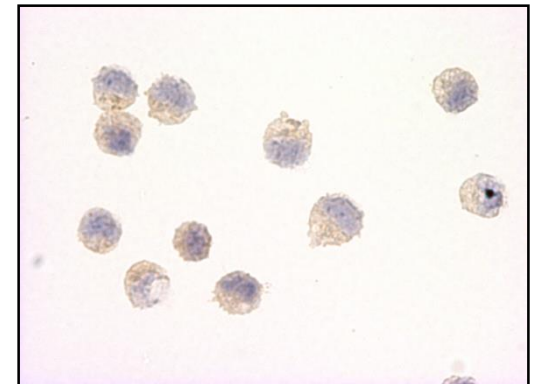
Expression of Bcl-2



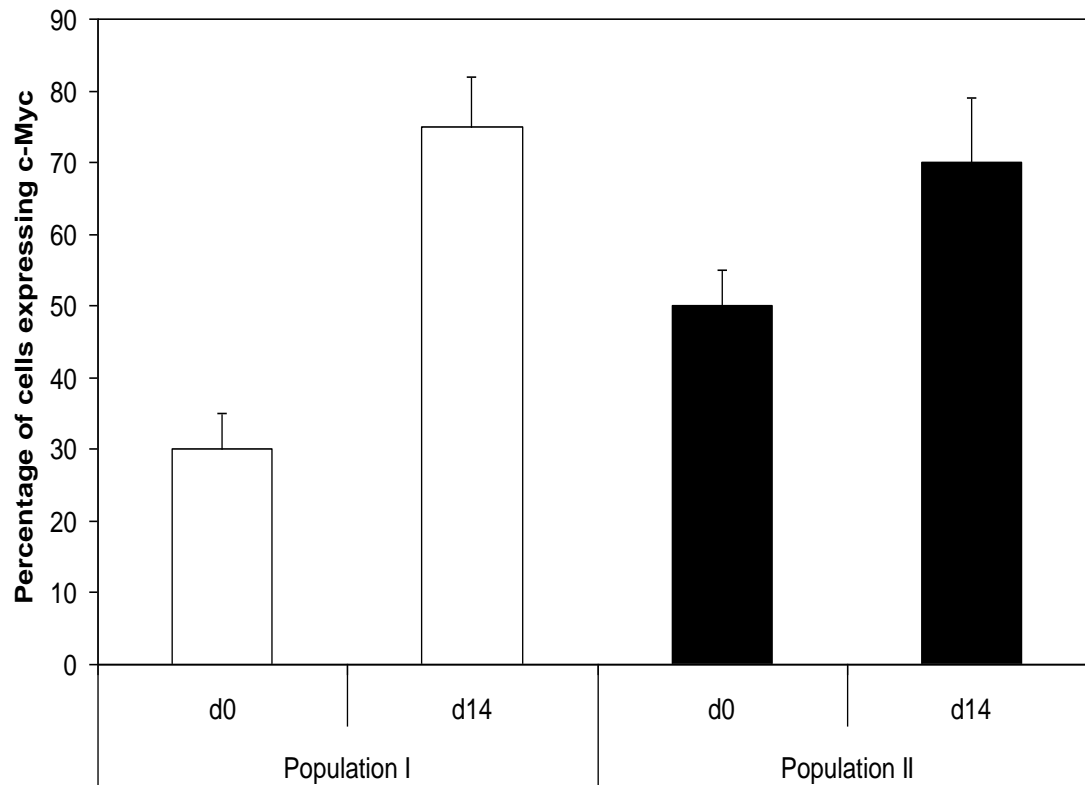
Day 0



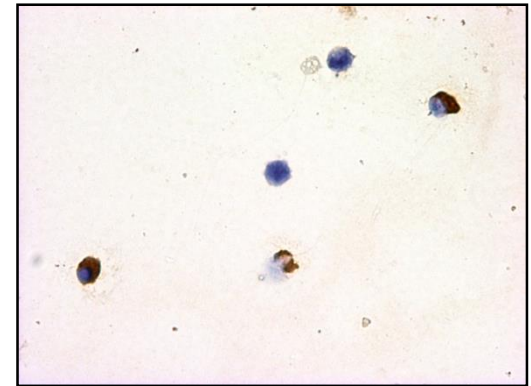
Day 14



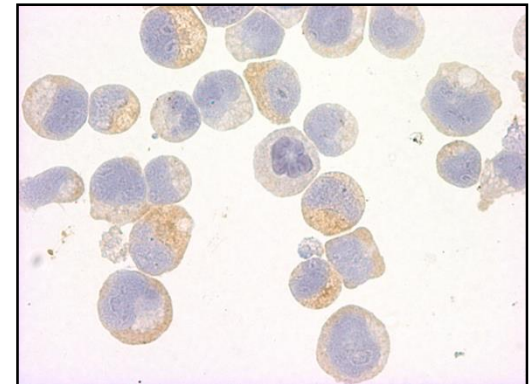
Expression of c-Myc



Day 0



Day 14

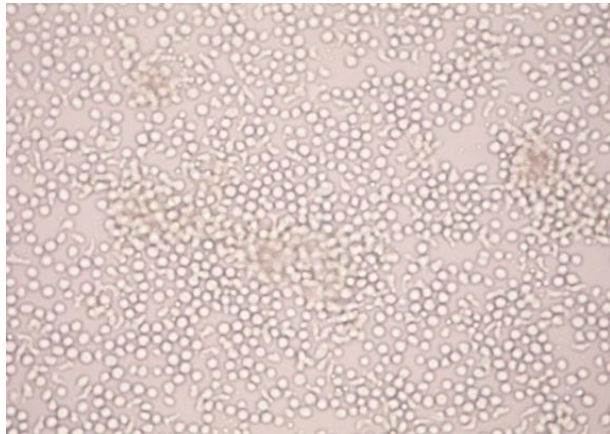


Stimulatory cytokines

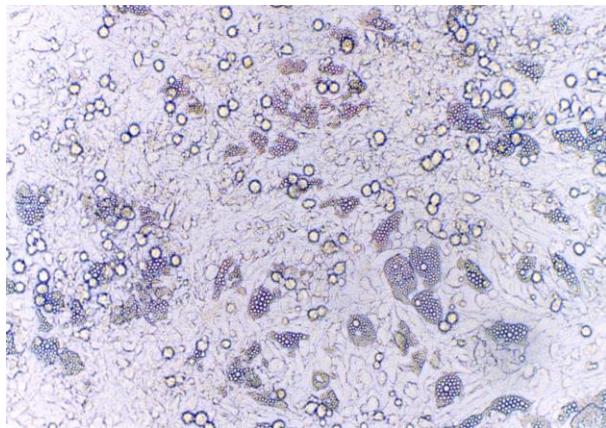
- Favor expression of cell cycle promoters
- Down-regulate expression of cell cycle inhibitors
- Favor expression of anti-apoptotic proteins
- Favor expression of proliferation stimulators

Strategies for ex vivo expansion of HSC

HSC expansion in culture

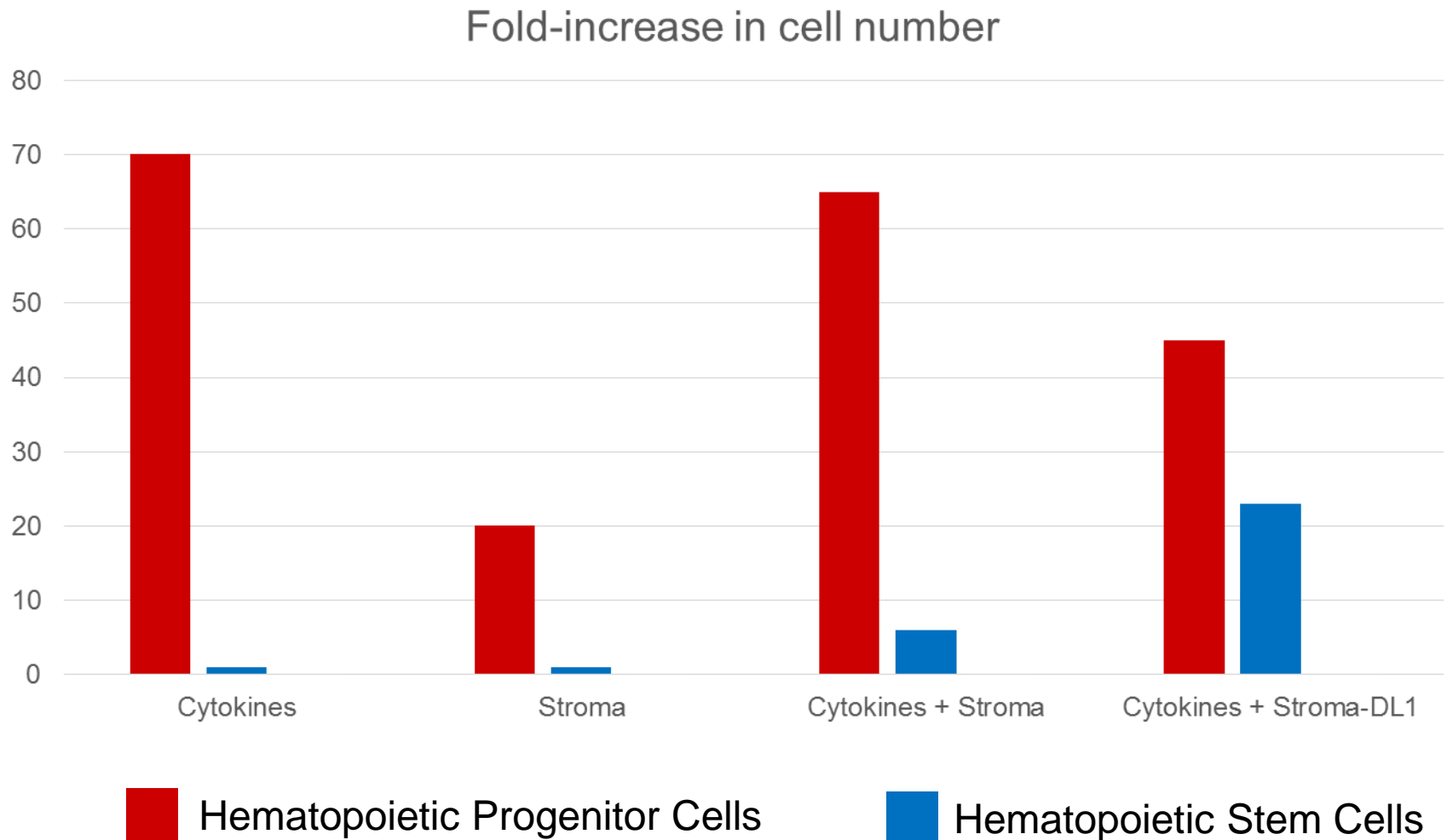


Liquid suspension cultures of HSC-enriched cell populations in the presence of recombinant stimulatory cytokines



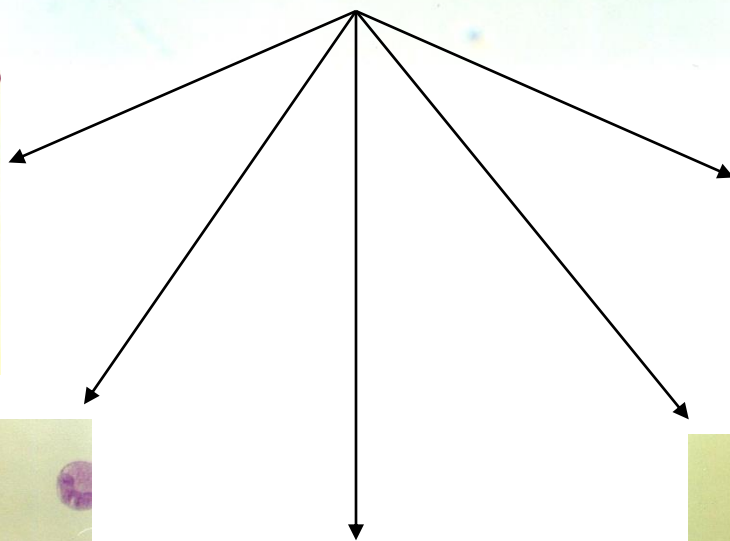
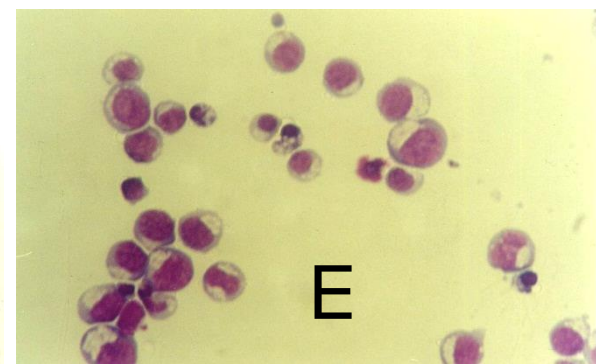
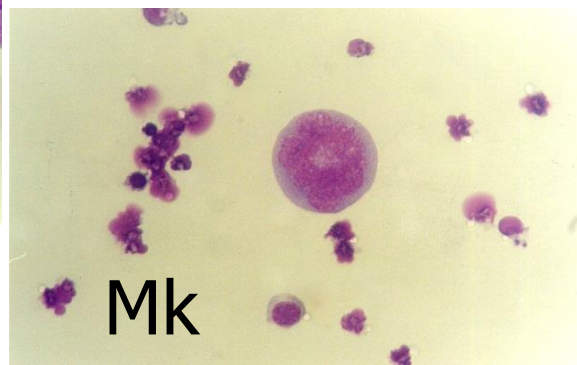
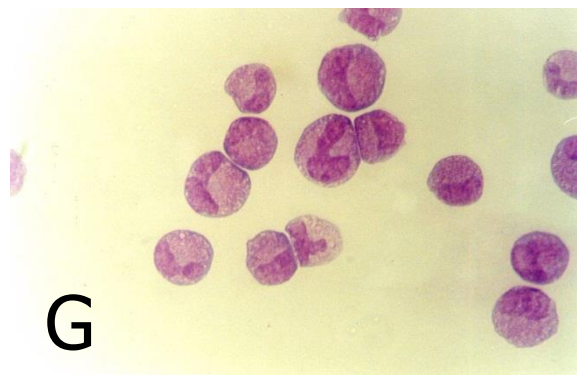
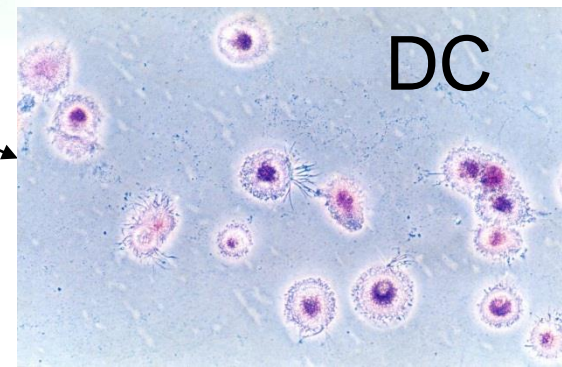
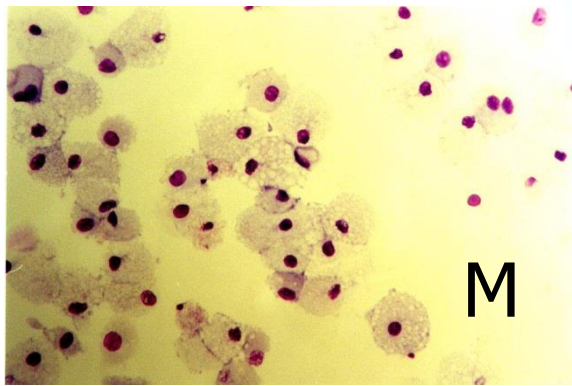
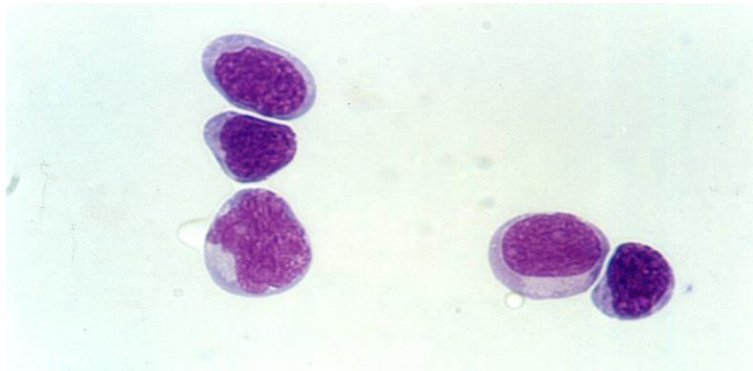
Liquid cultures of HSC-enriched cell populations in the presence of recombinant stimulatory cytokines and stromal cells

Ex vivo expansion of HSC

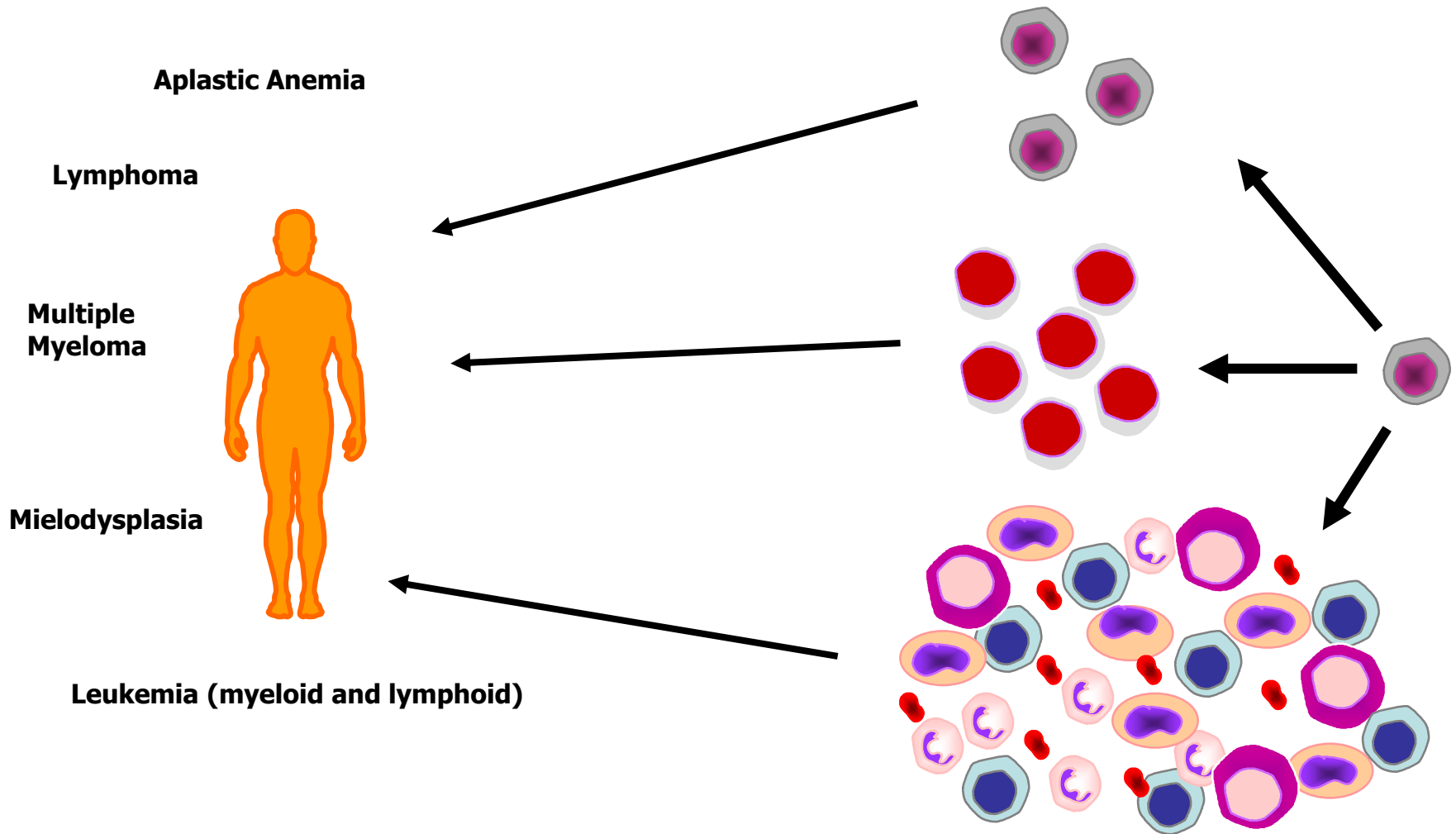


HSC differentiation

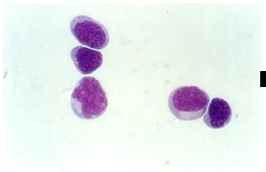
Multipotency vs Plasticity



HSC-based cell therapy for hematologic disorders



HSC



CD34⁺ CD38⁻ Lin⁻

¿Non hematopoietic cells?

¿Neural cells?

Experimental Design

HSC



Liquid culture in 3 phases:

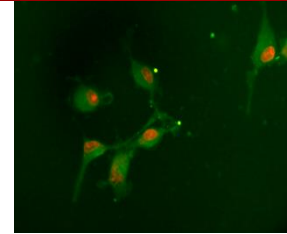
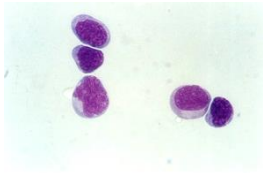
proliferation – priming - differentiation

CD34⁺ CD38⁻ Lin⁻

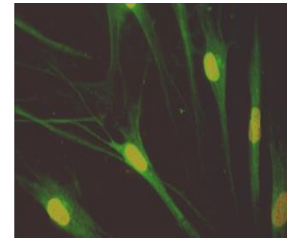


25 days

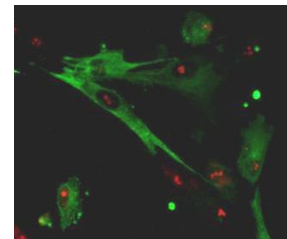
Expression of neural proteins



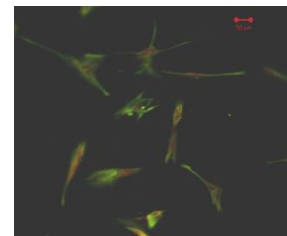
Nestin



MAP2

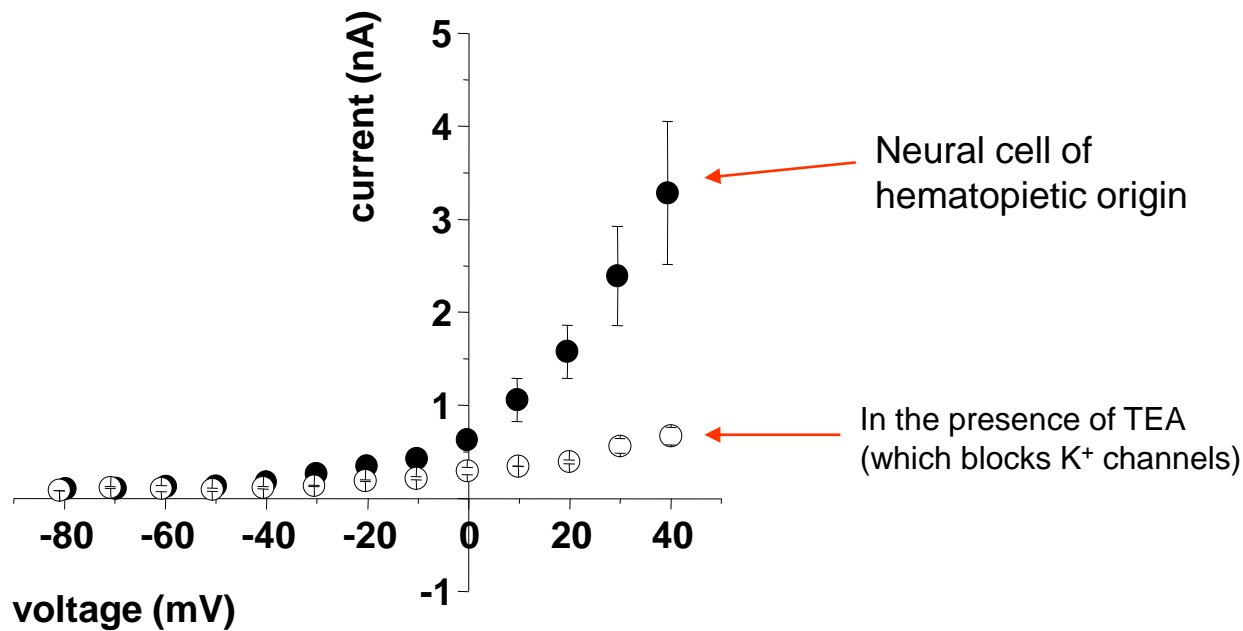
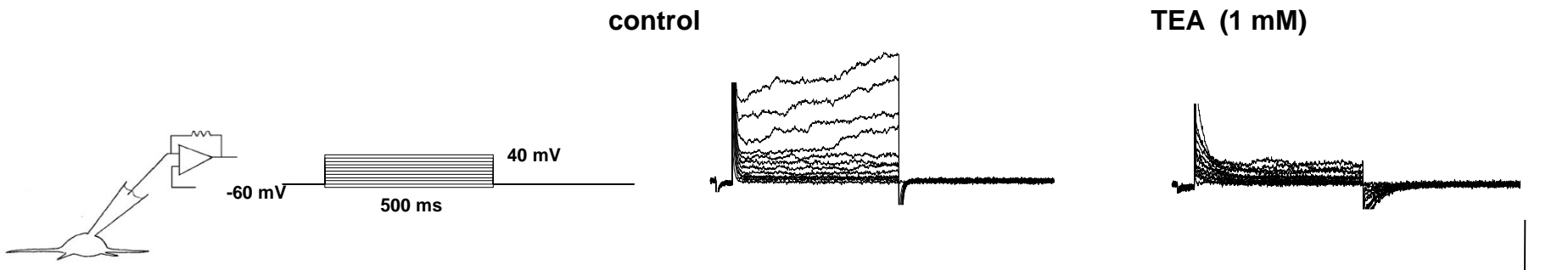


NeuN



NF

Patch clamp on neural-like cells originated from HSC



In vitro manipulation of HSC

- By using particular cytokine combinations, UCB-derived HSC can be selectively induced to differentiate into specific hematopoietic lineages
- Under the appropriate culture conditions, UCB-derived HSC can give rise to non-hematopoietic cells that show morphological, molecular and functional features of neural cells

HSC-based cell therapy for non-hematologic disorders?

