造血干细胞
（Hematopoietic Stem Cells）

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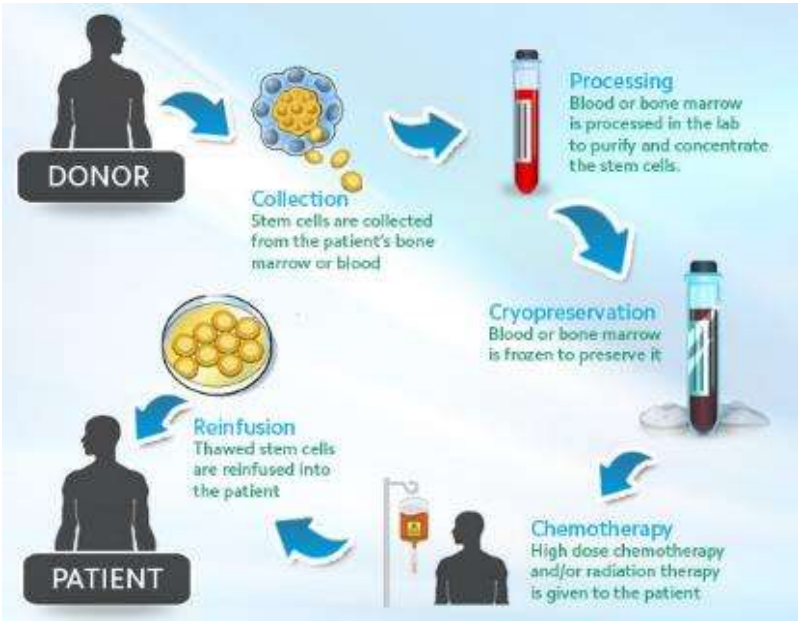
Bone marrow transplantation



Cord blood transplantation

THE NEW ENGLAND JOURNAL OF MEDICINE Oct. 26, 1989

HEMATOPOIETIC RECONSTITUTION IN A PATIENT WITH FANCONI'S ANEMIA BY MEANS OF UMBILICAL-CORD BLOOD FROM AN HLA-IDENTICAL SIBLING

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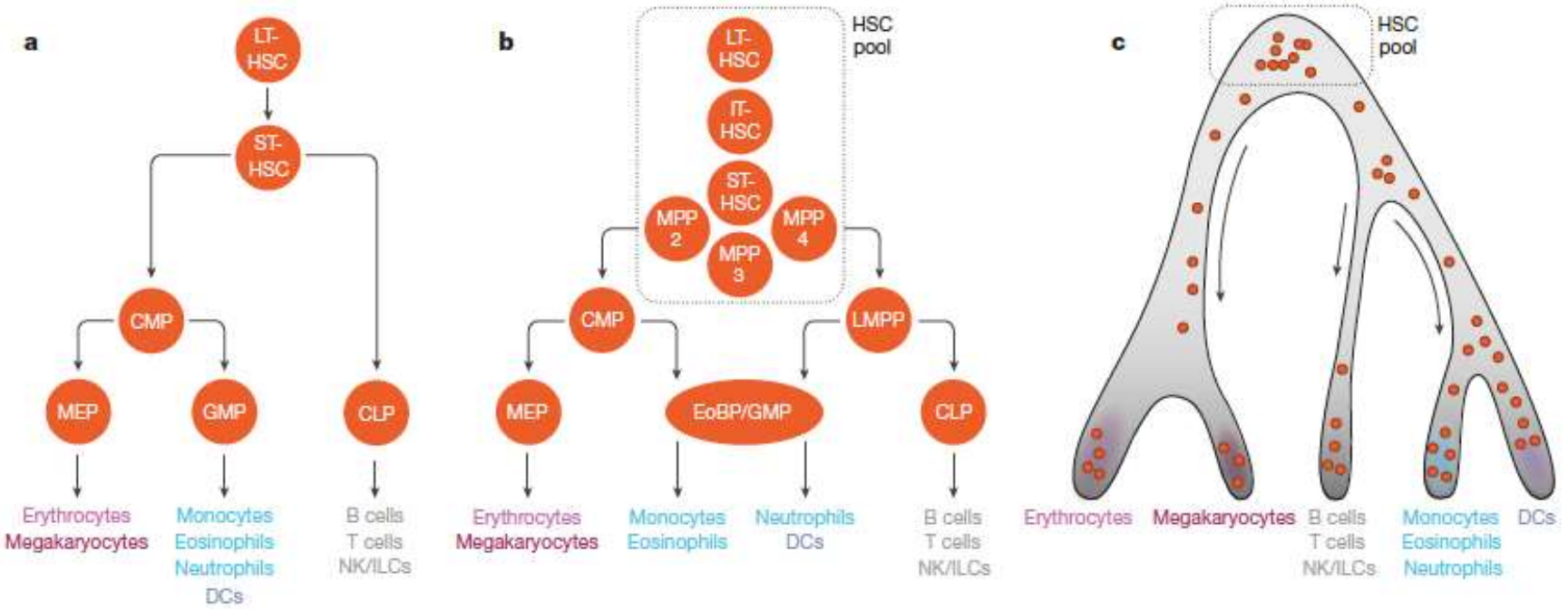
Table 3. Blood-Cell Counts before and after Transplantation.

DAY	HEMOGLOBIN	LEUKOCYTES	GRANULOCYTES	LYMPHOCYTES	PLATELETS	RETICULOCYTES
	g/dl	*no. of cells $\times 10^{-9}$ per liter*				
−20	6.8	3.1	0.25	2.8	18	10
0	9.7	0.8	0.0	0.8	120	0
8	10.9	0.4	0.0	0.4	80	0
15	11.6	0.4	0.0	0.4	39	0
22	7.8	0.9	0.3	0.6	50	5
29	8.5	1.0	0.3	0.5	105	17
36	9.4	1.7	0.6	0.5	55	36
43	11.3	5.1	2.4	1.9	31	90
50	8.9	3.4	1.5	0.7	62	162
57	8.9	5.6	3.2	1.0	174	63
90	11.3	5.1	4.0	1.1	296	50
120	13.0	3.9	2.3	1.1	265	40
160	12.0	3.7	1.4	1.6	293	45
240	12.3	5.2	2.7	1.6	354	50
282	12.2	4.8	2.3	1.2	315	—

Comparisons between cord blood and bone marrow transplantation

Advantages and Disadvantages of Using Cord Blood for Hematopoietic Cell Transplantation Compared with Bone Marrow	
Advantages	***Disadvantages***
Ease and safety of obtaining cells without harm to baby or mother	Slower time to neutrophil, platelet, and immune cell recovery
Efficient storage as units of HLA-typed cells in cryopreserved form in CB banks	Higher rate of graft failure
Available for immediate use after storage	Limited number of CB cells collected from babies at birth
Elicitation of a lower level of GVHD than BM cells	

Definition of hematopoietic stem cells (HSCs)



Self-renewal
Multilineage differentiation

Laurenti and Göttgens, Nature, 2018

RADIATION RESEARCH 14, 213-222 (1961)

A Direct Measurement of the Radiation Sensitivity of Normal Mouse Bone Marrow Cells[1]

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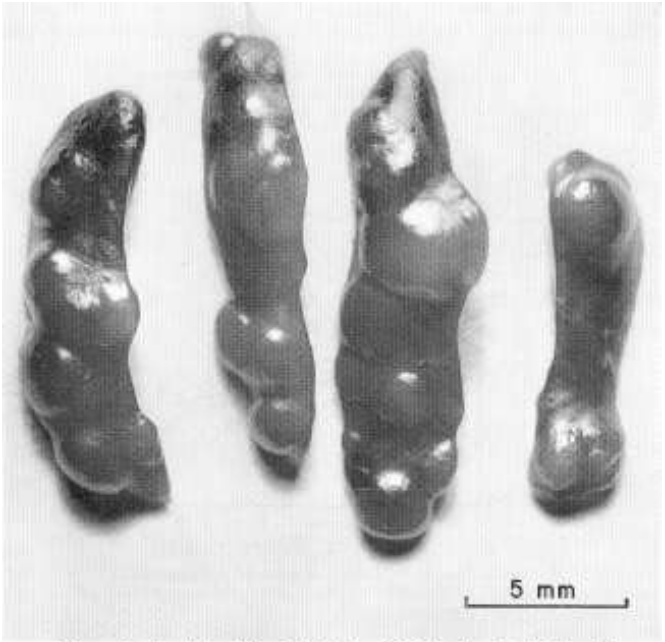


FIG. 1. Spleens of irradiated mice 10 days after injection of 6 X 10^4 nucleated cells. The nodules on which the assay is based are readily seen.

Table 1

Colony with abnormal karyotype	No. of metaphases scored	Abnormal chromosomes present	Frequency of abnormal chromosomes in cells having given number of chromosomes: 35 or less	36	37	38	39	40	Total No. of cells with given chromosome condition	Per cent of cells with at least one marker characteristic of colony
A	100	Minute plus long	6	0	4	8	63	0	81	99
		Minute only	0	5	1	1	9	2	18	
		Long only	0	0	1	2	0	0	3	
		None	0	0	0	0	0	1	1	
		Total cells	6	2	6	11	72	3		
B	100	Dicentric	0	2	4	5	14	70	95	95
		None	0	0	0	2	2	1	5	
		Total cells	0	2	4	7	16	71		
C	100	Minute plus dicentric	0	0	4	6	7	74	91	97
		Minute only	0	1	0	1	0	4	6	
		Dicentric only	0	0	0	0	0	0	0	
		None	0	0	0	1	0	2	3	
		Total cells	0	1	4	8	7	80		
D	73	Minute	6	2	6	6	10	40	73	97
		None	0	0	0	0	0	2	2	
		Total cells	6	2	6	6	10	51		

phase cells of any given colony so examined failed to reveal a readily identifiable chromosome aberration, that colony was scored as containing no obvious marker. Since female donor marrow was used, the presence, in the cells of the colonies examined, of the third unpaired small chromosome characteristic of the male karyotype[2,3] was avoided; this facilitated identifying any odd minute chromosome encountered as a radiation-induced marker.

A total of 42 colonies obtained from 36 animals were examined in the foregoing manner. Of these colonies, 4 contained cells with an obviously abnormal karyotype.

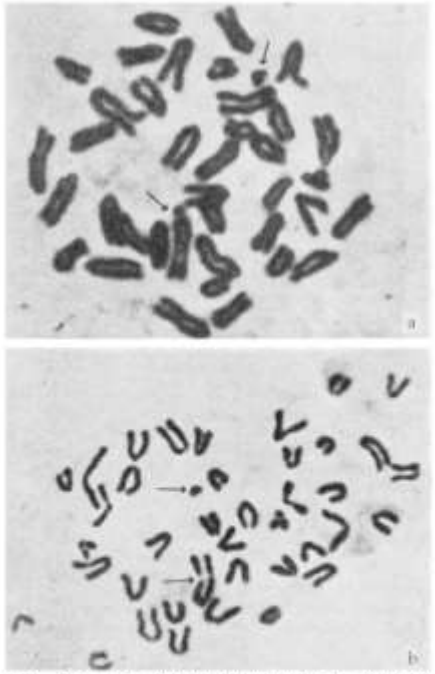


FIG. 2. Representative chromosome complements from two of the colonies listed in Table 1. The abnormal chromosomes are indicated by arrows. (× 1,450)
a, Colony *A*, the chromosome count is 39 instead of the normal 40; *b* colony *C*

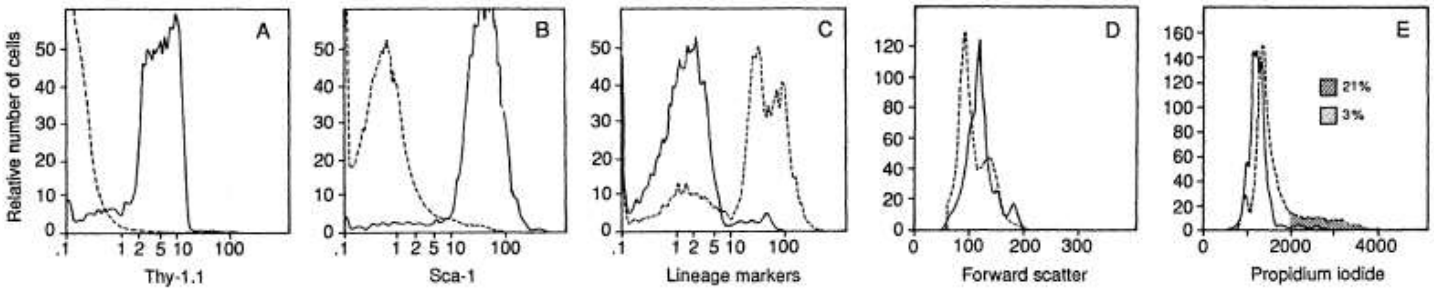
Table 1 summarizes the types of chromosome markers encountered and the frequencies of these markers within the cells of each colony. For example, mitotic figures from colony *A* showed three characteristic abnormalities: (1) a modal chromosome number of 39; (2) an abnormally small chromosome; (3) an abnormally long sub-telocentric chromosome (Fig. 1, top). Eighty-one of 100 metaphase cells examined manifested all three abnormalities; and 99 per cent of the cells contained at least one of these distinguishing characteristics. The occasional inability to score one or the other of the two markers is probably the result of the following technical difficulties: (1) cytoplasmic rupture and chromosome loss; or (2) excessive overcrowding and overlapping of chromosomes in less-than-optimally spread metaphase cells. In the latter circumstance, whenever it was impossible to delineate with certainty one or other of the marker chromosomes, the cell was scored as lacking that particular marker. Of the 3 cells from colony *A* which were recorded as containing 40 chromosomes, two were very difficult to count because of overcrowding of the chromosomes, and the chromosome number recorded for them may represent a miscount. Similar considerations are applicable to the interpretation of the data compiled for the other 3 marked colonies. A representative complement from colony *C* is shown in Fig. 1 (bottom). It is characterized by an abnormally small chromosome and a long chromosome which is either a dicentric or has a prominent secondary constriction.

Experiments were performed to test for the presence of pre-existing abnormal karyotypes among the colony-forming cells of normal donor marrow. Groups of Swiss mice were exposed to total-body doses of 900 rads; and, following irradiation, each mouse received 5 × 10^4 nucleated bone-marrow cells from female donors of the same strain. No further irradiation was given. Of 55 colonies examined from these controls, all contained, exclusively, mitoses with the normal female diploid complement of 40 chromosomes. This finding rendered unlikely the existence, within the donor marrow, of uniquely and uniformly marked colony-forming cell aggregates. The 4 abnormal karyotypes were thus the result of chromosome damage by ionizing radiation.

The results given in Table 1 show that when recognizable marker chromosomes are present in the cells of a colony, an overwhelming majority of the cells contain the same markers. Since normal mouse marrow contains no uniquely and uniformly marked colony-forming cell aggregates, and since chromosome breakage by radiation is a random process, rendering highly improbable the generation of an identical abnormality in each of the cells of a hypothetical colony-forming aggregate, it can be concluded that all the cells in these marked colonies were derived from a single cell in which a chromosome aberra-

Purification and Characterization of Mouse Hematopoietic Stem Cells

GERALD J. SPANGRUDE, SHELLY HEIMFELD, IRVING L. WEISSMAN



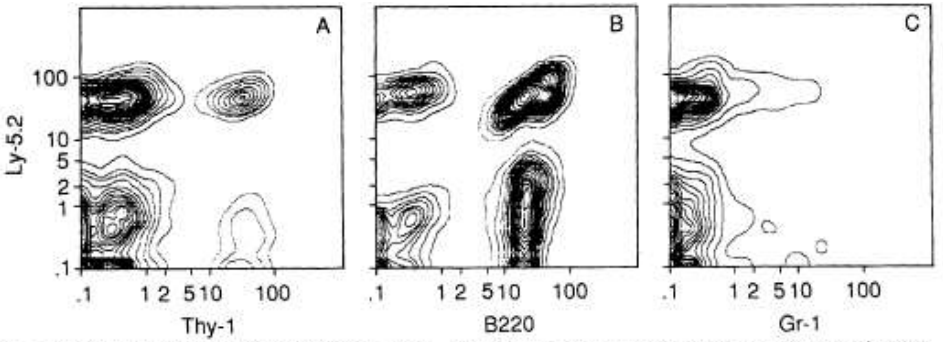


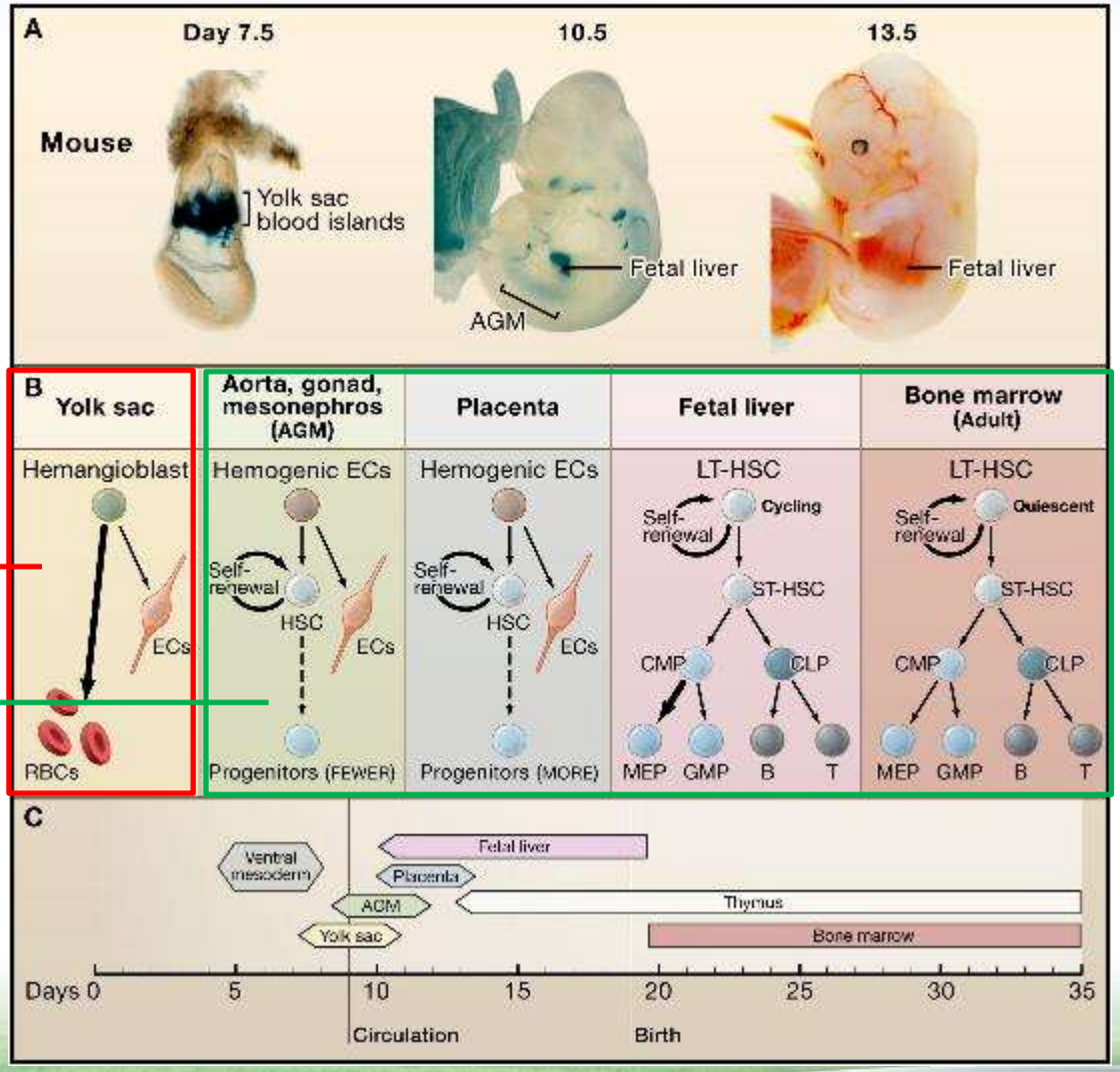
Fig. 4. Multiple hematolymphoid repopulation by purified stem cells. Limiting numbers of hematopoietic stem cells reconstitute multiple hematolymphoid lineages. Forty Thy-1^{lo}Lin$^-$Sca-1^+ cells (C57BL/6–Ly-5.2) were transferred intravenously into lethally irradiated (900 rads) Ly-5 congenic mice (C57BL/Ka, Ly-5.1) along with 200 host-derived stem cells. At various times thereafter, donor-derived (Ly-5.2^+) cells were detected in the peripheral blood and phenotyped by two-color FACS analysis. Six weeks after reconstitution, 50 percent of the peripheral blood leukocytes in this mouse were derived from the 40 donor hematopoietic stem cells. These included (**A**) 60 percent of the circulating T cells (Thy-1^+) cells, (**B**) 50 percent of the B cells ($B220^+$ cells), and (**C**) 50 percent of the neutrophils (Gr-1^+ cells).

Spangrude et al., Science, 1988

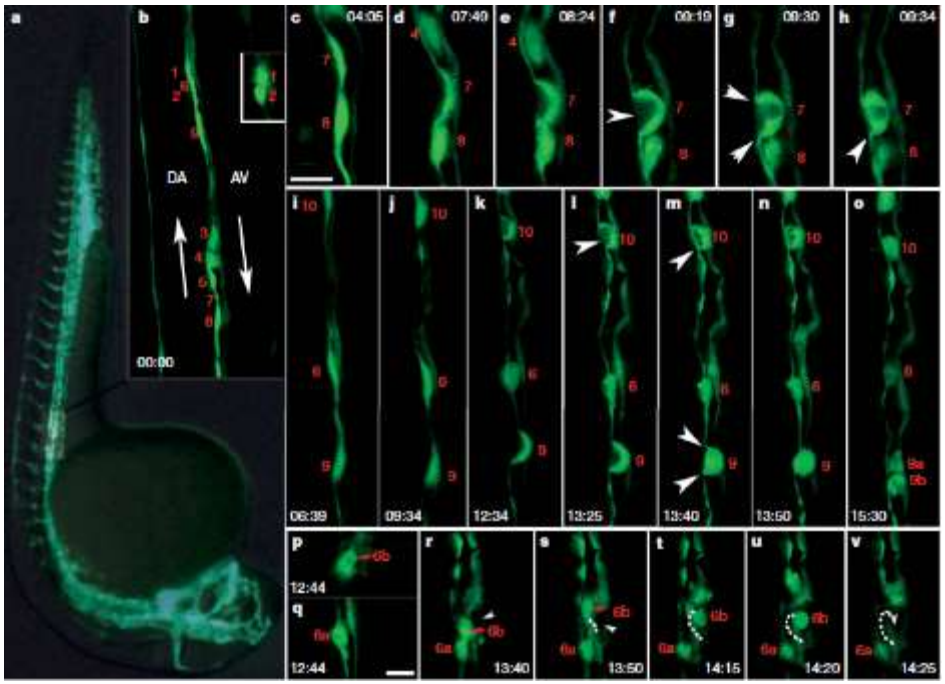
Mouse hematopoiesis

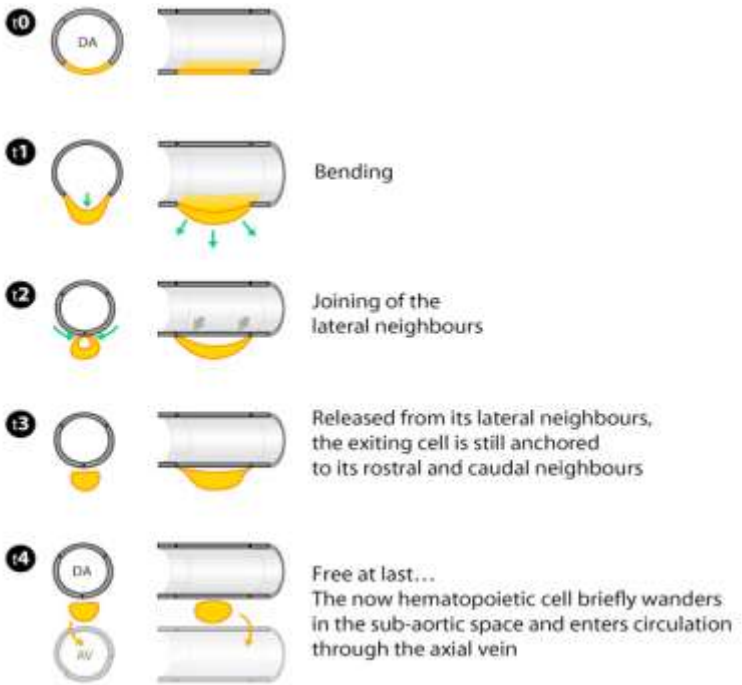
Primitive Hematopoiesis

Definitive Hematopoiesis



Endothelial-to-hematopoietic transition (EHT)

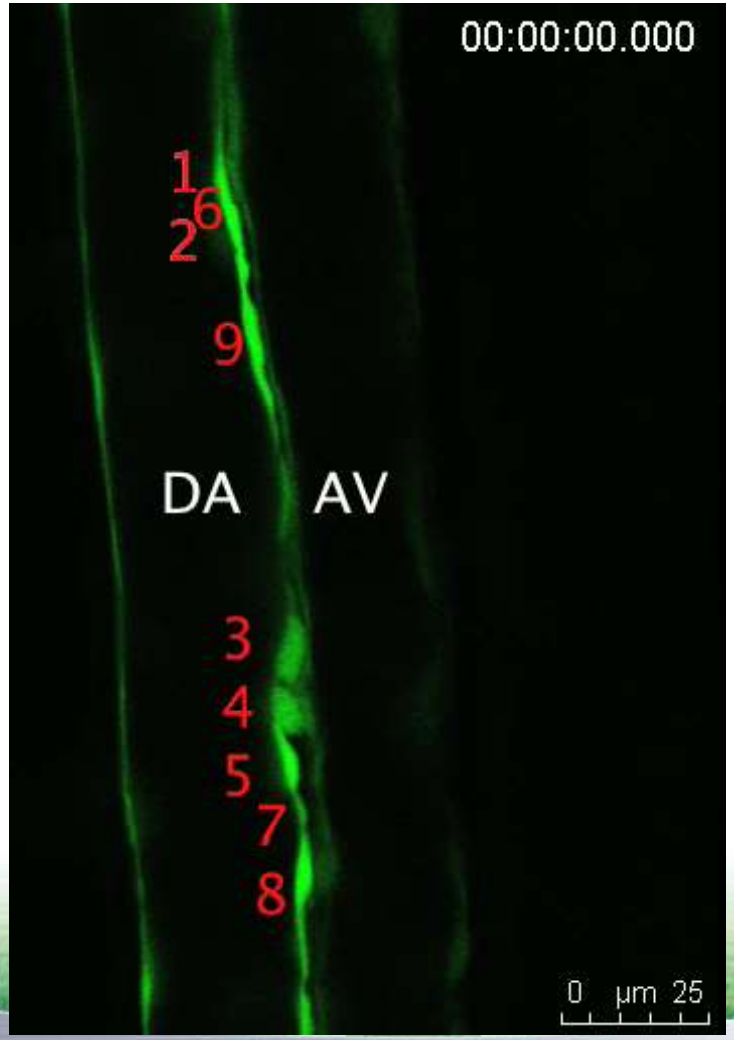




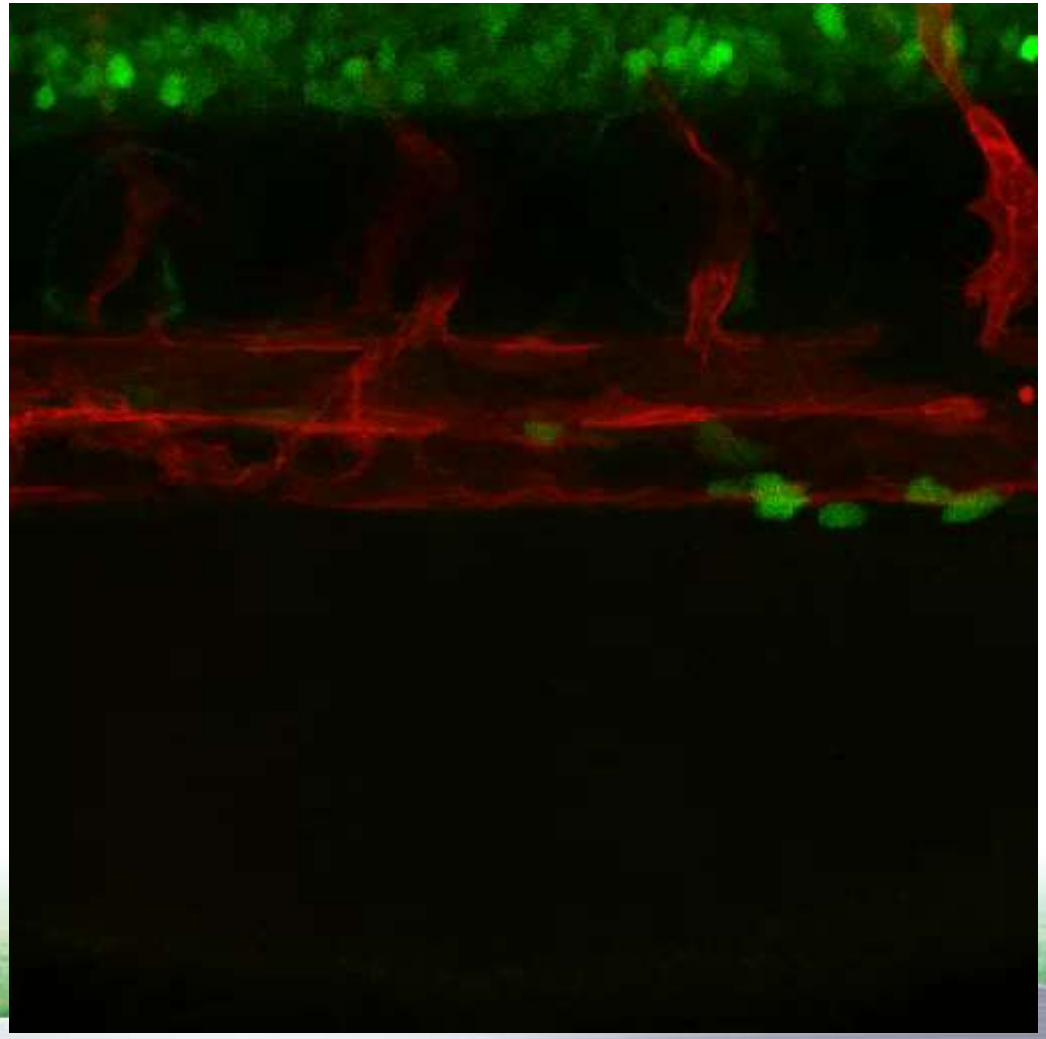
Kissa *et al*., *Nature*, 2010

Endothelial-to-hematopoietic transition (EHT)

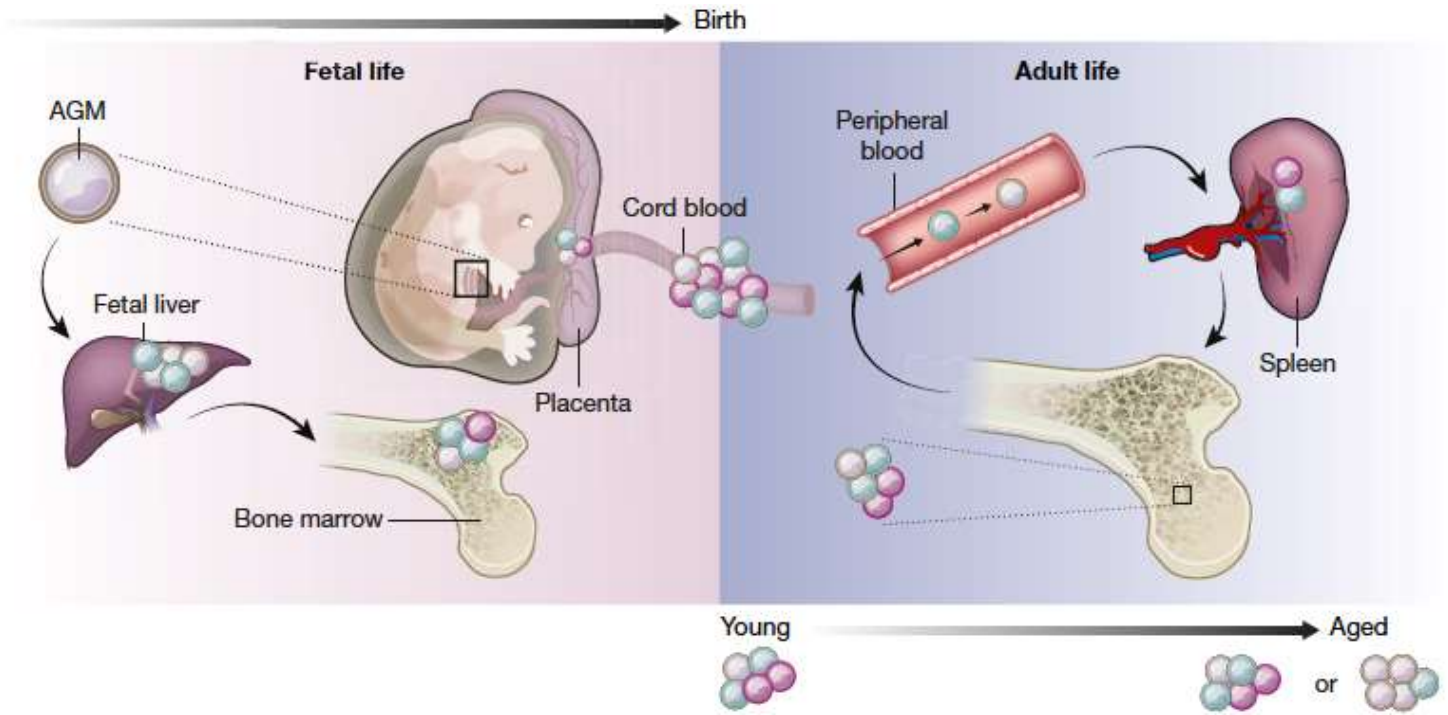
Kdr-EGFP



Kdr-mTomato;Cmyb-EGFP

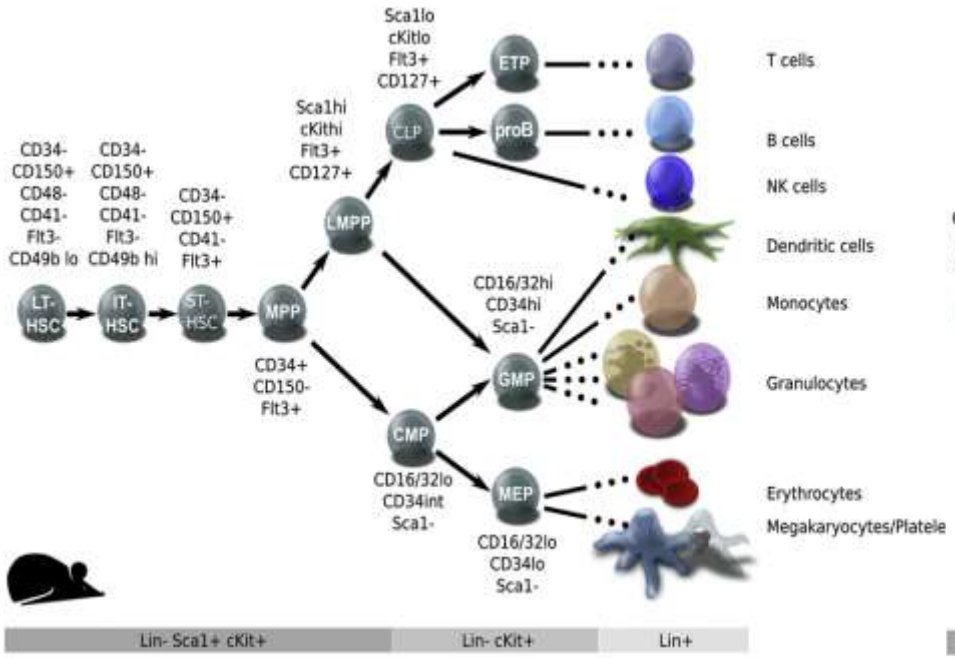


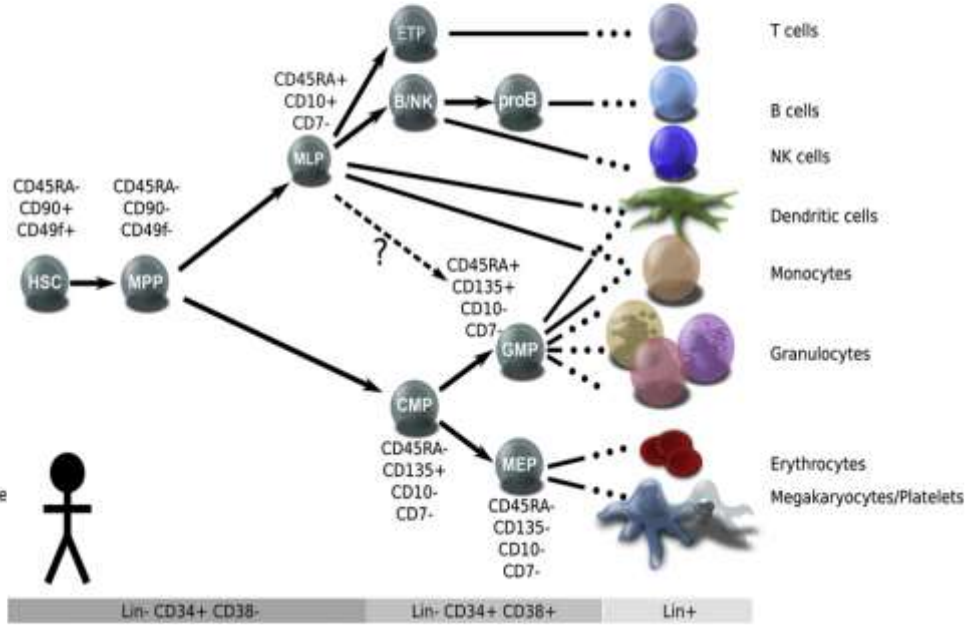
HSC life cycle



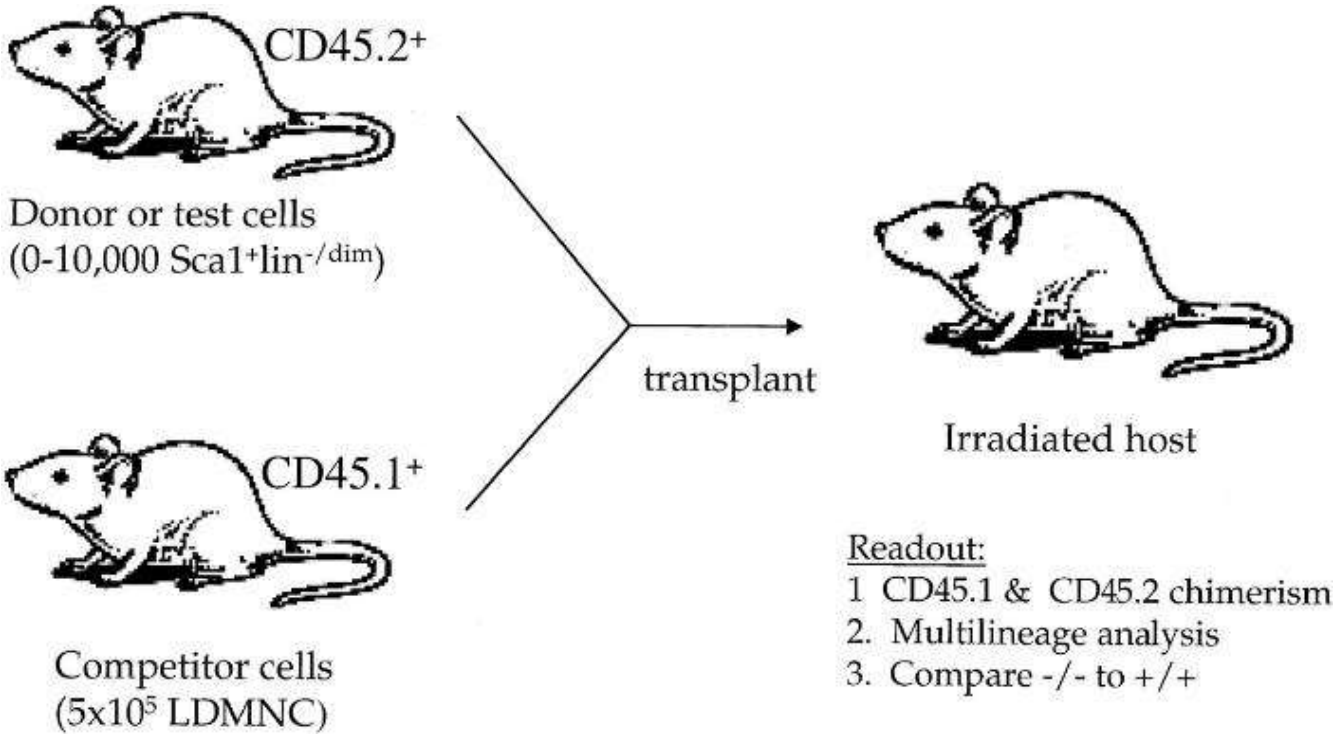
Laurenti and Göttgens, Nature, 2018

Phenotypic markers differ between mouse and human HSCs





Functional analysis of mouse HSCs: Competitive long-term repopulation analysis

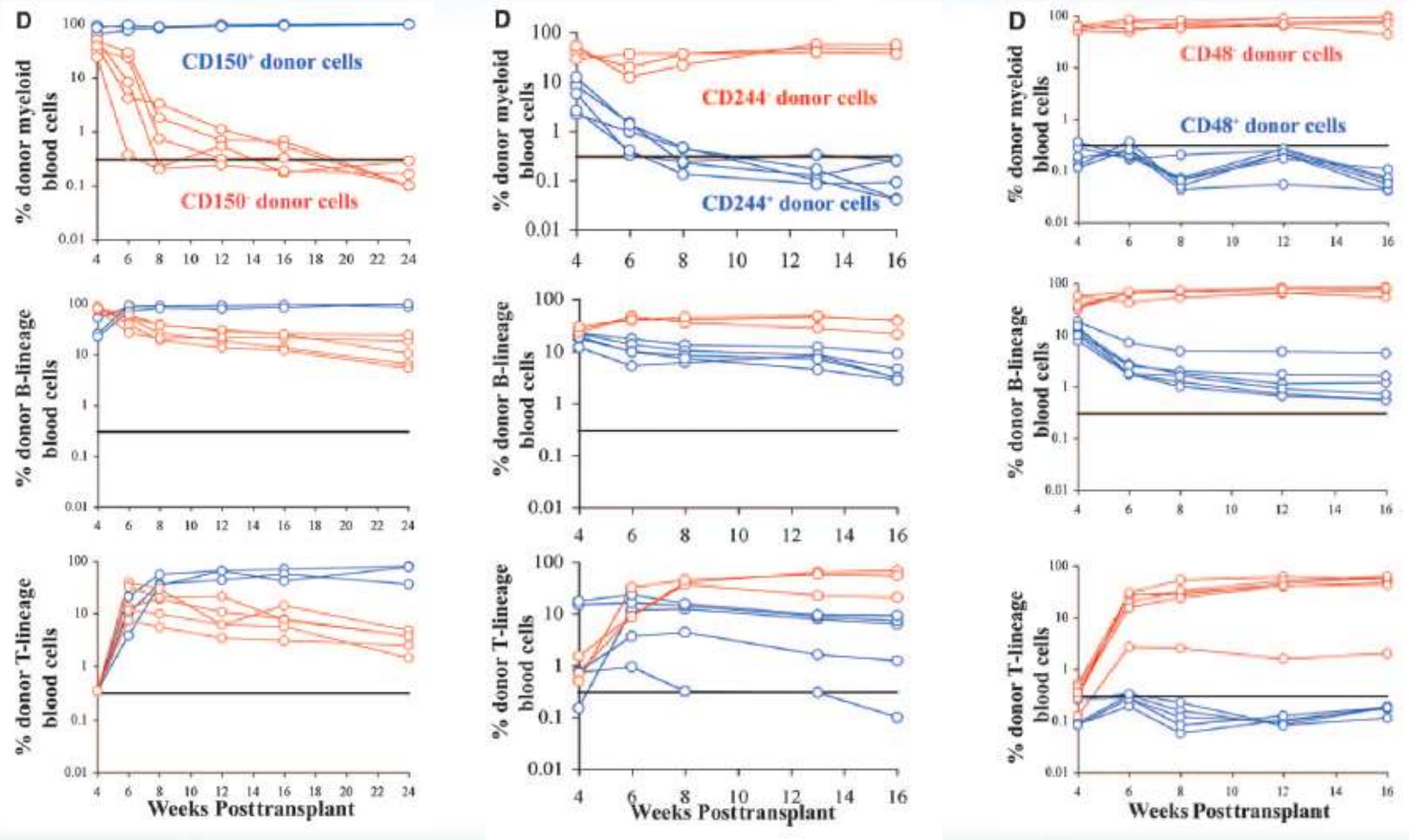


Phenotypic markers of mouse HSCs

Markers	references
Lin^{-} $Thy\text{-}1^{Low}$ $Sca\text{-}1^{+}$	Spangrude et al., 1988
$CD34^{-/Low}$ Lin^{-} $Sca\text{-}1^{+}$ $c\text{-}kit^{+}$	Osawa et al., 1996
Side Population (high Hoechst-efflux ability)	Goodell et al., 1996
*Tip-SP Lin^{-} $Sca\text{-}1^{+}$ $c\text{-}kit^{+}$	Matsuzaki et al., 2004
$CD150^{+}$ CD244 $CD48^{-}$	Kiel et al., 2005
BrdU or Histone 2B-GFP-retaining, $CD150^{+}$ $CD48^{-}$ $CD34^{-}$ Lin^{-} $Sca\text{-}1^{+}$ $c\text{-}kit^{+}$	Wilson et al., 2008 Foudi et al., 2009

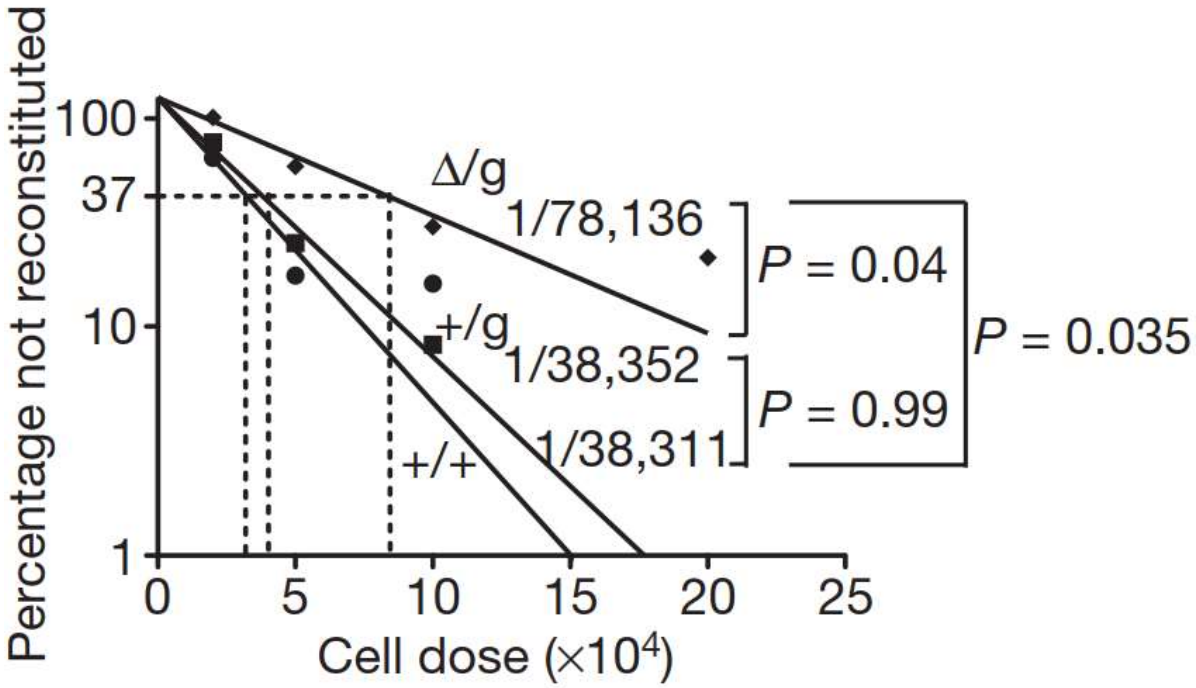
*Tip-SP: The highest Hoechst-efflux fraction in the Side Population

Determining phenotypic markers of mouse HSCs

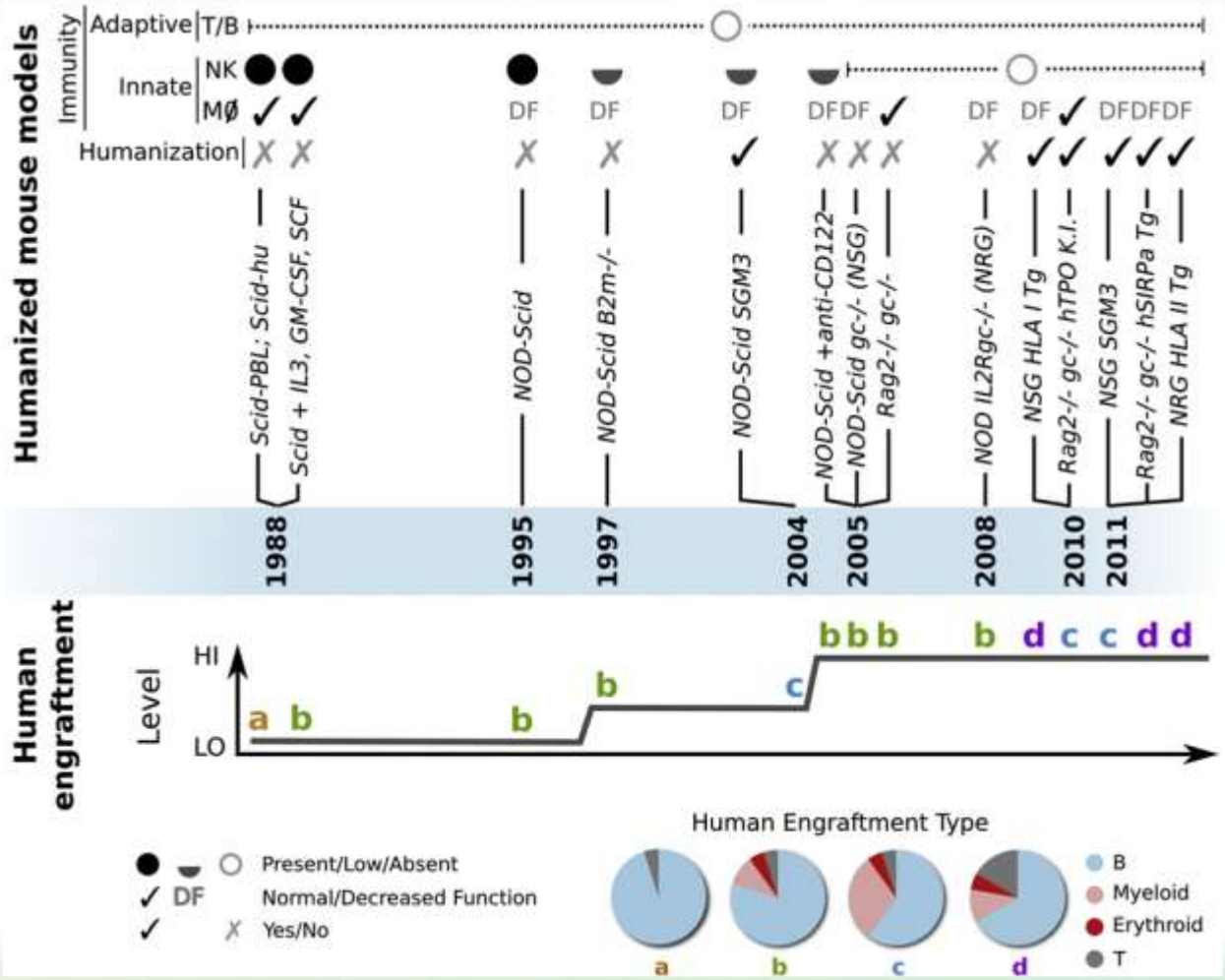


Kiel et al., Cell, 2005

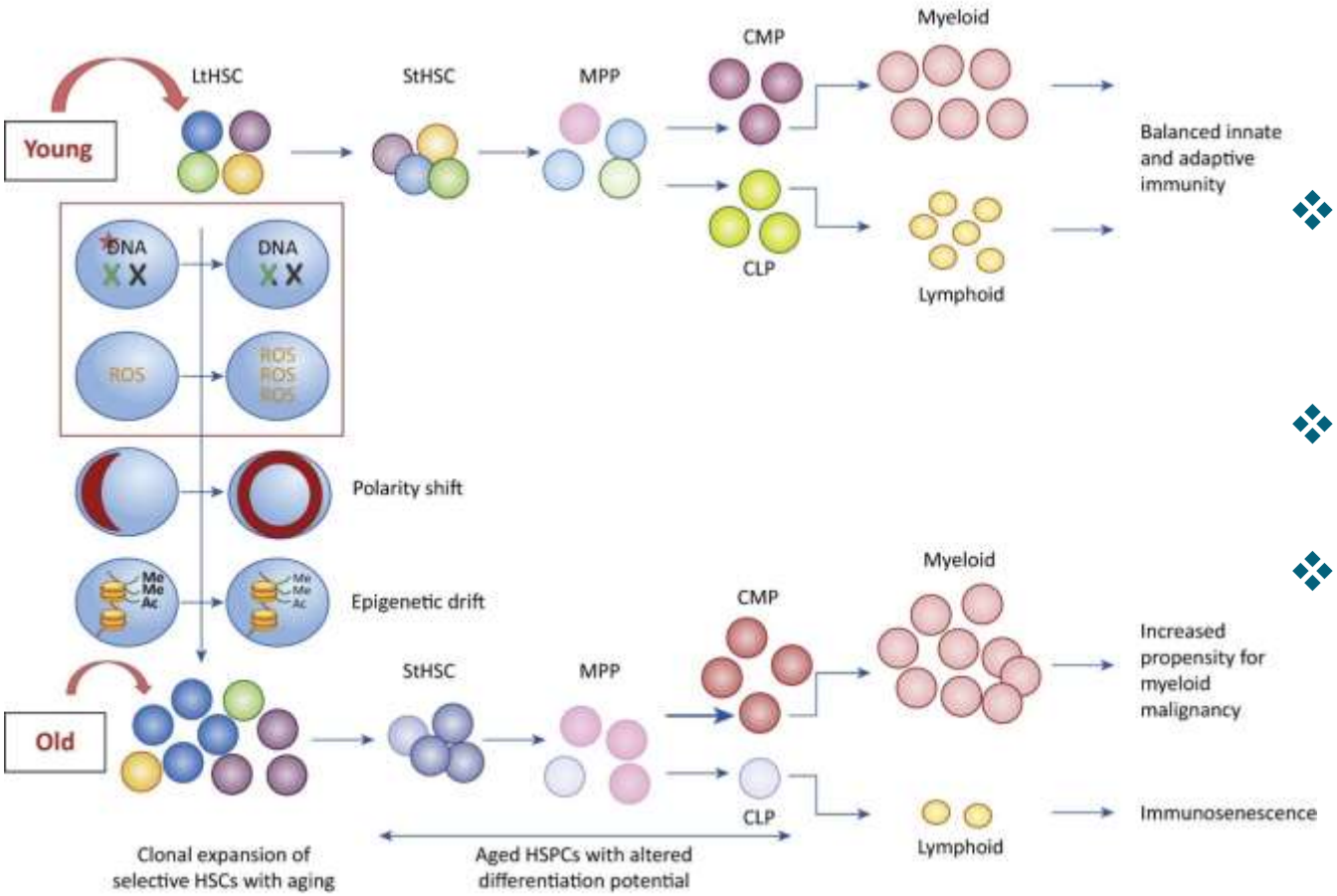
Functional analysis of mouse HSCs: Limiting dilution analysis



Functional analysis of human HSCs



Aging hallmarks of HSCs



- Increased frequency of phenotypic HSCs
- Myeloid-biased hematopoiesis
- Clonal expansion

Akunuru *et al*., Trends in Molecular Medicine, 2016

Four strategies to expand HSCs in vitro

1. Differentiation of ESCs of iPSCs into HSCs;
2. Reprogramming of mature blood cells into HSCs;
3. Chemical screening;
4. Reconstruction of the bone microenvironment.

Take home message

- Hematopoiesis can be classified into primitive hematopoiesis (primitive erythrocytes formation) and definitive hematopoiesis (HSCs formation);
- HSCs are blood cell progenitors that can self-renewal and give rise to all of the blood lineages;
- HSCs emerge through trans-differentiation of endothelial cells in the AGM region during embryonic development;
- Mouse and human HSCs have distinct phenotypic markers, but their functions can be definitively tested by transplantation analysis;
- Aging hallmarks of HSCs and their expansion strategies.