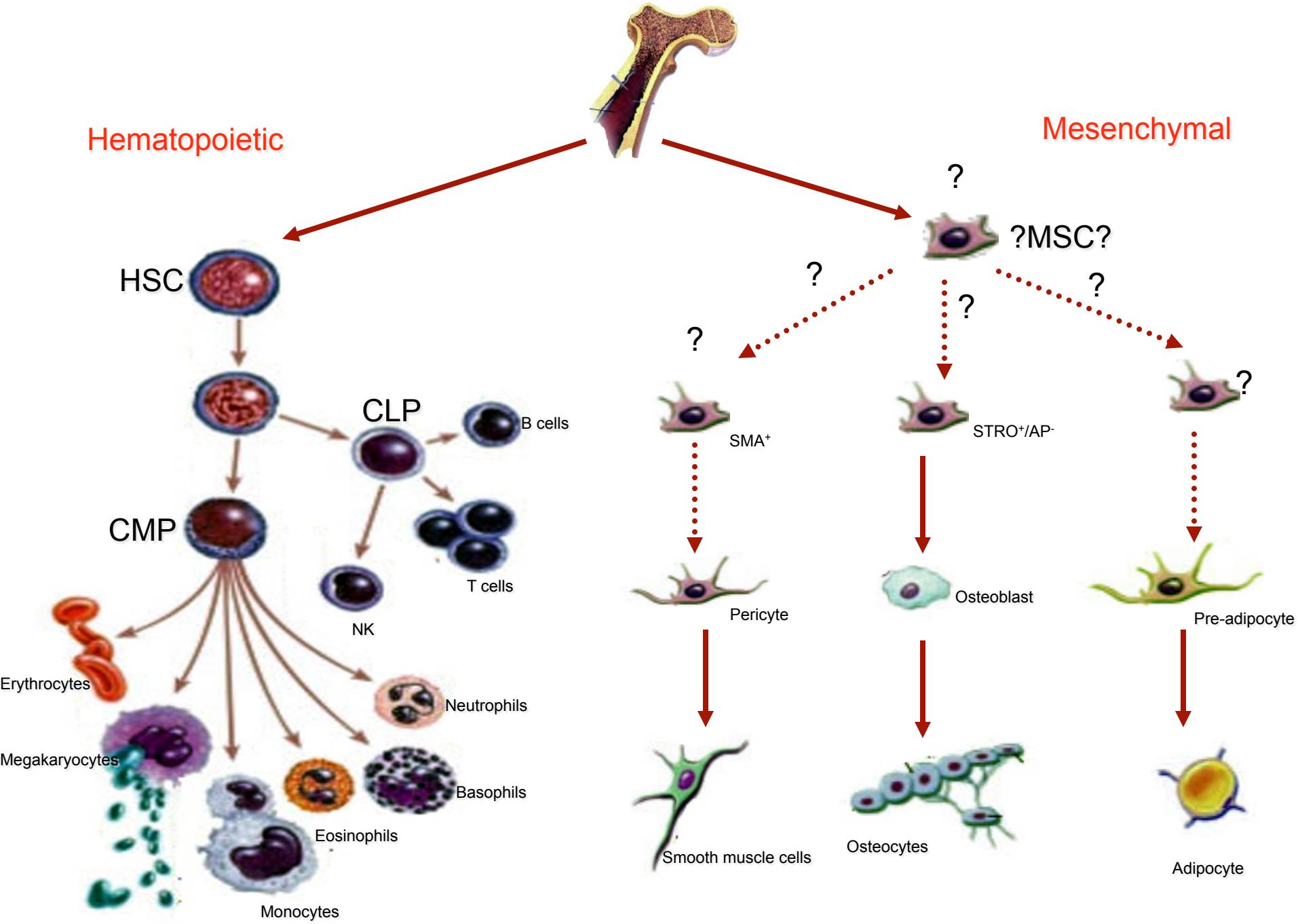
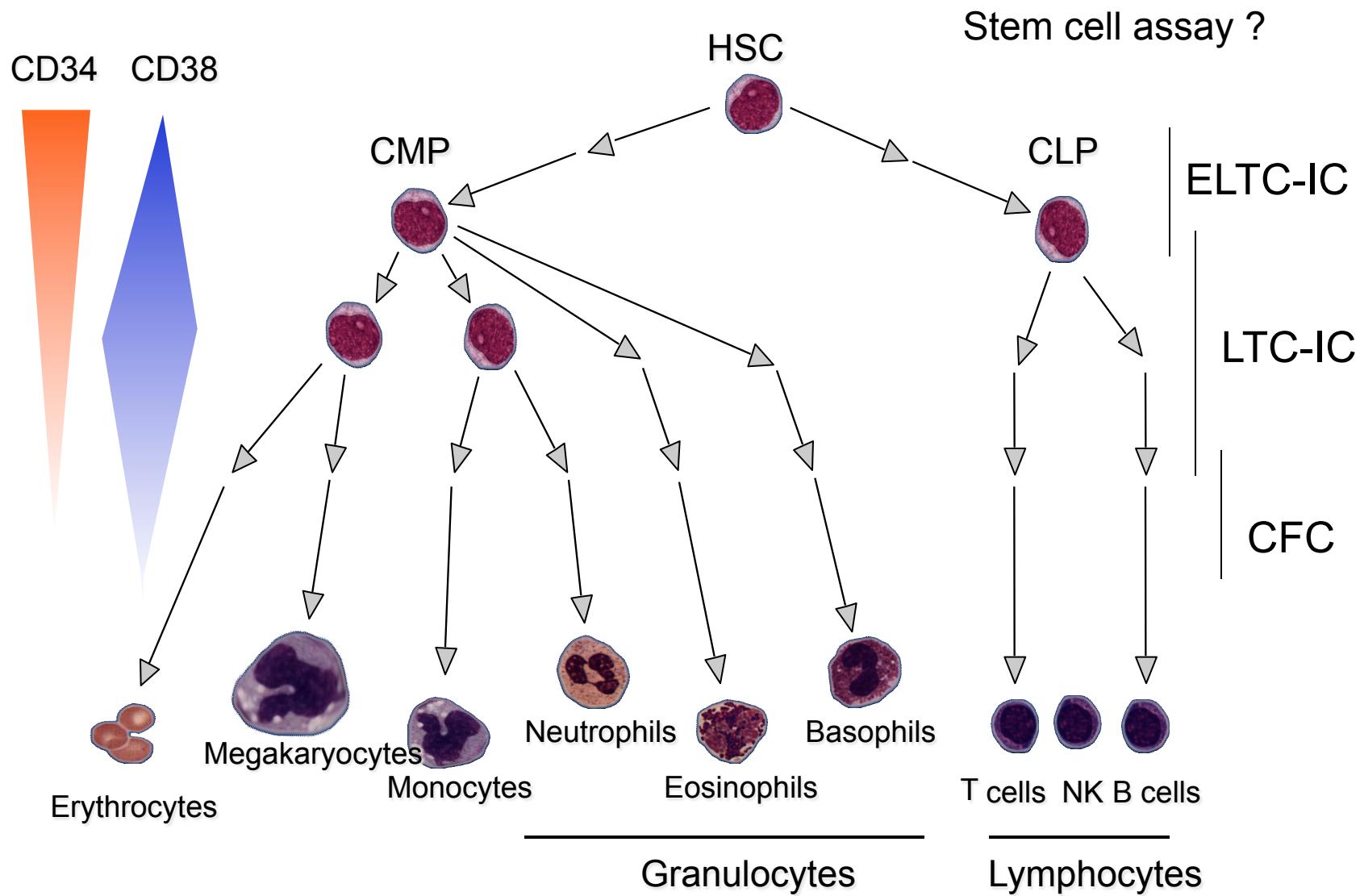


Cellules souches cancéreuses

Prof. Dominique Bonnet
Cancer Research UK, London Research Institute



The “Hematopoietic” (Blood) System



Cellules Souches Hematopoietiques

Les cellules souches hématopoïétiques sont caractérisées par leur capacité *in vivo* de repopuler entièrement le système sanguin

1. Capable de prolifération et différenciation
2. Capable d'auto-renouvellement

LEUCEMIE AIGUE MYELOIDE

- Production excessive de blastes leucémiques ayant une capacité de prolifération limité
- Concept de la présence d'une sous-population leucémique avec prolifération importante et capacité d'autorenouvellement existe afin de maintenir la maladie

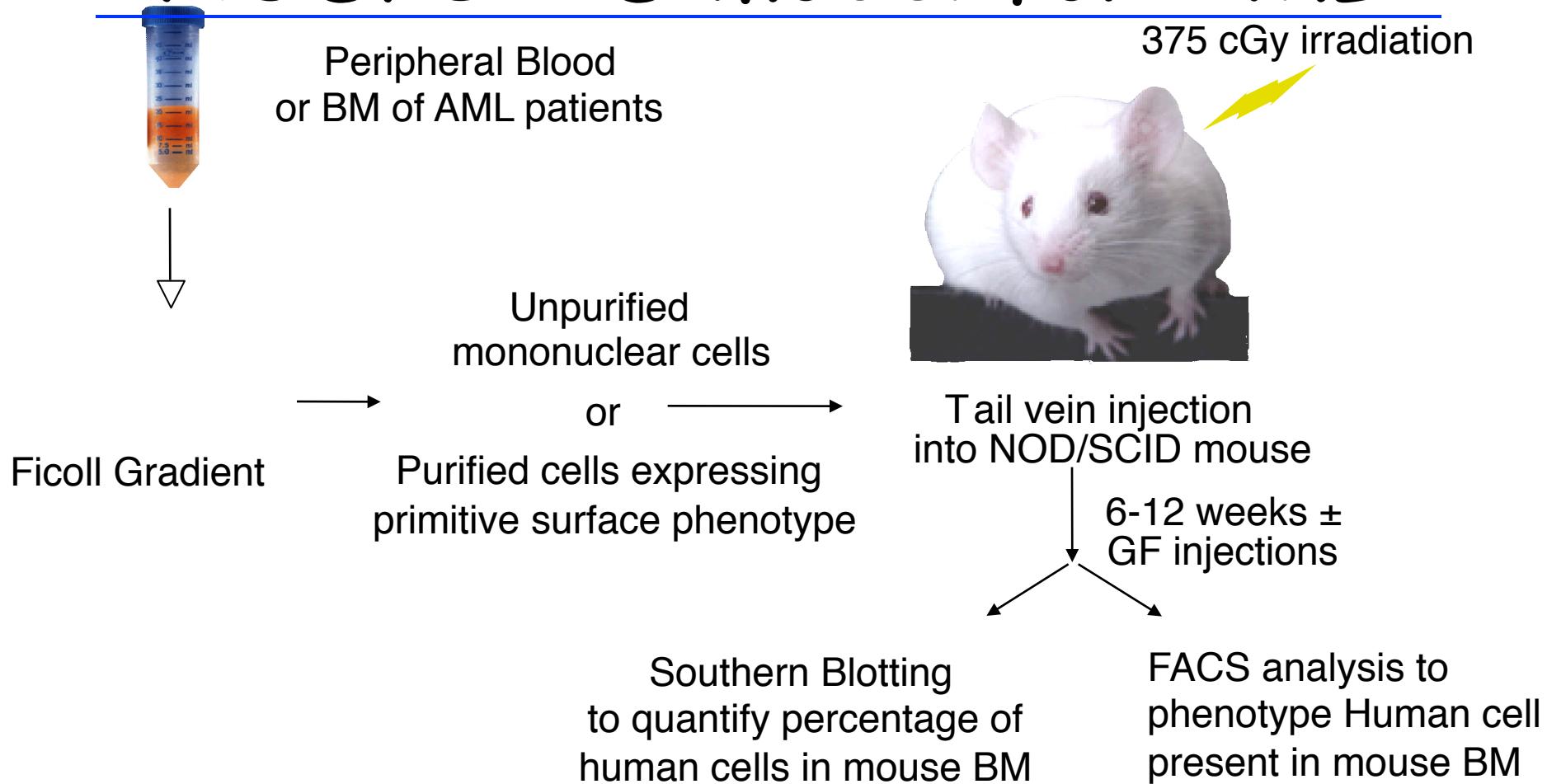
Evidence de l' existence de cellules souches leucémiques

- ✓ Une faible proportion de blastes forment des colonies *in vitro* (1 cellule sur 10^2 forme des CFU-LAM)
- ✓ Ces CFUs ont un faible taux d'auto-renouvellement
- ✓ Donc, existence d'une population leucémique plus immature pour maintenir la leucémie

Questions majeures

- ✓ Quelles cellules leucémiques est capable d'initier et maintenir la maladie ? – besoin d'un test fonctionnel
- ✓ Quelle cellule normale est à l'origine des CSL- besoin d'un test permettant de purifier les cellules leucémiques et les comparer avec les cellules normales

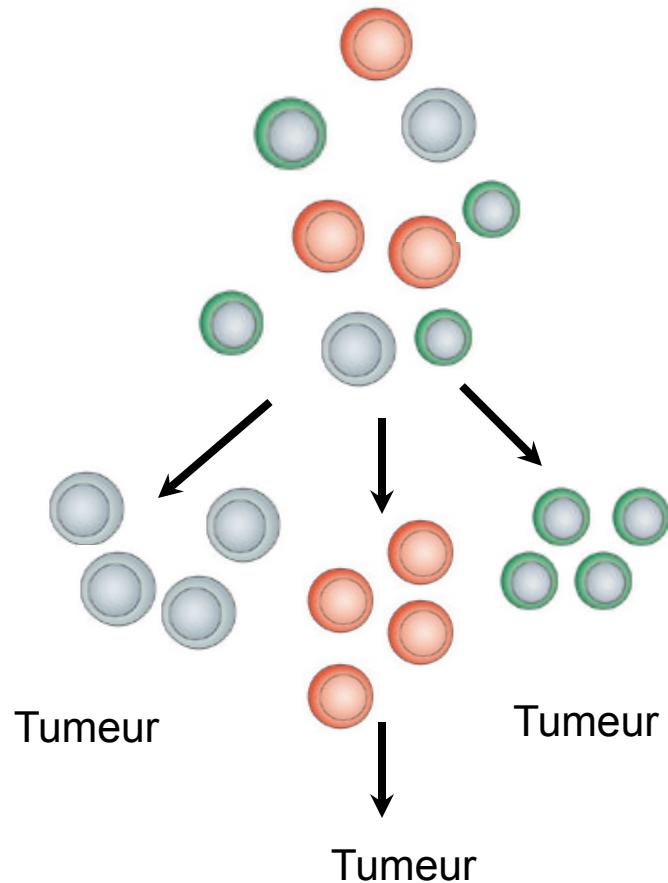
NOD/SCID model for AML



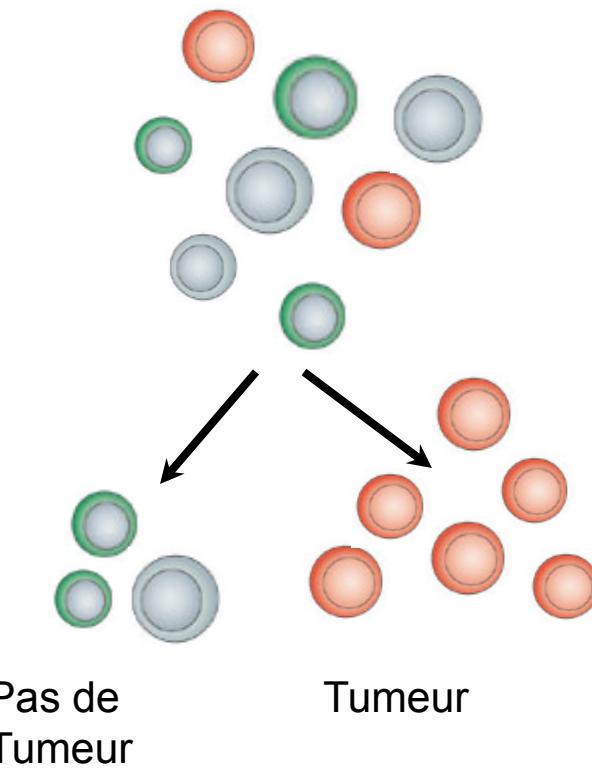
- Model that faithfully reproduces the AML disease in mice
- Enables characterisation of engrafted cells

Modèle de prolifération tumorale

Modèle Stochastique



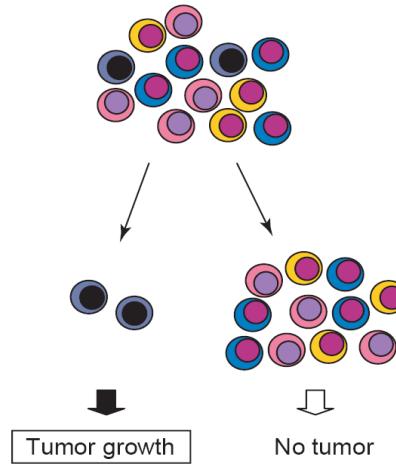
Modèle des cellules souches leucémiques



Etude fonctionnelle *in vivo* pour étudier le système hématopolétique



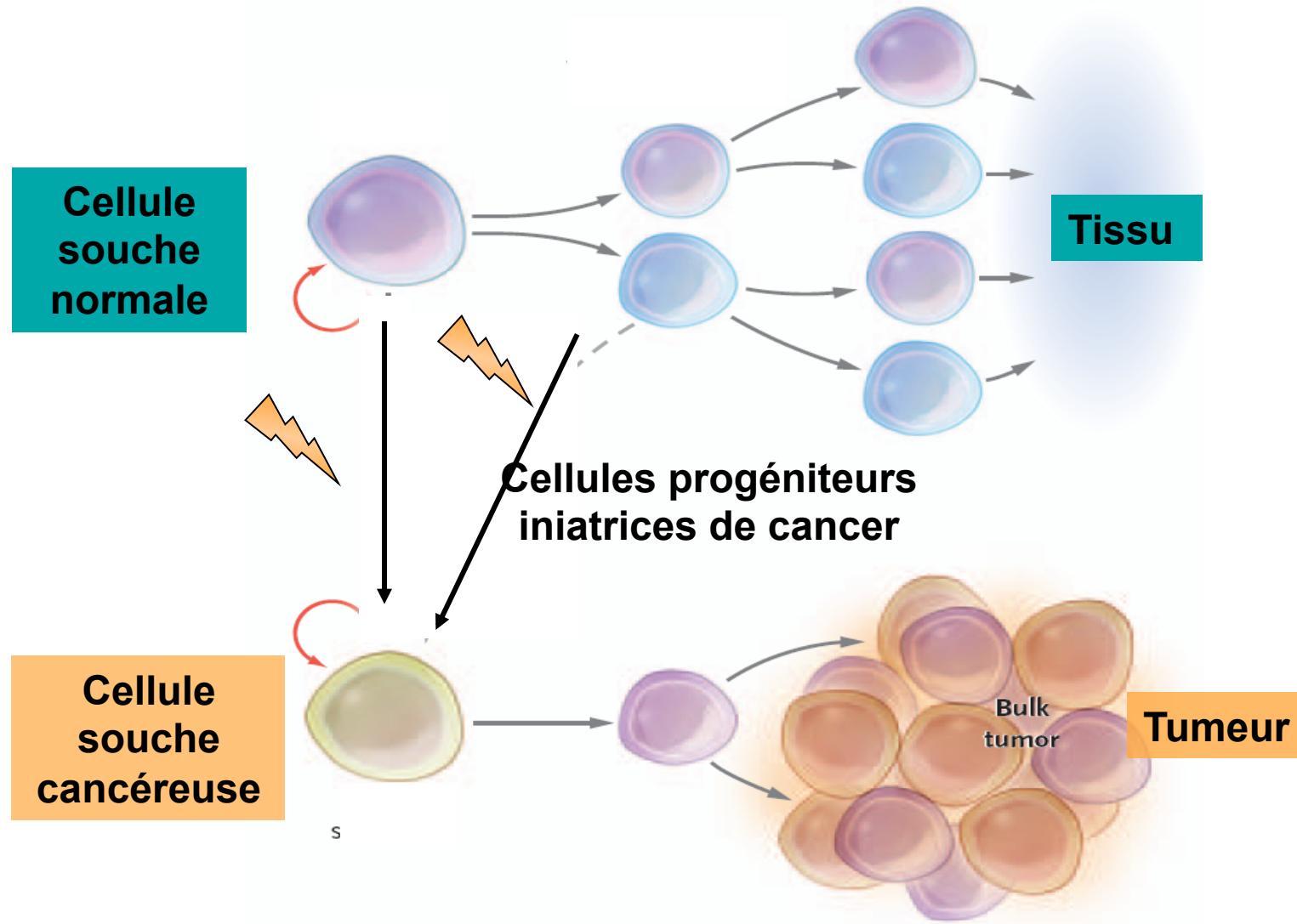
Existence de cellules souches leucémiques



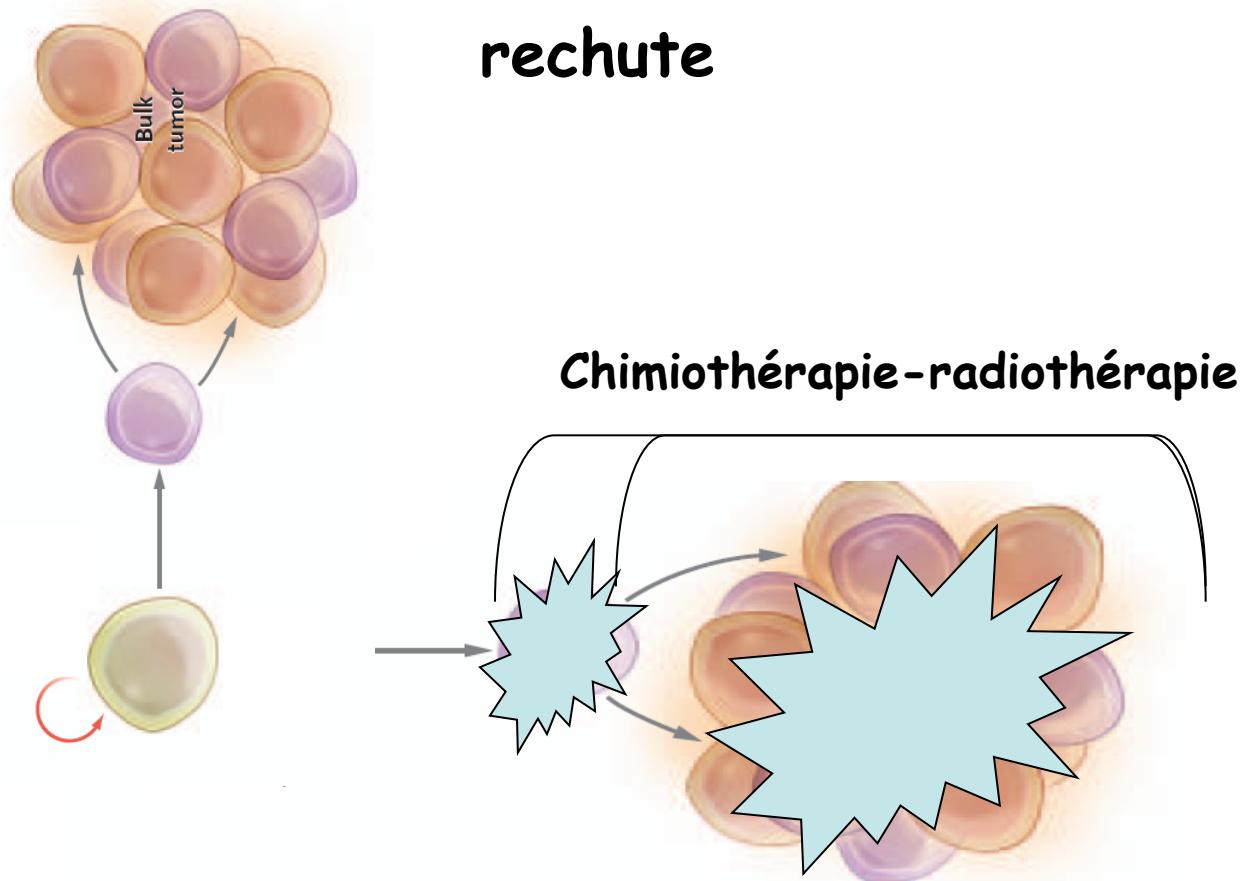
Le clone leucémique est organisé comme une hiérarchie dans laquelle un petit nombre de progéniteurs prolifèrent et refournissent en continu la population de cellules leucémiques non proliférantes



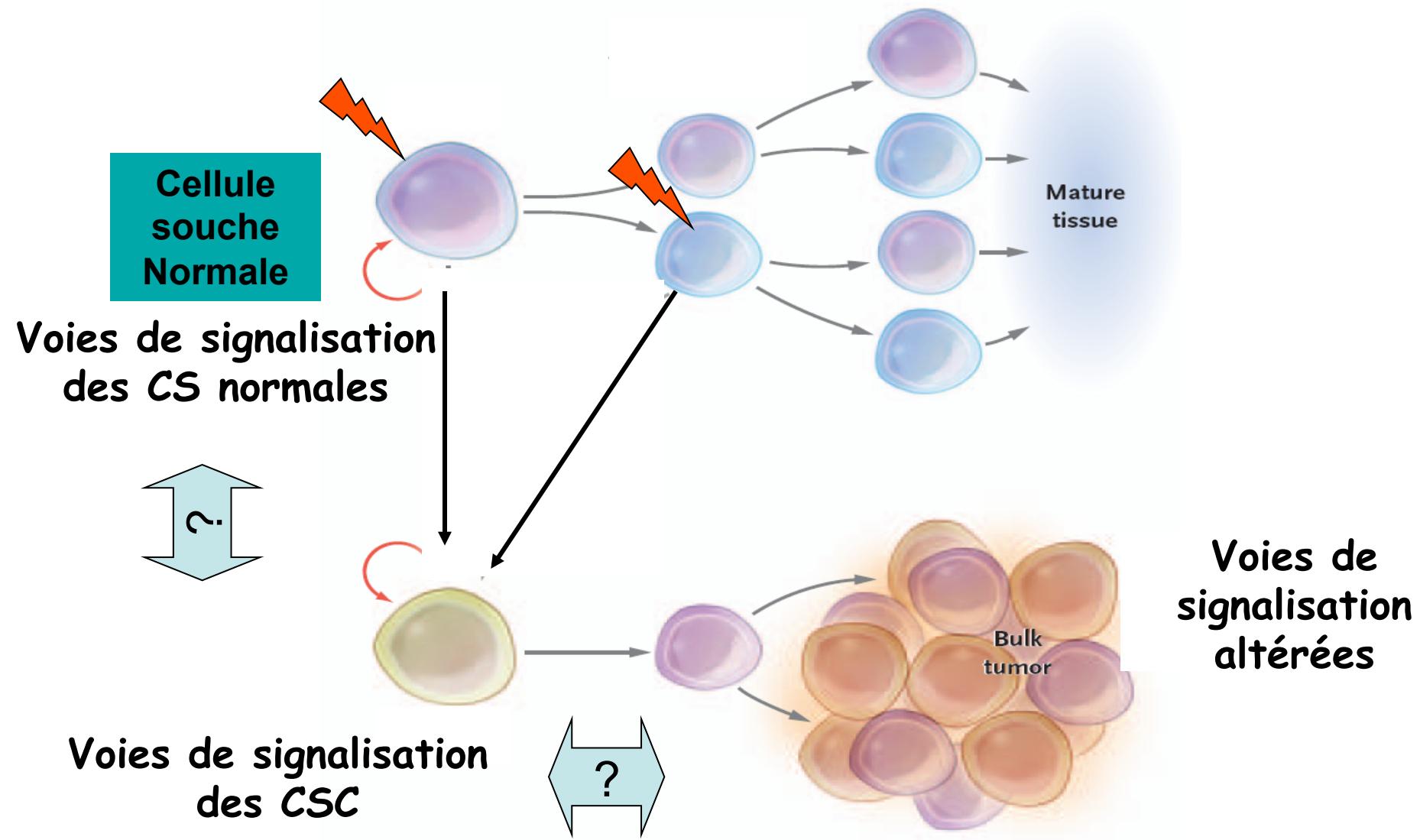
Concept des cellules souches cancéreuses (CSCs)



Impactes des CSCs pour les thérapies ciblées



Mécanismes moléculaires des cellules souches cancéreuses



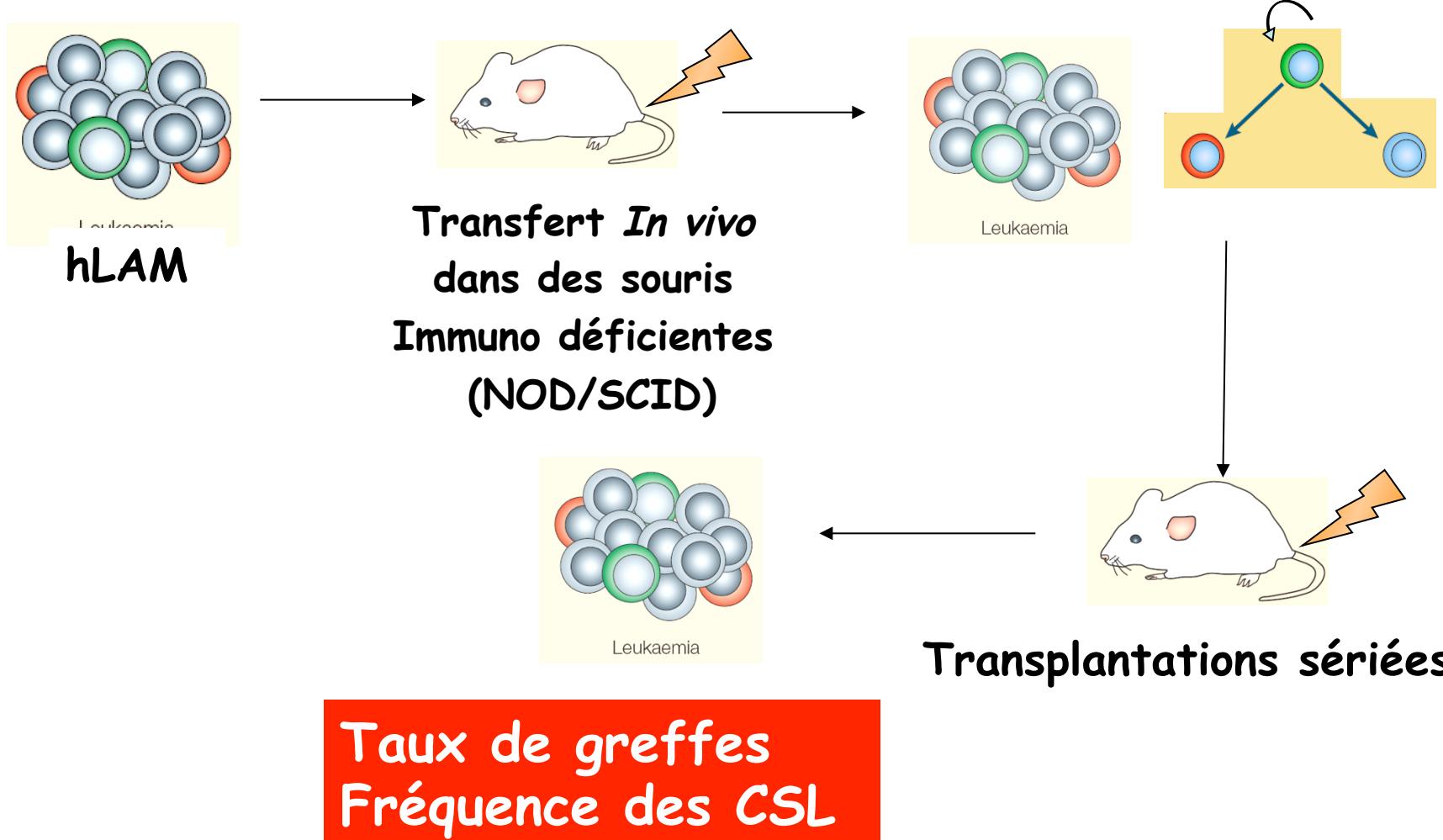
Première étape

Caractérisation des cellules souches cancéreuses

- ❖ Auto-renouvellement
 - ❖ Multipotentialité
 - ❖ Tumorigénicité
- In vitro*
- In vivo*

Hommes: cellules de malades, tests *in vitro*, xenogreffes
Modèles animaux: transgeniques ou greffés

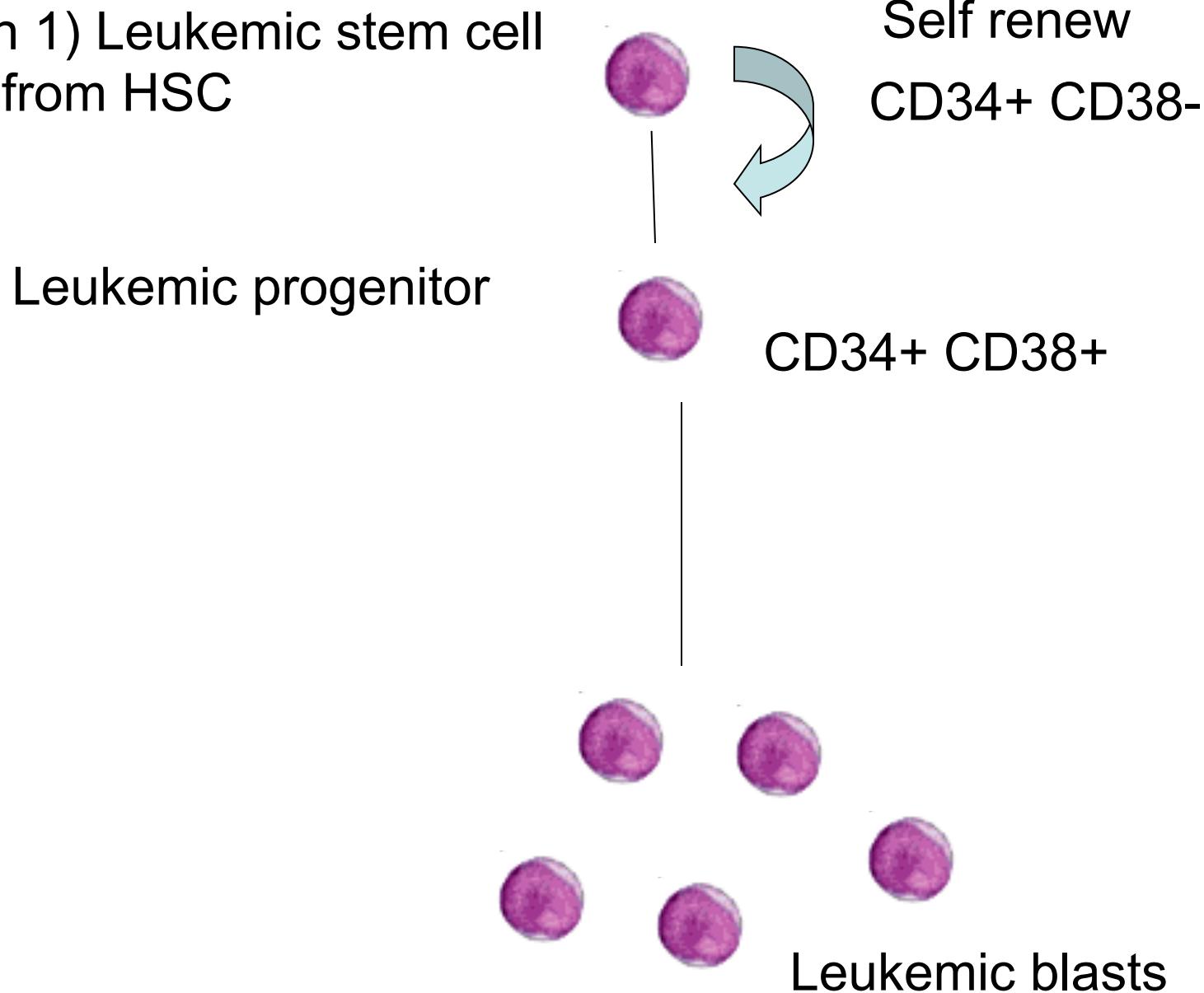
Leucémie: Tests *in vivo* pour confirmer la présence des CSLs humaines



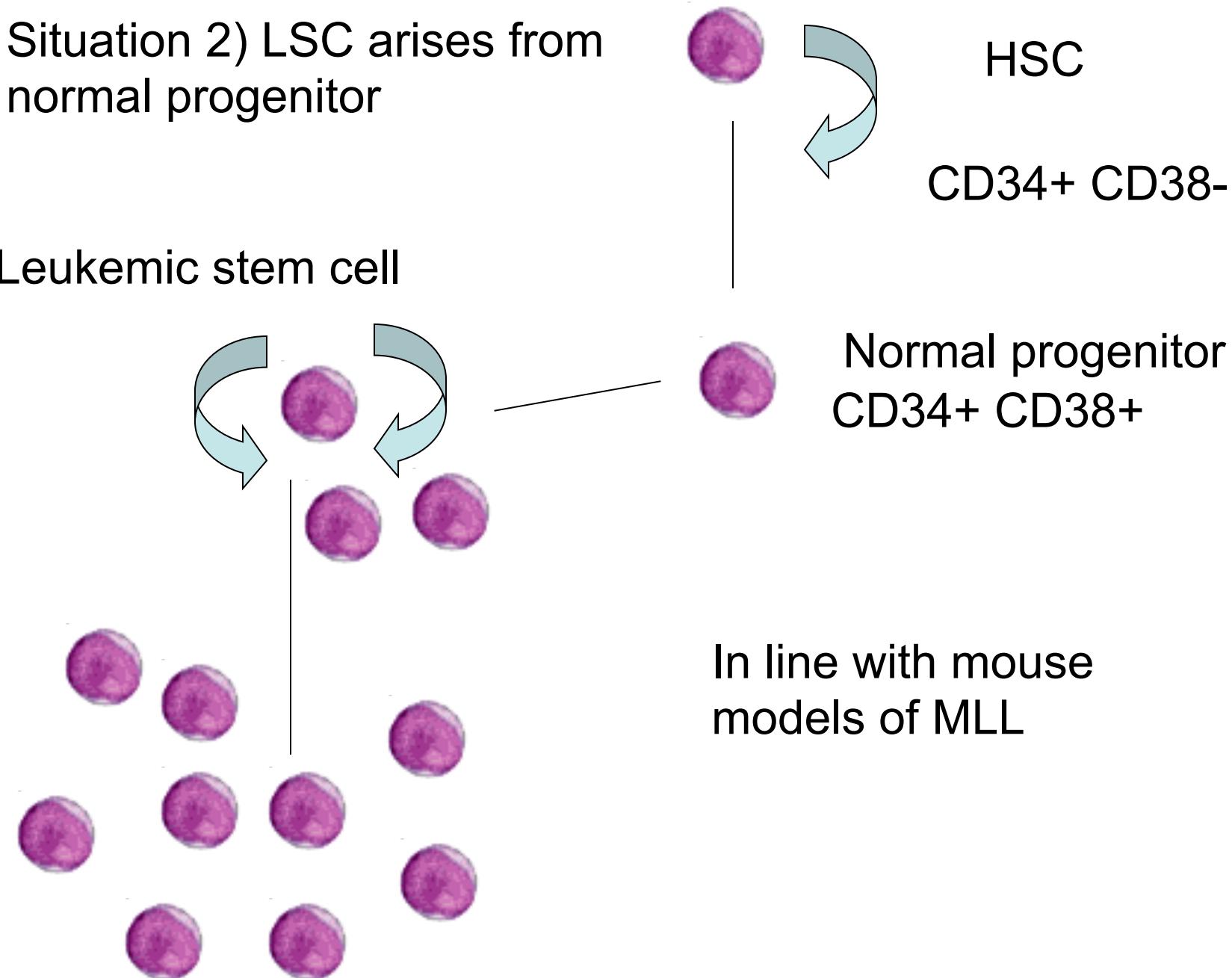
Test de dilution limite : les CSL sont présentes à une fréquence faible de 0.2 to 100.10^6 de cellules mononucléées

Hétérogénéité des cellules souches leucémiques

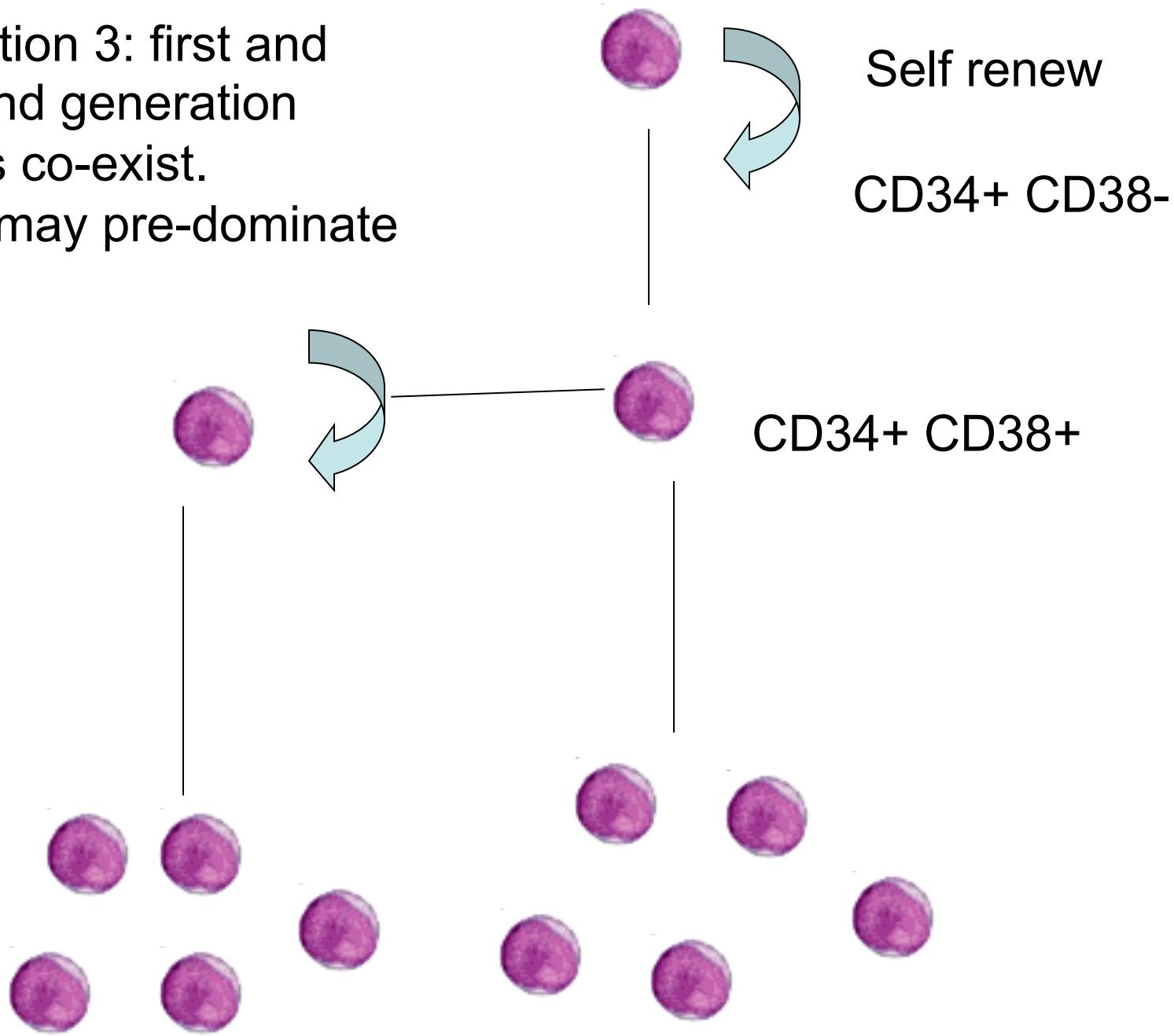
Situation 1) Leukemic stem cell
derived from HSC



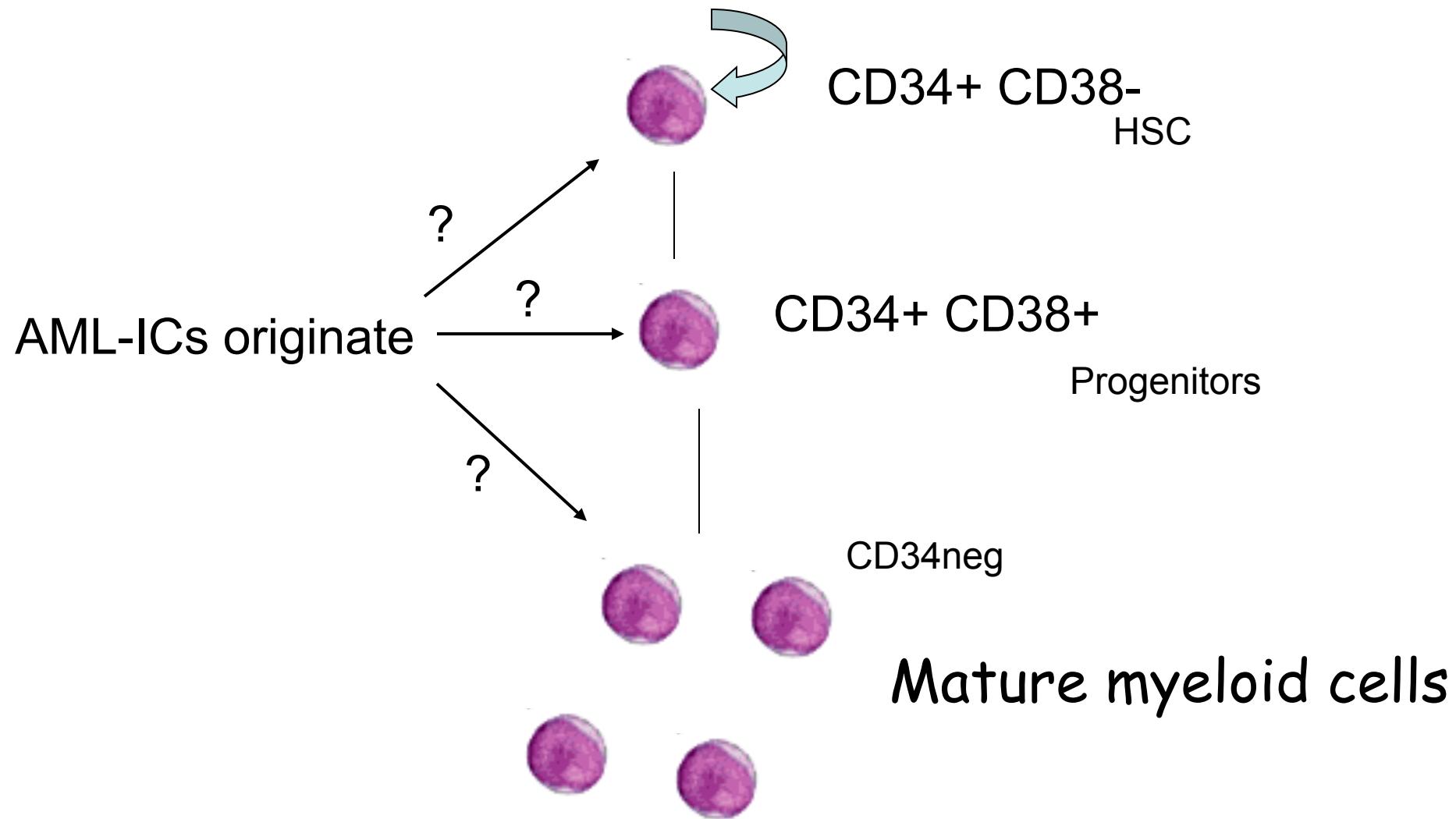
Situation 2) LSC arises from normal progenitor



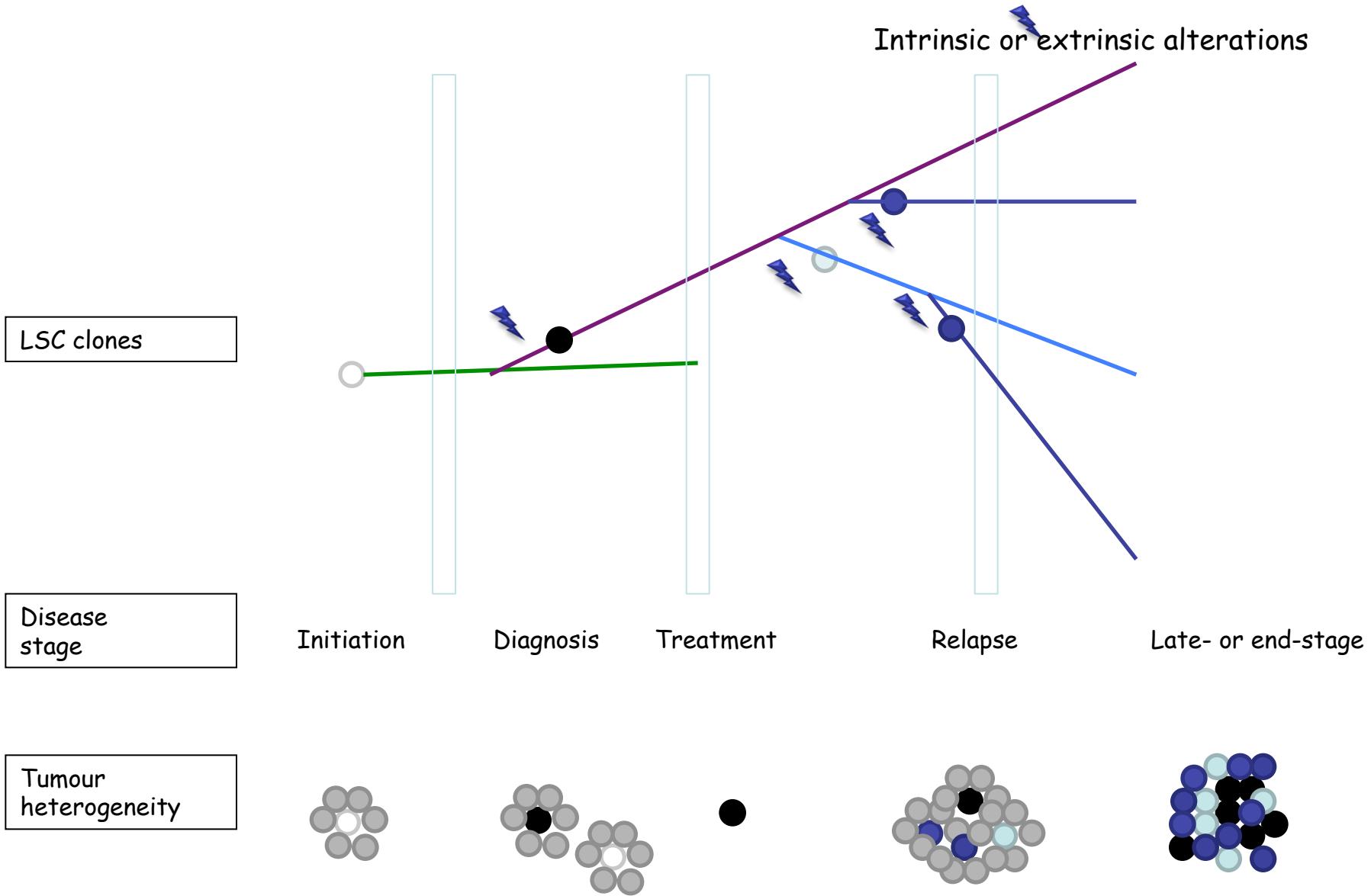
Situation 3: first and second generation LSCs co-exist.
One may pre-dominate



Can we conclude on the origin of AML-ICs based on phenotype ?



What happen during the disease progression ?



Role de la "Niche" hématopoïétique dans le maintien des cellules souches leucémiques

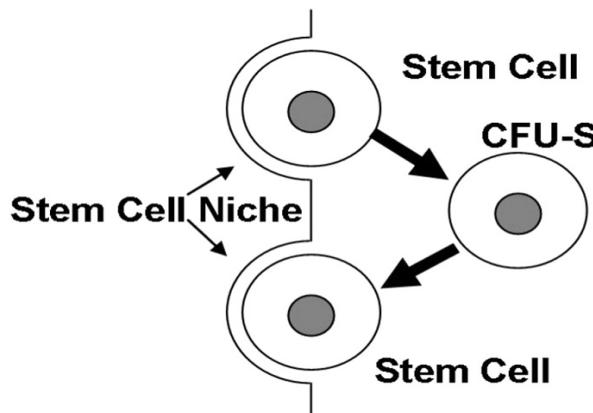
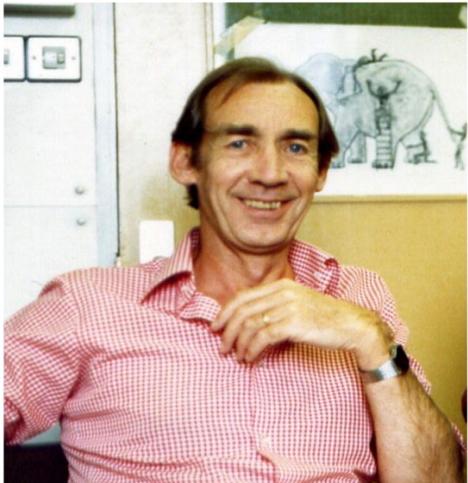
History of the Stem Cell Niche

- ✓ R Schofield introduces the concept of “stem cell niche” in 1978 (*R. Schofield. Blood Cells, 1978; 4: 7*).

- ✓ First proven in Drosophila
 - Testicular GSC by A. Spradling
 - Ovarian GSC by M. Fuller
 - C. Elegans by Kimble.

- ✓ Recent advancement in intra-vital imaging of the stem cell niche and used of transgenic mouse models shed new light on the role of different component of the niche in the bone marrow.

Concept of the stem cell niche



Review by Papayannopoulou, T. et al.
Blood 2008

R. Schofield

- 1- anatomic defined site
- 2- a location where stem cells could sustained and self-renew
- 3- a place where differentiation was inhibited
- 4- a limited space that control of number of stem cells
- 5- a place where more mature cells can revert into stem cells

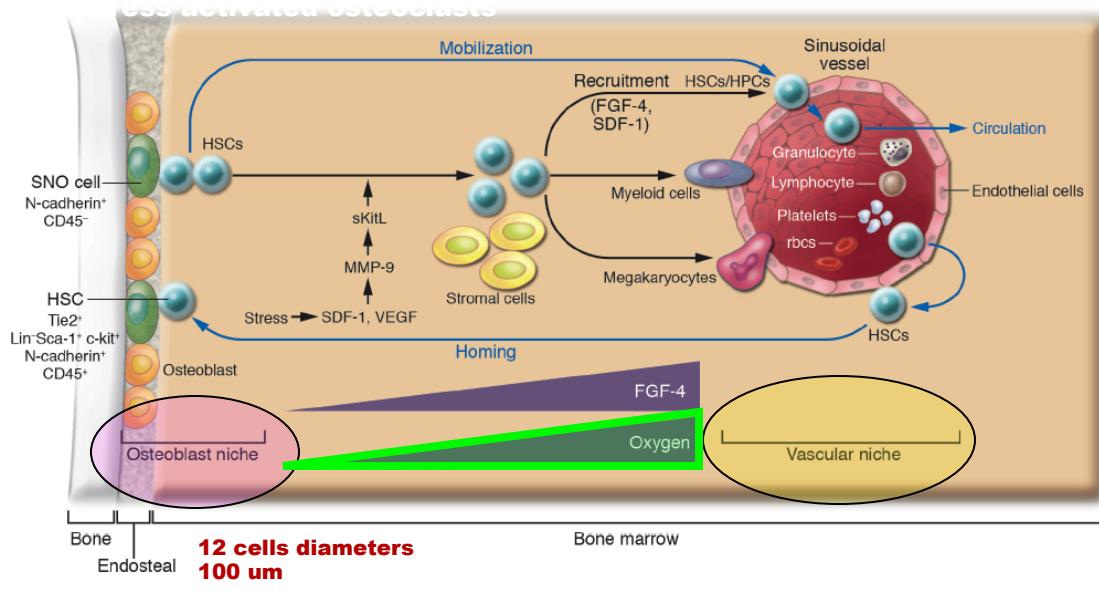
The stem cell niche model

The stem cell niches in bone

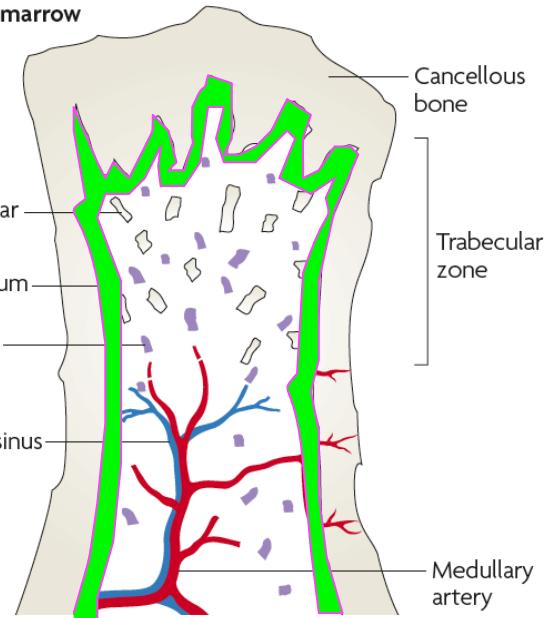
Tong Yin¹ and Linheng Li^{1,2}

The Journal of Clinical Investigation <http://www.jci.org> Volume 116 Number 5 May 2006

The stem cell niche is hypoxic



a Bone marrow

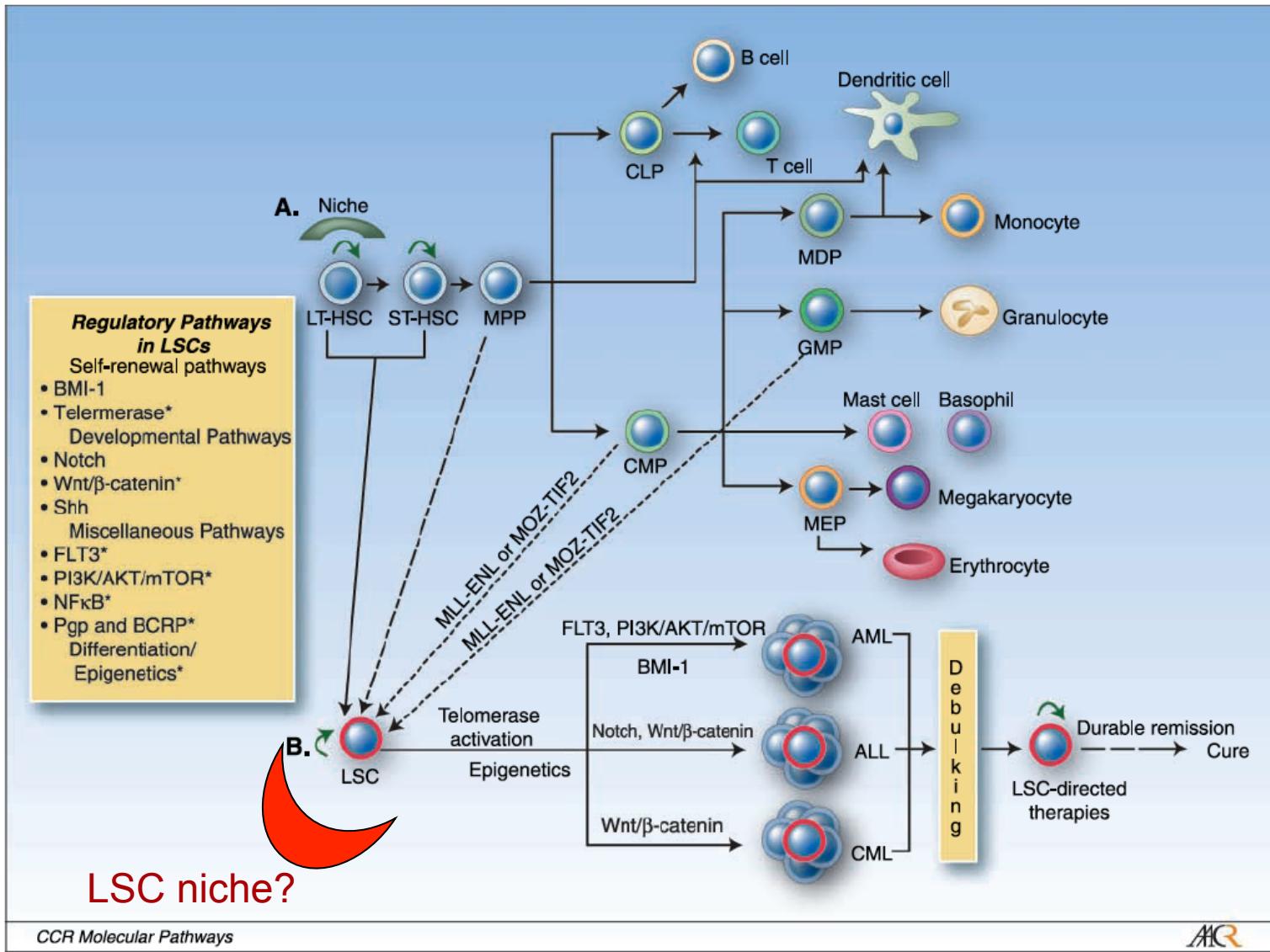


Uncertainty in the niches that maintain haematopoietic stem cells

Mark J. Kiel and Sean J. Morrison

Nature Reviews Immunology | AOP, published online 7 March 2008;

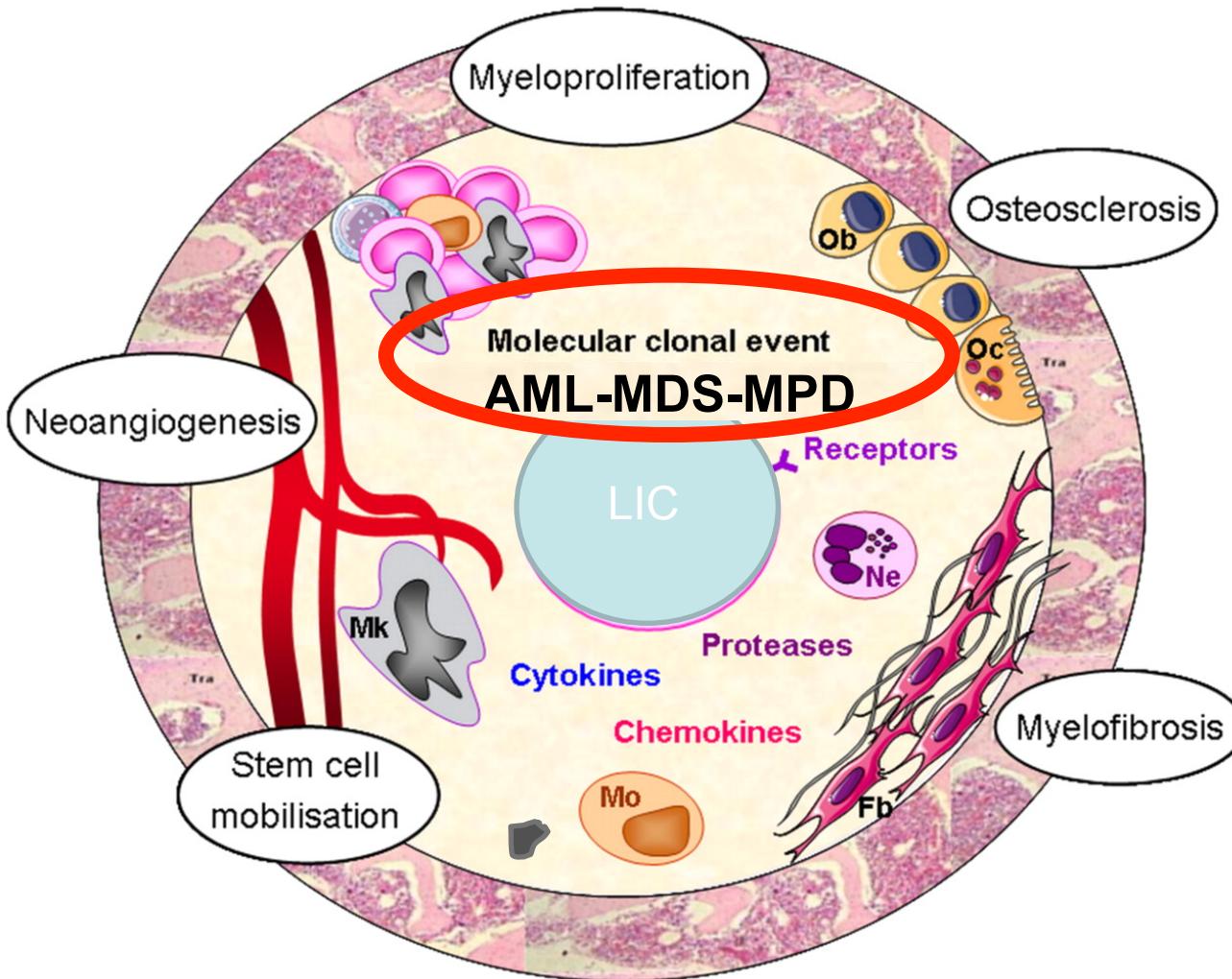
Mechanisms that regulate LSCs ?



What is the rationale for a LSC Niche ?

- No good *in-vitro* model to study AML progenitor / stem cells: a functional indication ?
- HSCN : the ideal place to escape anti-mitotic treatments (quiescence, self-renewing site)
- BM failure : a direct consequence of a competition for the niche(s) ?

LICs in their niche: What are the determinant factors ?



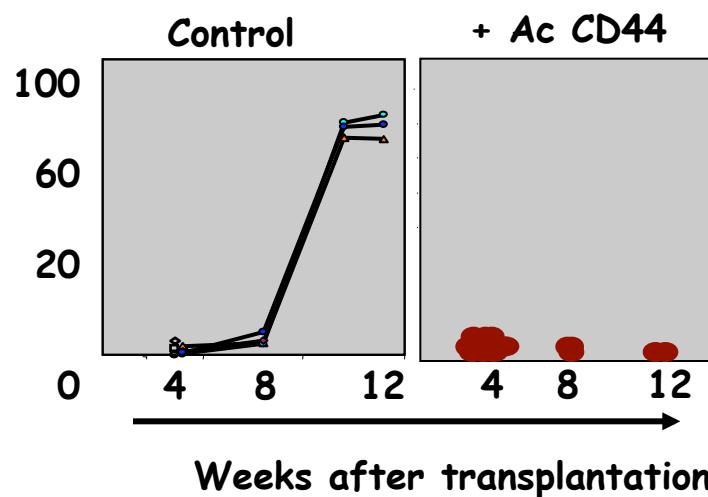
anti-CD44Ab eradicates the human leukemic clone

human LAM cells



Control and Anti-CD44
treated mice

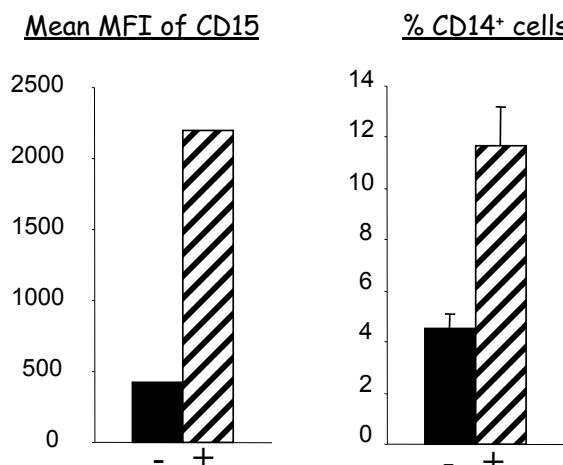
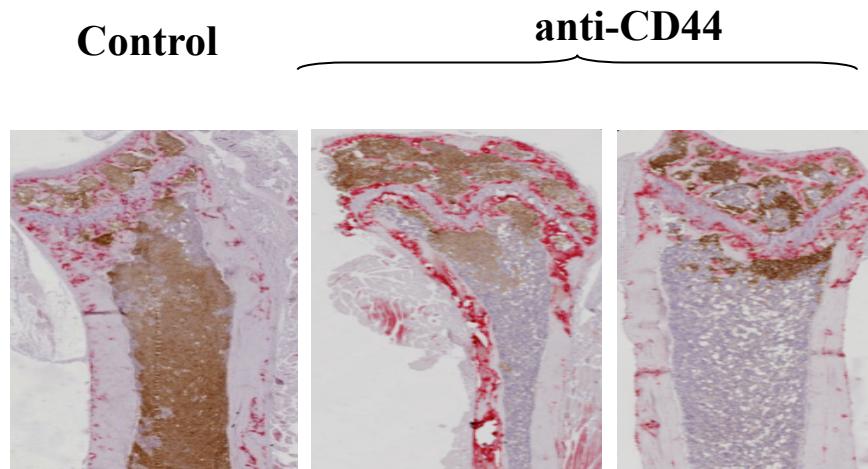
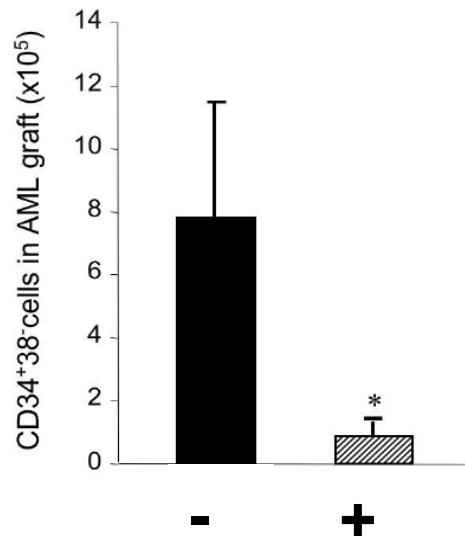
3 times/wk for 1 month



BM cells



Anti-CD44 mAb diminue la masse tumorale leucémique dans les xénogreffes



Au travers de la différenciation des cellules leucémiques

J DICK
F SMADJA

Identification des CSCs (tumeurs solides)

Propriétés de cellules souches cancéreuses

❖ auto renouvellement

❖ Multipotentialité

capacité à donner des cellules cancéreuses phénotypiquement diverses avec un potentiel prolifératif limité

❖ Tumorigenicité

cellule capable d'initier et de maintenir la croissance d'un clone malin *in vivo*

Identification des CSCs dans les tumeurs solides

BRAIN

Singh SK, Cancer Research, 2003
Yuan X, Oncogene, 2004
Galli R, Cancer Research, 2004

BREAST

Al-Hajj M, PNAS, 2003
Ponti D, Cancer Research, 2005

PROSTATE

Collins AT, Cancer Research, 2005
Patrawala L, Oncogene, 2006

SKIN

Fang D, Cancer Research, 2005

HEAD AND NECK

Prince ME, PNAS, 2007

COLON

Ricci-Vitiani L, Nature, 2007
O'Brien C, Nature, 2007

LUNG

Cancer Research, 2007

LIVER

Mol Cancer Res, 2008

PANCREAS

Li C, Cancer Research, 2007

Isolement de la population cellulaire

Biopsies tumorales (tumeur primaire ou métastases)



dissociation par digestion enzymatique et filtration

Suspension cellulaire



- ❖ Expression de marqueurs
- ❖ Side population
- ❖ Formation de sphères

Populations purifiées enrichies en CSC

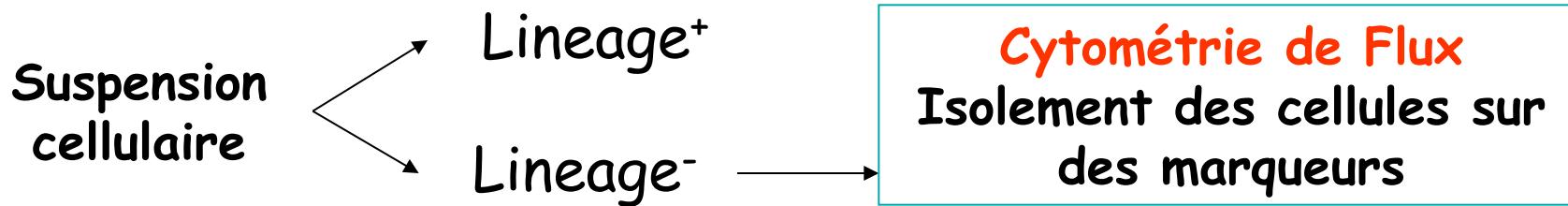
Identification de marqueurs d'enrichissement en CSC

Tumour type	CSC marker	Tumour cells expressing CSC marker, %	Minimal number of cells expressing CSC markers for tumour formation	Injected in Matrigel	Transplantation site	Strain	Refs
Breast	CD44 ⁺ /CD24 ^{low}	11–35	200	+	Mammary fat pad	NOD-SCID	19
Breast	CD44 ⁺ /CD24 ⁻	ND	2×10^3	–	Mammary fat pad	NOD-SCID	77
Breast	ALDH1 ⁺	3–10	500	+*	Mammary fat pad	NOD-SCID	33
Brain	CD133 ⁺ (GBM)	19–29	100	–	Brain	NOD-SCID	20
	CD133 ⁺ (MB)	6–21	100	–	Brain	NOD-SCID	20
Brain	CD133 ⁺	2–3	500	–	Brain	nu/nu	24
Colon	CD133 ⁺	1.8–25	200	+	Kidney capsule	NOD-SCID	26
Colon	CD133 ⁺	0.7–6	3×10^3	–	Subcutaneous	SCID	27
Colon	EpCAM ^{hi} /CD44 ⁺	0.03–38	200	+	Subcutaneous	NOD-SCID	32
Head and neck	CD44 ⁺	0.1–42	5×10^3	+	Subcutaneous	Rag2/ γ ^{-/-} DKO, NOD-SCID	117
Pancreas	CD44 ⁺ /CD24 ⁺ /ESA ⁺	0.2–0.8	100	+	Pancreas	NOD-SCID	65
Pancreas	CD133 ⁺	1–3	500	–	Pancreas	NMRI-nu/nu	28
Lung	CD133 ⁺	0.32–22	10^4	–	Subcutaneous	SCID	42
Liver	CD90 ⁺	0.03–6	5×10^3	–	Liver	SCID/Beige	53
Melanoma	ABCBS5 ⁺	1.6–20	10^6	–	Subcutaneous	NOD-SCID	35
Mesenchymal	Side population (Hoechst dye)	0.07–10	100	–	Subcutaneous	NOD-SCID	118

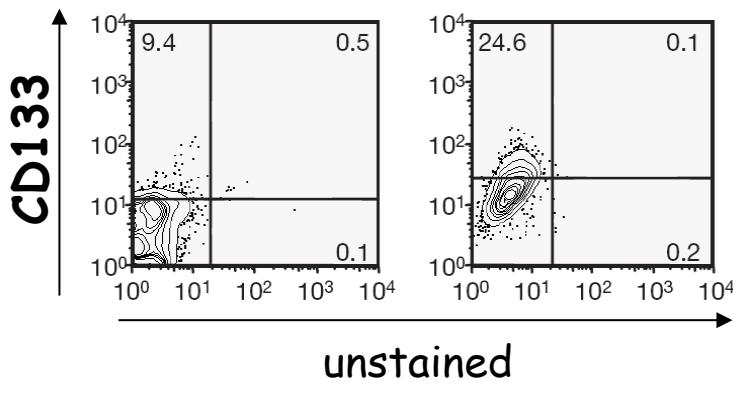
*Also injected with fibroblasts. ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; EpCAM, epithelial cell adhesion molecule; ESA, epithelial specific antigen; GBM, glioblastoma multiforme; MB, medulloblastoma, ND, not determined; NOD-SCID, non-obese diabetic-severe combined immunodeficient; Rag2/ γ ^{-/-}DKO, Rag 2 common cytokine receptor γ -chain double knockout.

Isolement de la population cellulaire

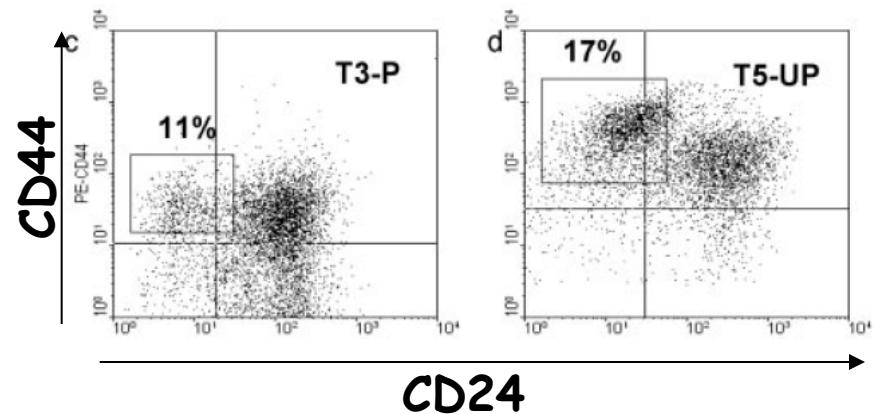
Expression de marqueurs



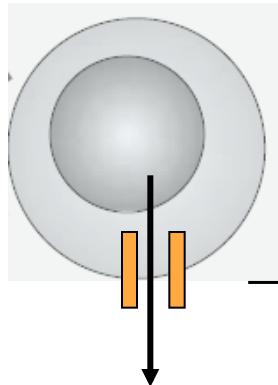
Colon (*O'Brien, Nature, 2007*)
1,8 to 24,6 % CD133⁺



Sein (*Al-Hajj, Cancer Res, 2003*)
11 to 35 % CD44⁺ CD24⁻



Isolement de la population de CSC Side Population (SP)



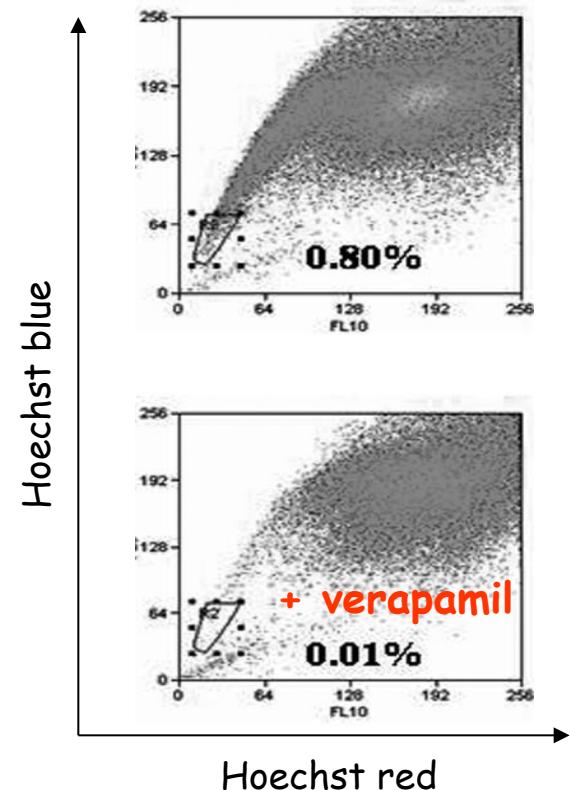
ATP binding cassette (ABC) transporters

ABCB1 (MDR1, P-gp)

ABCG2 (or BCRP, MXR)

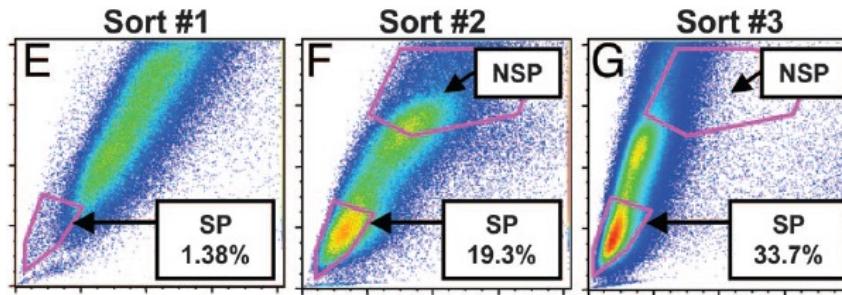
→Resistance aux drogues

Exclusion d'un colorant Hoechst 33342 Isole
une population de cellules sans colorant
Goodell et al., 1996



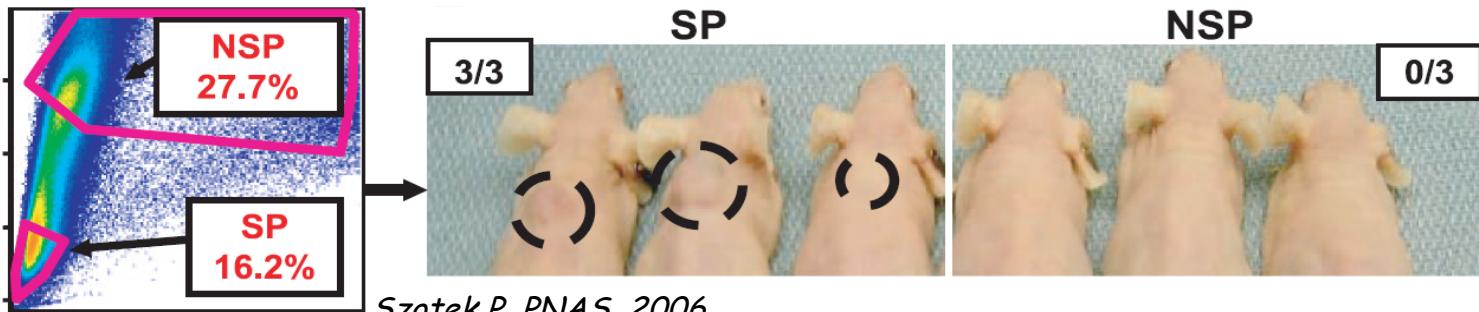
Isolement de la population cellulaire Side Population (SP)

- Purification des cellules SP et SP negatives (NSP)



- Tumorigenicity *in vivo*

Tumor Appearance 10 Weeks



Ovarian cancer side population defines cells with stem cell-like characteristics.

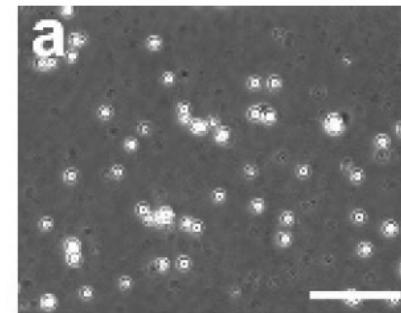
Isolement de la population cellulaire

Formation des sphères

Culture en suspension liquide

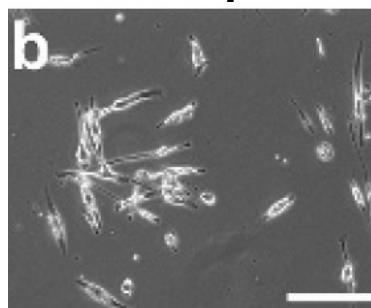
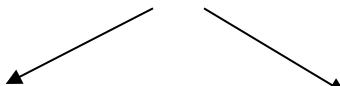
Facteurs de croissance

Nombre faible de cellules en culture

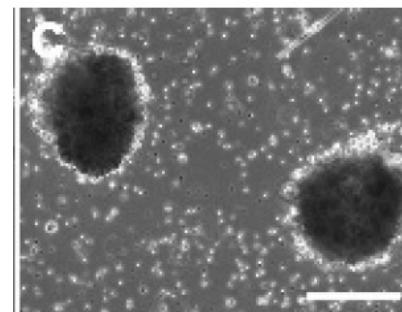


Cellules avec des propriétés distinctes

La majorité des cellules sont adhérentes avec perte de prolifération



Une minorité forme des sphères d'origine clonale



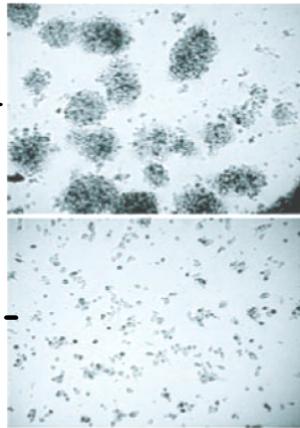
Isolation of cell population

Sphere formation assay

Primary sphere formation assay

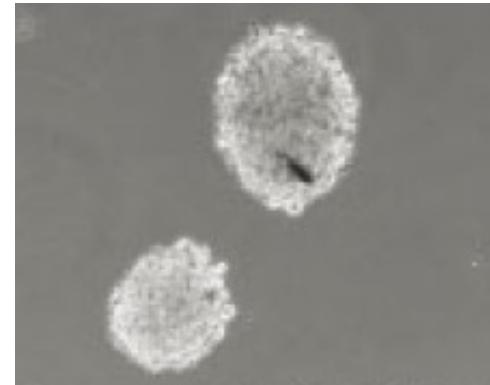
Neurospheres

CD133⁺

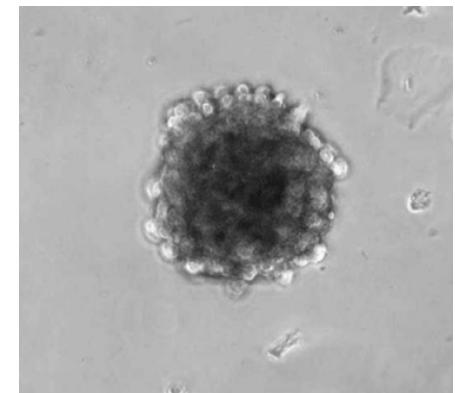


CD133⁻

Mammospheres



Sarcospheres



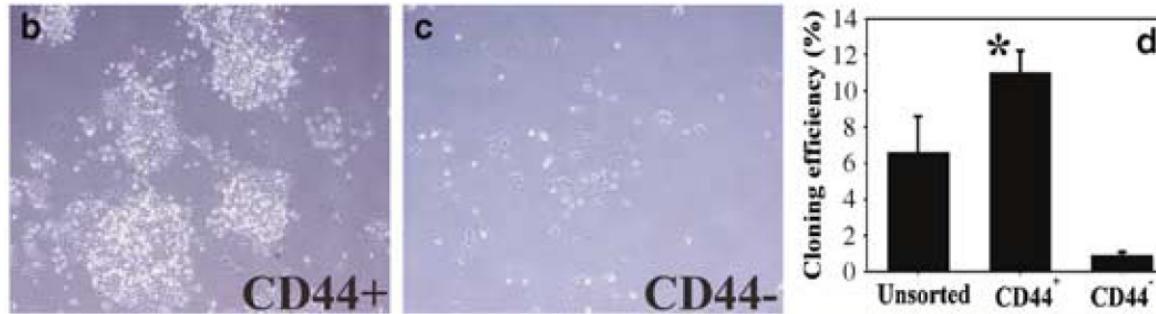
Isolement de la population cellulaire

Formation des sphères

Test primaire de formation de sphères

Efficacité à former des sphères

Prostate
cancer



Patrawala L, Oncogene, 2006

Tumor type	Primary sphere (clonogenic) frequency
Medulloblastoma ($n = 3$)	13.8%, 14.8%, 25.1%
Pilocytic astrocytoma ($n = 3$)	0.3%, 1.3%, 1.5%
Glioblastoma ($n = 1$)	11.4%
Anaplastic ependymoma	8%

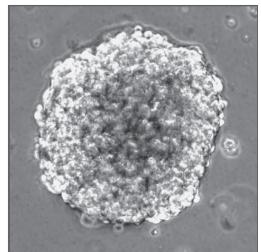
Singh SK, Cancer Res, 2003

Isolement de la population cellulaire

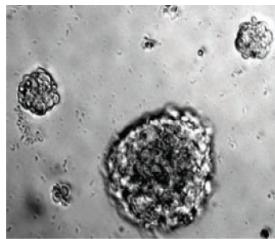
Formation des sphères

Pouvoir d'autorenouvellement

Test secondaire de formation de sphere



Dissociation



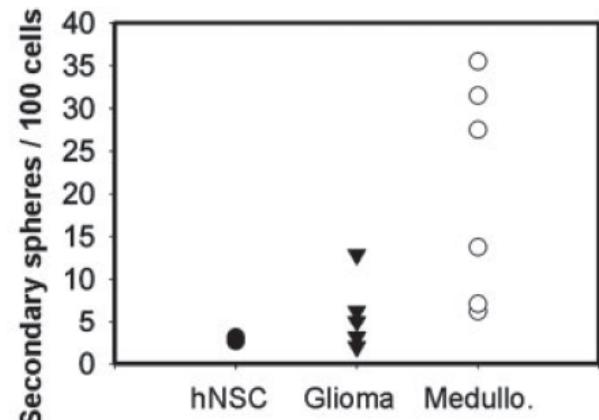
n passages

*Expansion
In vitro*

Primary sphere

Secondary sphere

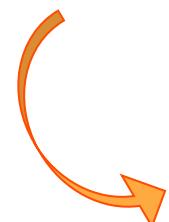
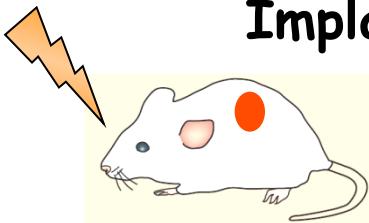
La capacité de faire des sphères au fur et à mesure des passages témoigne du pouvoir d'auto renouvellement et de l'agressivité de la tumeur. (*Singh SK, Cancer Res, 2003*)



Characterisation des CSCs *in vivo*

Modèles XENOGREFFES
souris sNOD/SCID

- Injection
- Sous cutanée
- Injection orthoptique
- Implantation de petites pièce tumorales



- Niche (interactions avec la matrice extracellulaire)
- Modalités d'injections des cellules: nombre de cellules, endroit de la greffe, facteurs adjuvants

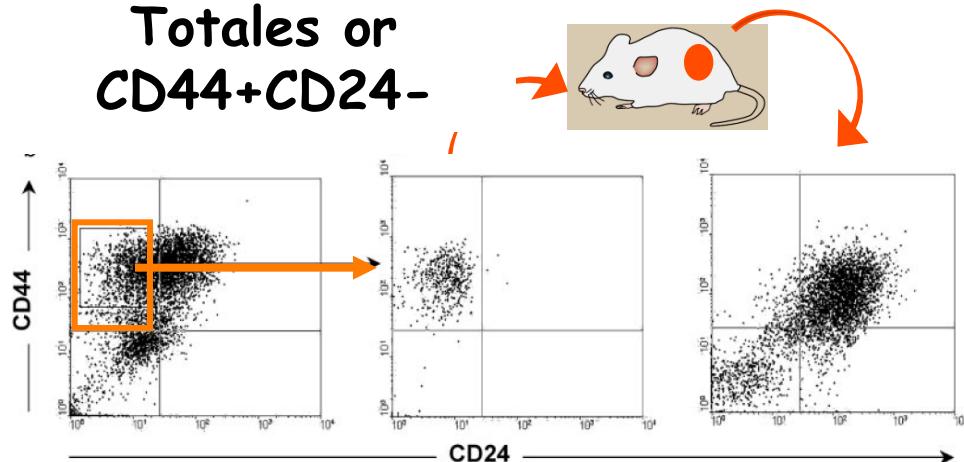
- ❖ Tumorigenicité
- ❖ Auto-renouvellement
- ❖ Multipotentialité

Cellules souches cancéreuses

Ex: cancer du sein

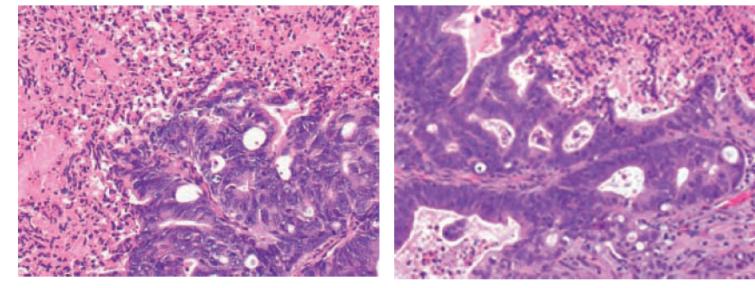
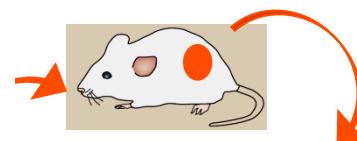
Cellules tumorales

Totales or
CD44⁺CD24⁻



Tumeur identique
immunophénotype et morphologie
que la tumeur initiale

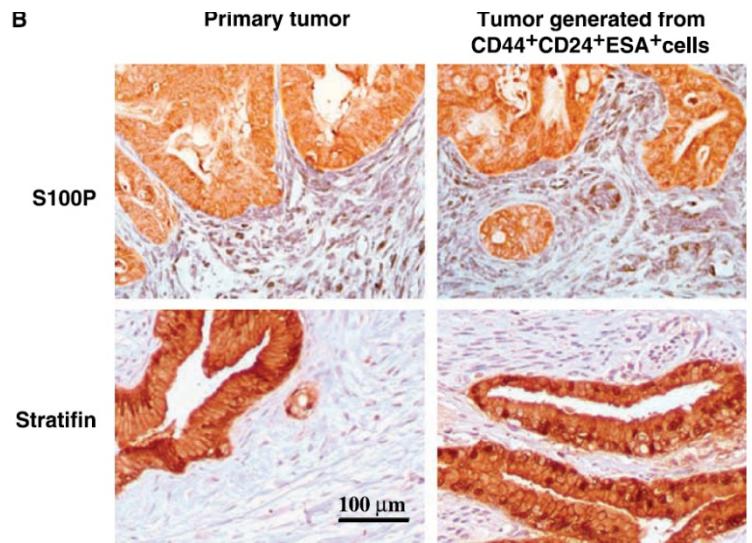
- 1. Tumorigenicité**
- 2. Multipotentialité**



Primary Tumor

Tumor generated from
CD44⁺CD24⁺ESA⁺cells

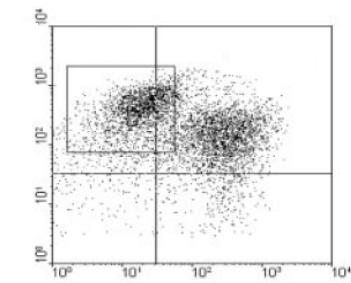
hematoxylin-eosin staining



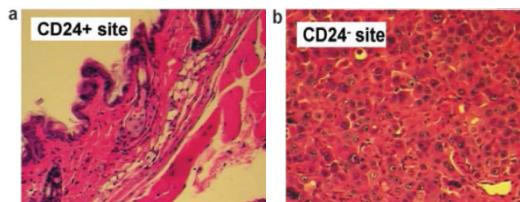
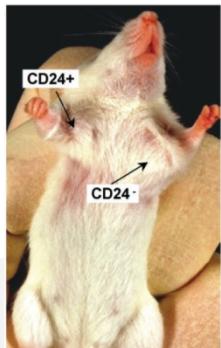
Differentiation marker expression

Caractérisation *In vivo* des CSCs

Tumorigenicité

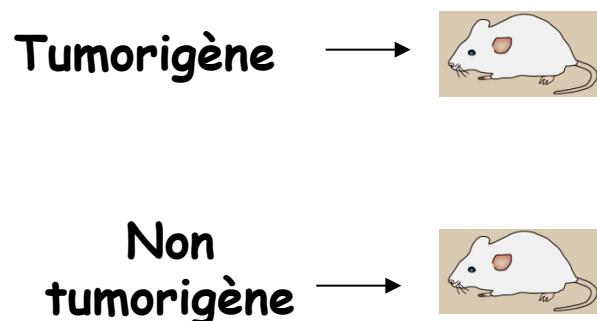


Isolement de la population par cytométrie

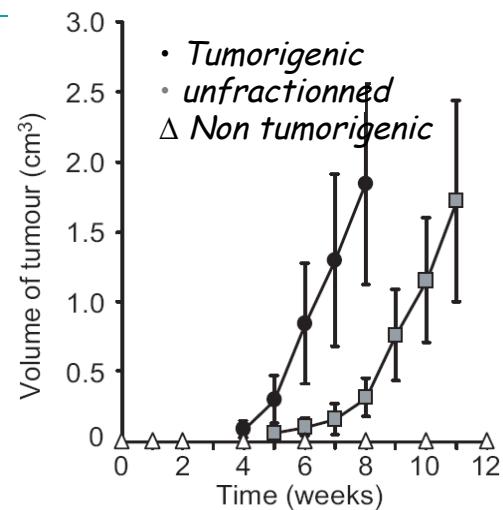


hematoxylin-eosin staining

	Tumors/injections						
	5×10^5	10^5	5×10^4	2×10^4	10^4	5×10^3	10^3
Unsorted	8/8	8/8	10/10		3/12		0/12
CD44 ⁺ CD24 ⁺	—	—	—	0/10	0/10	0/14	0/10
CD44 ⁺ CD24 ^{-/low}	—	—	—	10/10	10/10	14/14	10/10

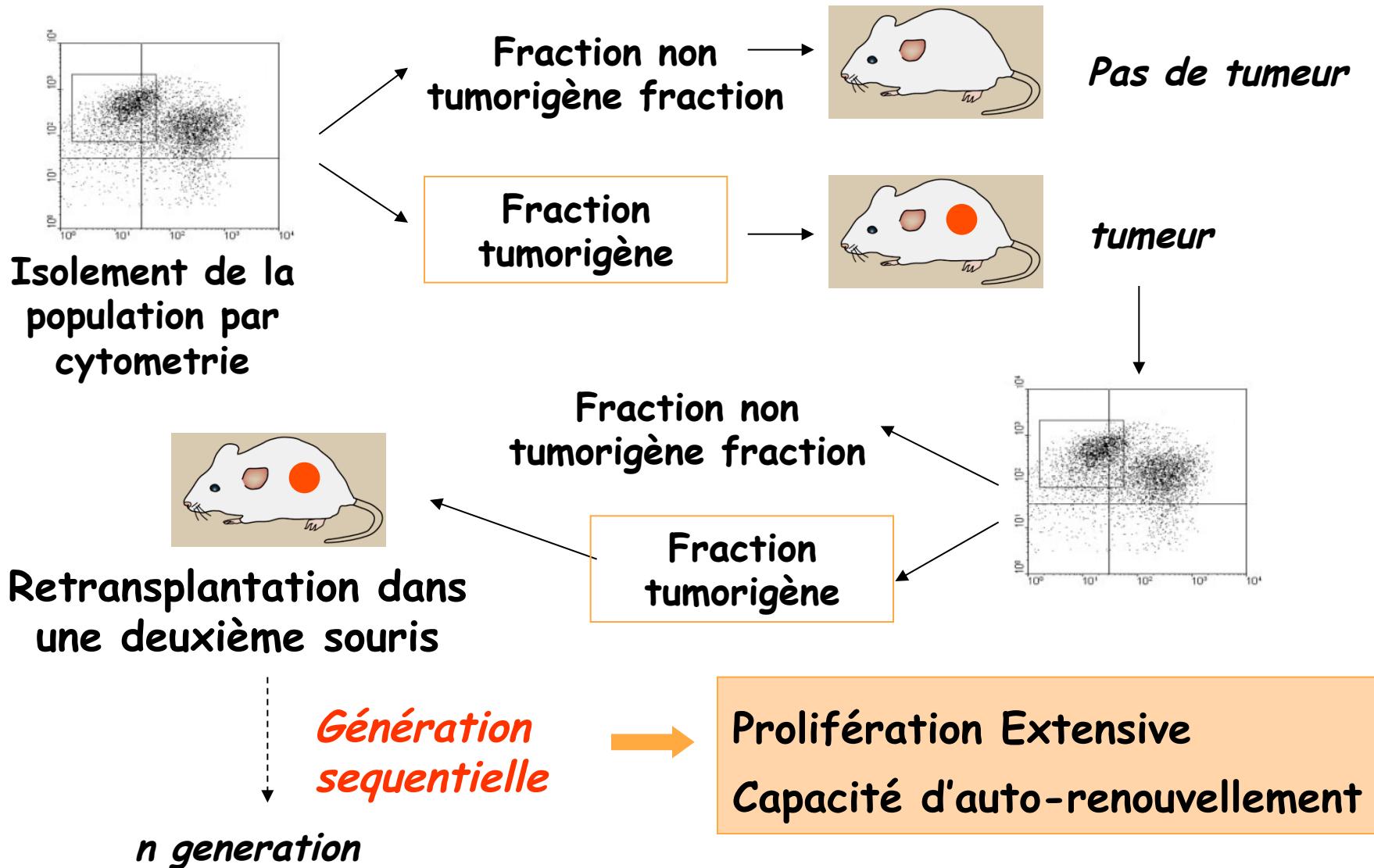


Capacité des cellules isolées à former des tumeurs



Caractérisation *In vivo* des CSCs

Auto renouvellement



Caractérisation des CSCs

Auto-renouvellement

Dilution Limite

Pour quantifier le nombre des CSCs

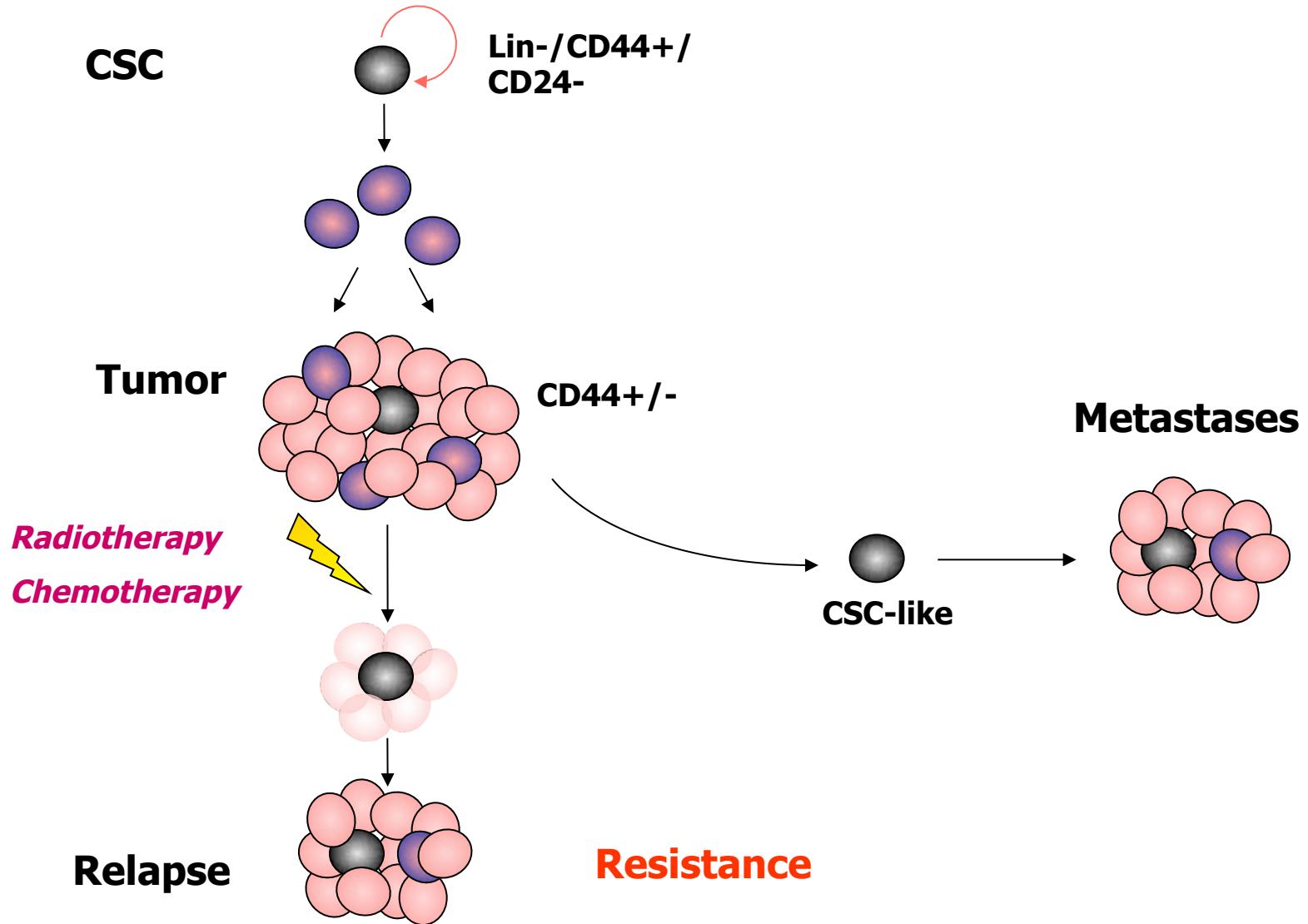
Colon cancer cell source	Cell dose	Total number of mice with tumours (%)
Bulk	1×10^4	0/8 (0)
	2.5×10^4	1/8 (12.5)
	5×10^4	4/10 (40)
	7.5×10^4	4/8 (50)
	1×10^5	10/10 (100)
	1×10^6	17/17 (100)
	2×10^6	8/8 (100)
CD133 ⁺	1×10^2	1/4 (25)
	5×10^2	5/6 (83.33)
	1×10^3	7/7 (100)
	5×10^3	8/8 (100)
	1×10^4	10/10 (100)
	2×10^4	9/9 (100)
CD133 ⁻	5×10^3	0/5 (0)
	1×10^4	0/6 (0)
	2×10^4	0/6 (0)
	5×10^4	0/8 (0)
	1×10^5	0/8 (0)
	2.5×10^5	1/9 (11.1)

1 per 5.7×10^4 cells

1 per 262 cells

Enrichissement
216 fois

O'Brien C, Nature, 2007



Cancer stem cell identification

Helps define diseases, novel molecular pathways and therapies

