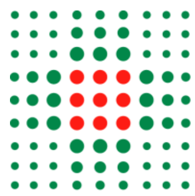


SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Ferrara

università di ferrara
DA SEICENTO ANNI GUARDIAMO AVANTI.

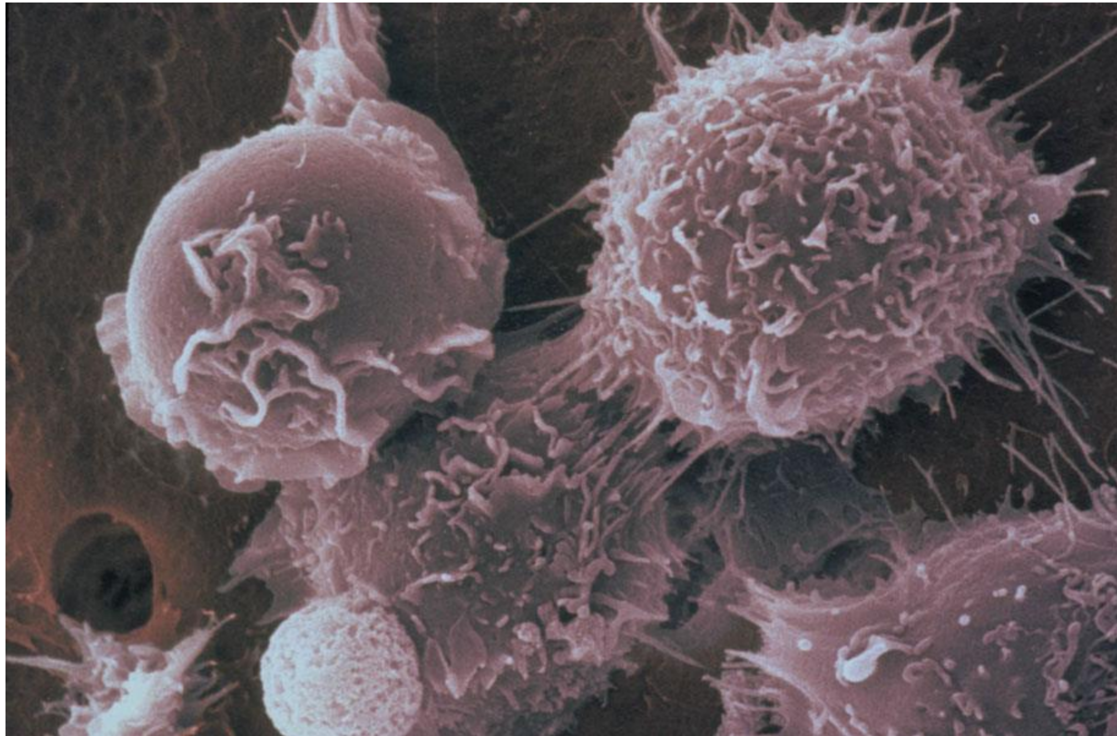
Isolamento e caratterizzazione dei precursori emopoietici

Prof. Gian Matteo Rigolin
Ematologia
Azienda Ospedaliero Universitaria
Arcispedale S. Anna Ferrara

CELLULA STAMINALE

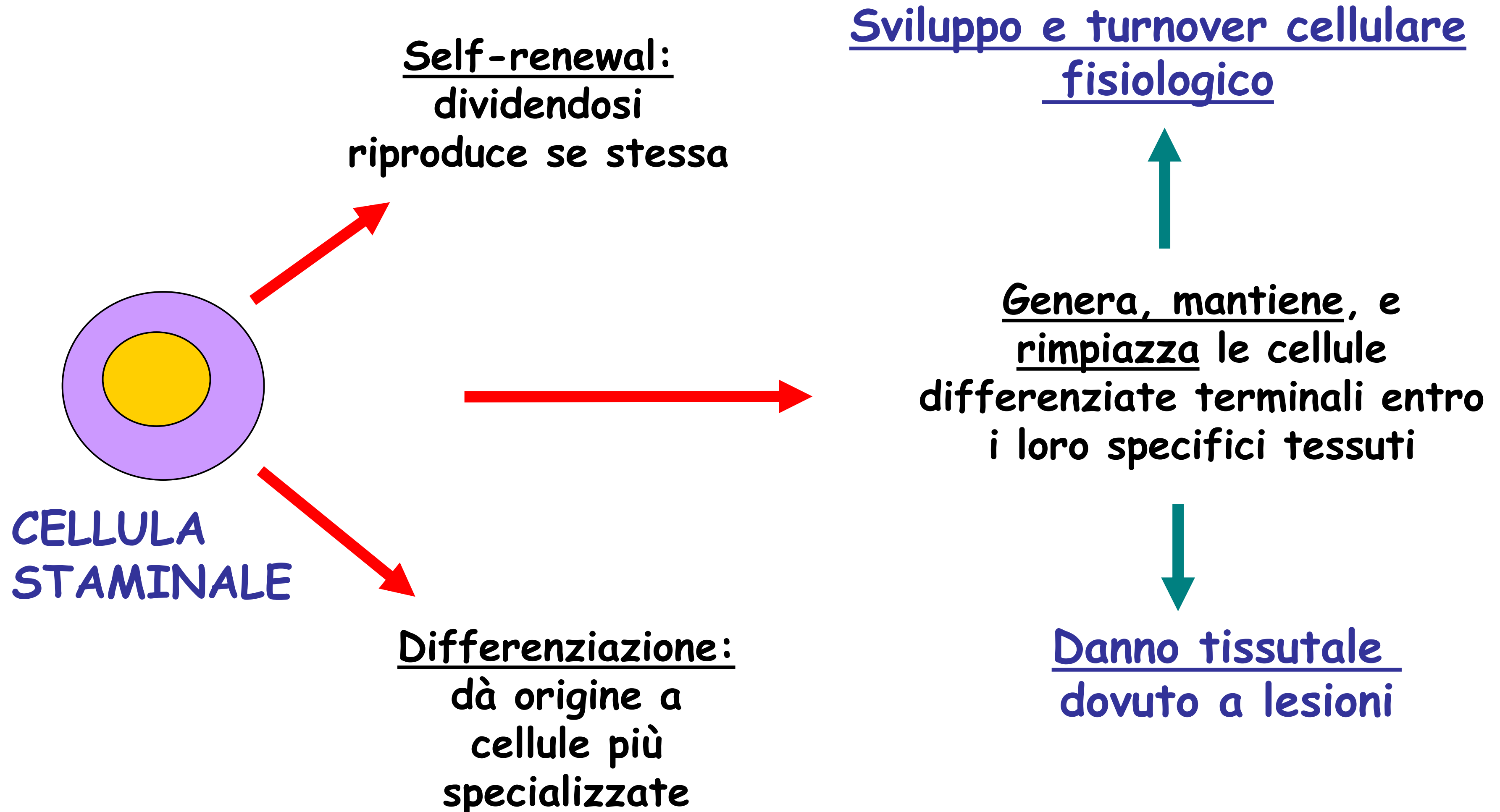
1. Stem cell definitions and distinctions
2. HSC concepts and their origin
3. Molecular regulation of hematopoiesis
4. Trafficking of primitive hematopoietic cells
5. Manipulating HSC for clinical use

CELLULA STAMINALE

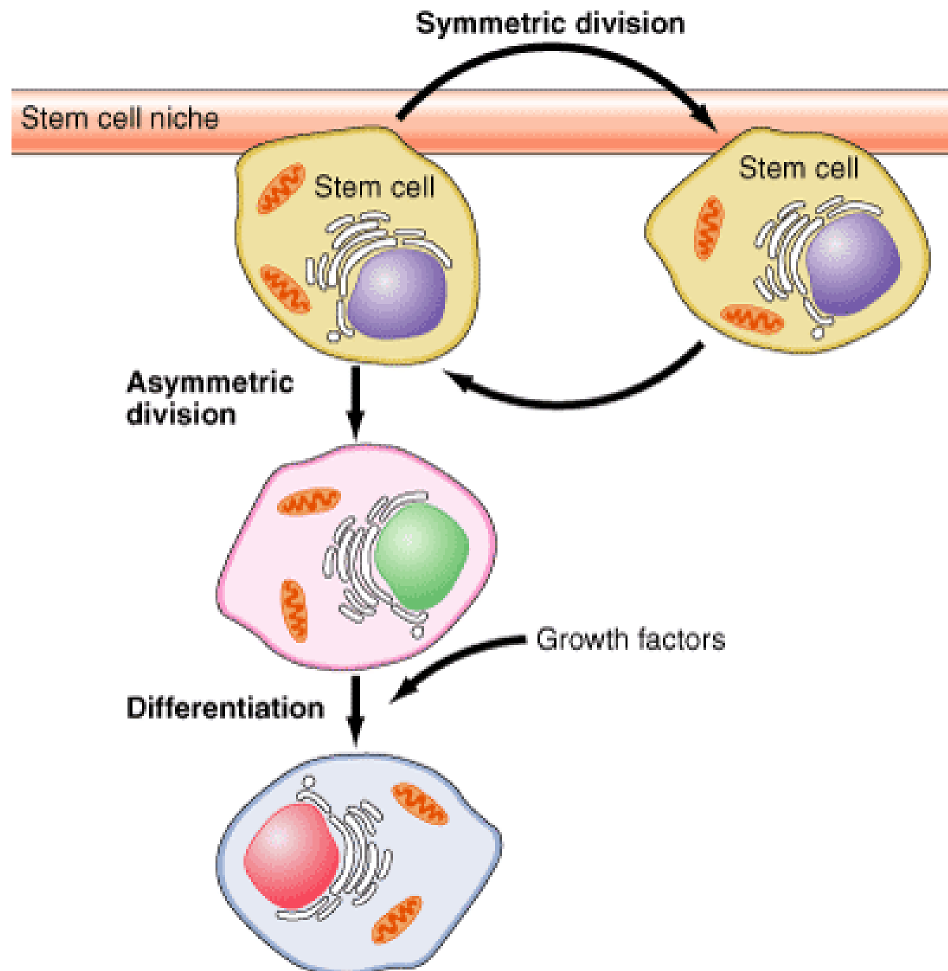


Anne McLaren Nature 414, 129-131:2001

CELLULA STAMINALE: definizione



Cellula staminale



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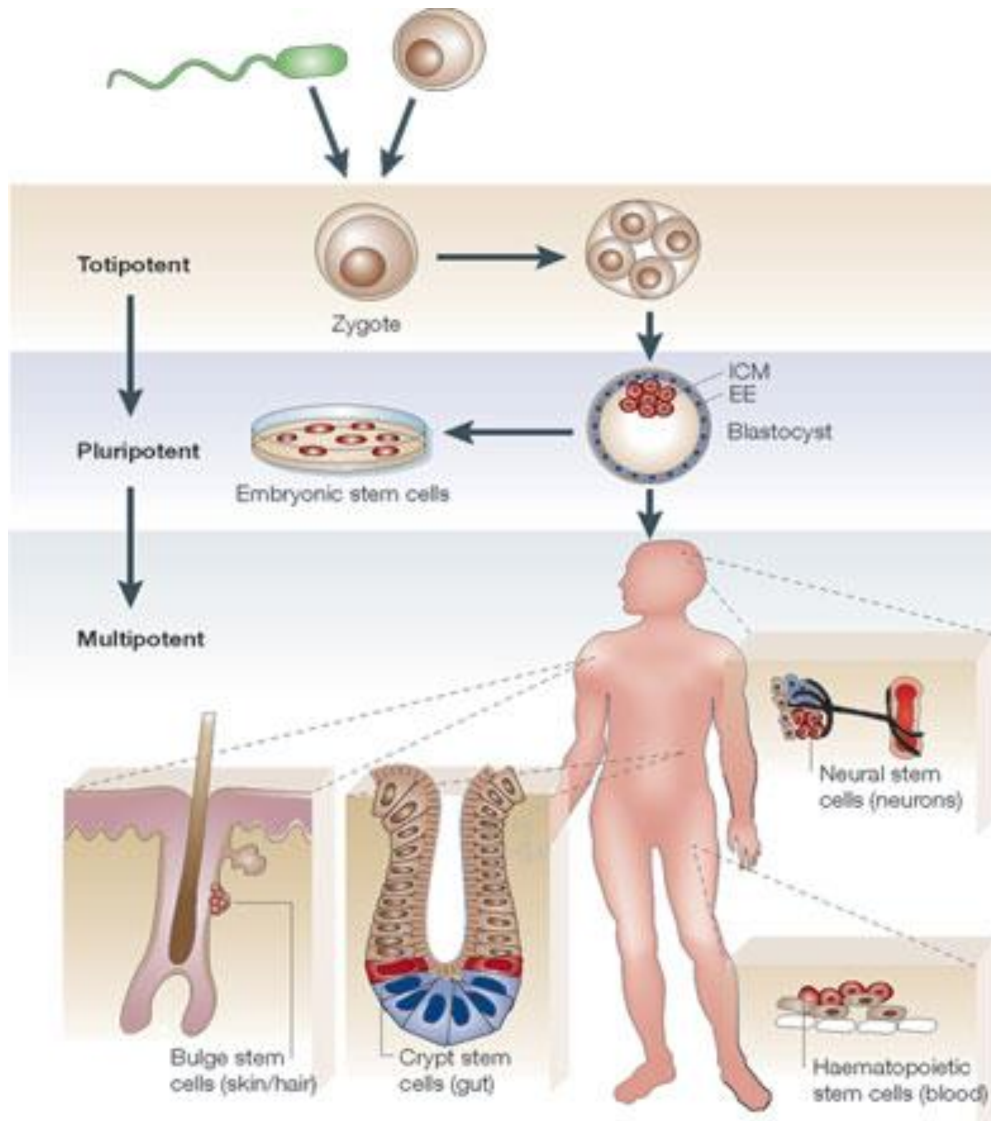
The stem-cell hierarchy

there are multiple different types of stem cells, each defined by their production ability.

Term	Definition	Example
Totipotency	Ability to form the embryo and trophoblast of the placenta	Fertilised oocyte or zygote
Pluripotency	Ability to differentiate into almost all cells of the three germ layers	Embryonic stem cells
Multipotency	Ability to differentiate into a limited range of cell lineages appropriate to the location	Adult, somatic, or tissue-based stem cells
Unipotency	Ability to generate one cell type	Type II pneumocyte

The stem-cell hierarchy



The totipotent zygote formed by the fusion of egg and sperm divides to form the inner cell mass (ICM) and the extra-embryonic (EE) tissue of the blastocyst.

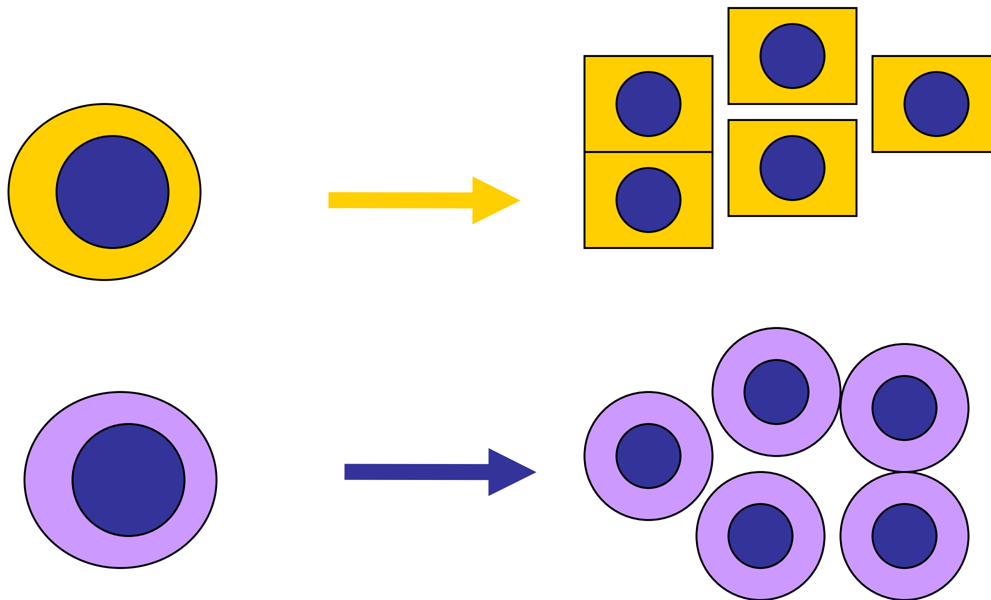
During the development of the embryo, the pluripotent stem cells in the ICM become increasingly restricted in their lineage potential and generate tissue-specific, multipotent stem cells

Eckfeldt CE et al, Nat Rev Molecular Cell Biology 6;726-737:2005.

Human stem cells: heterogeneity

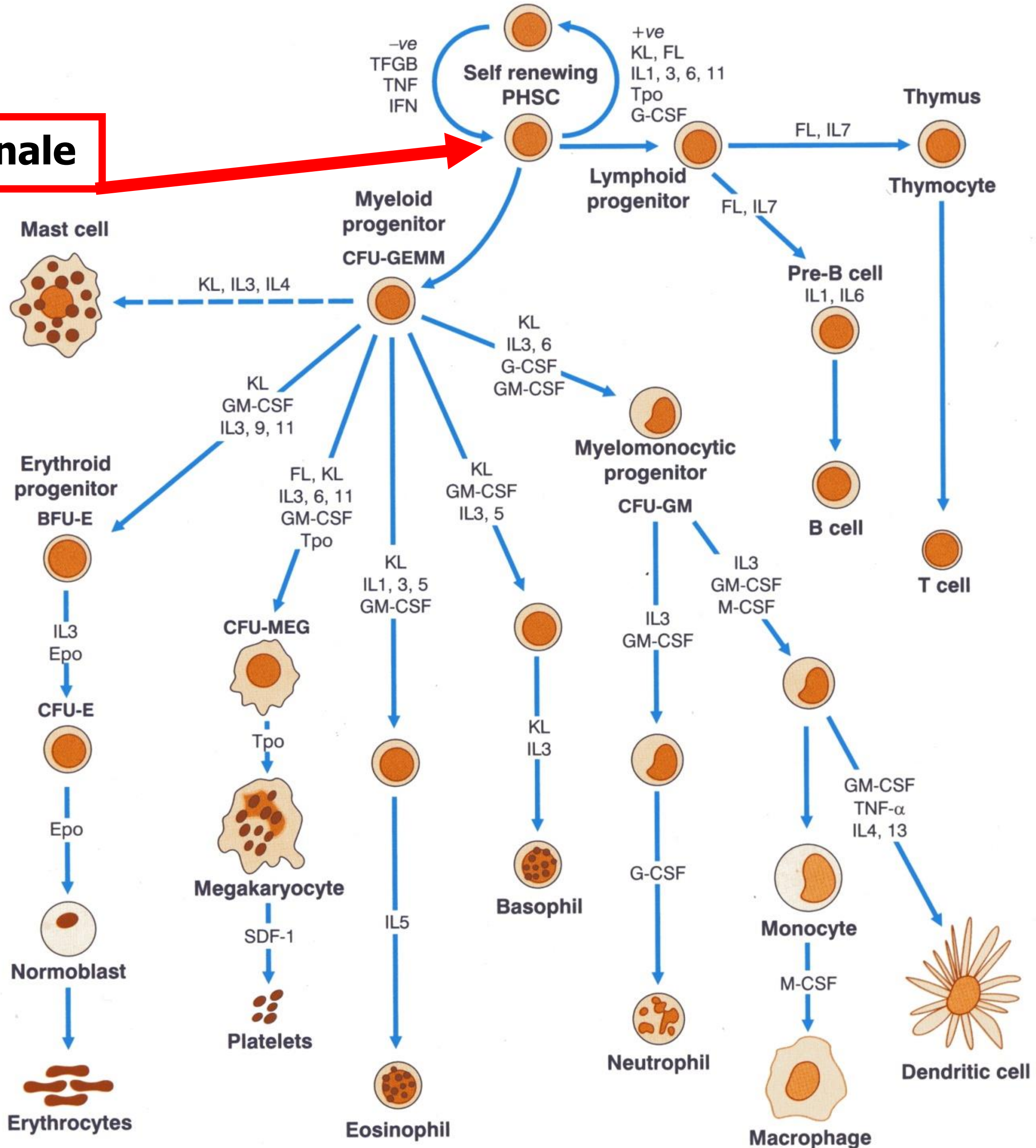
- Embryonic stem cells
- Adult stem cells intrinsic to various tissues
  - Hemopoietic stem cells
  - Mesenchimal stem cells
  - Neural stem cells
  - Hepatic stem cells
  - Pancreatic stem cells
  - Stem cells of the skin (Keratinocytes)
  - Epitelial stem cells of the lung
  - Stem cells of the intestinal epithelium
  - Endothelial stem cells
  - Skeletal muscle stem cells
  - Cardiac stem cells
  - Etc.

Models for differentiation of adult stem cells into solid-organ-specific cells



Distinct stem cells differentiate each into its own organ-specific cells

Cellula staminale



EMOPOIESI

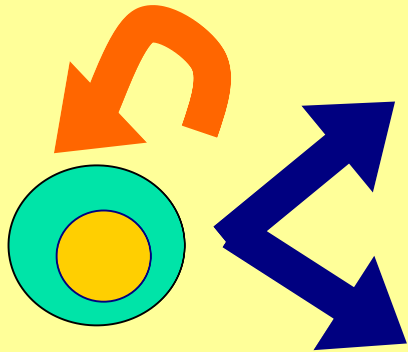
The cellular compartment model

- The compartment model has given rise to terms that are generally applied to cells of hematopoietic origin.

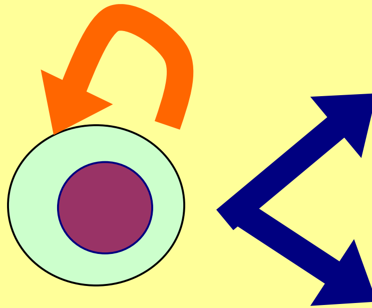
  - **Stem cells** are those that are multipotent and self-renewing.
  - **Progenitor cells** have limited ability to self–renew and are likely to be unipotential or of very limited multipotential.
  - **Precursor cells** are restricted to a single lineage, such as neutrophil precursors, and are the immediate precursors of the mature cells found in the blood.
  - **The mature cells** are generally short-lived and reprogrammed to be highly responsive to cytokines, while the stem cells are long-lived, cytokine-resistant and generally quiescent.

Stem cell renewal and differentiation

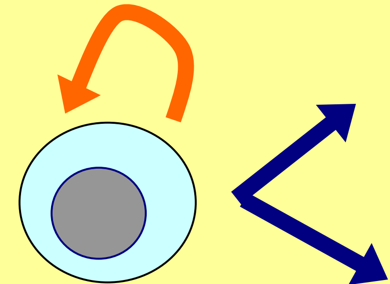
PROLIFERATIVE AND
RENEWAL POTENTIAL



STEM CELL



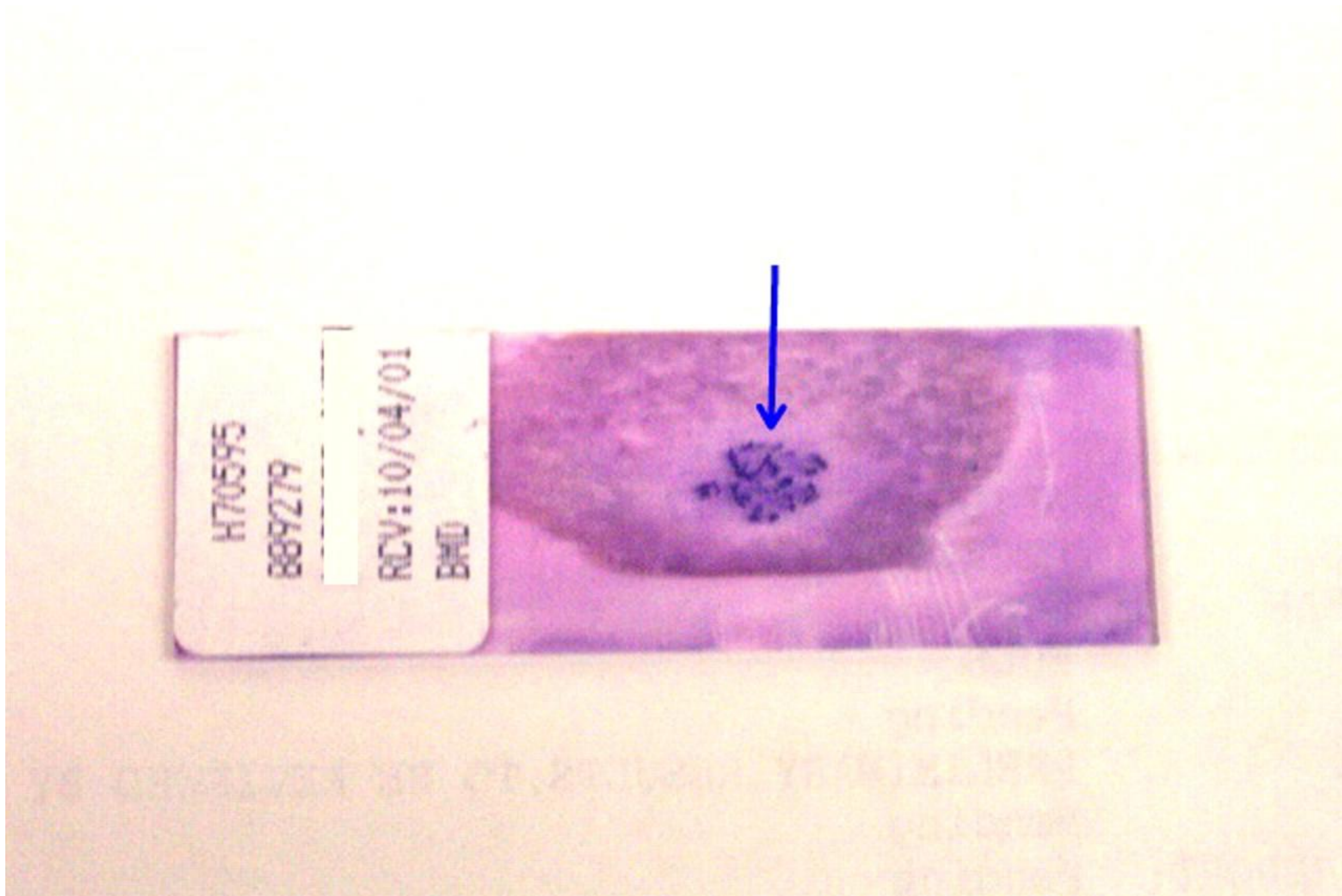
PROGENITOR
CELL



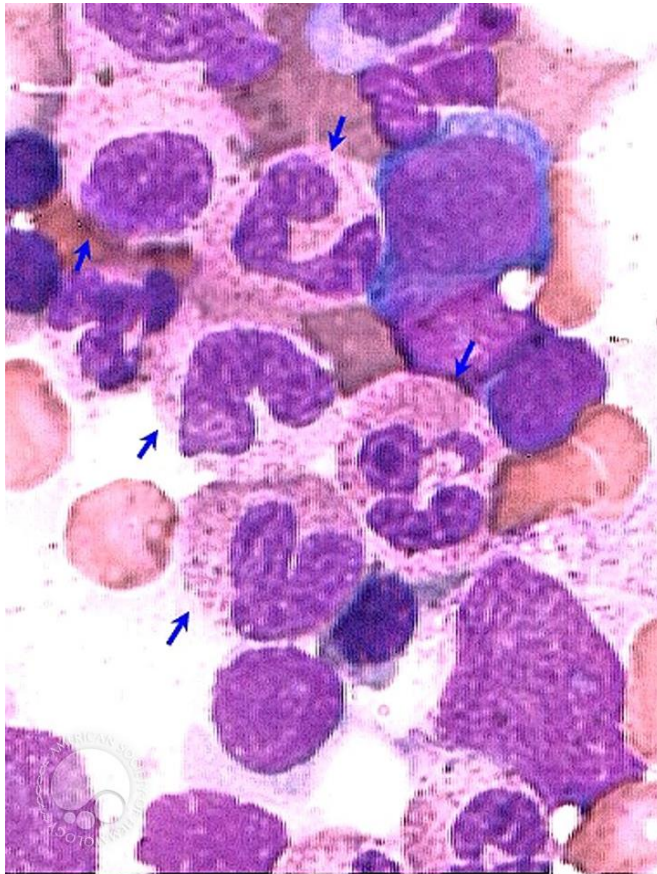
DIFFERENTIATED
CELL

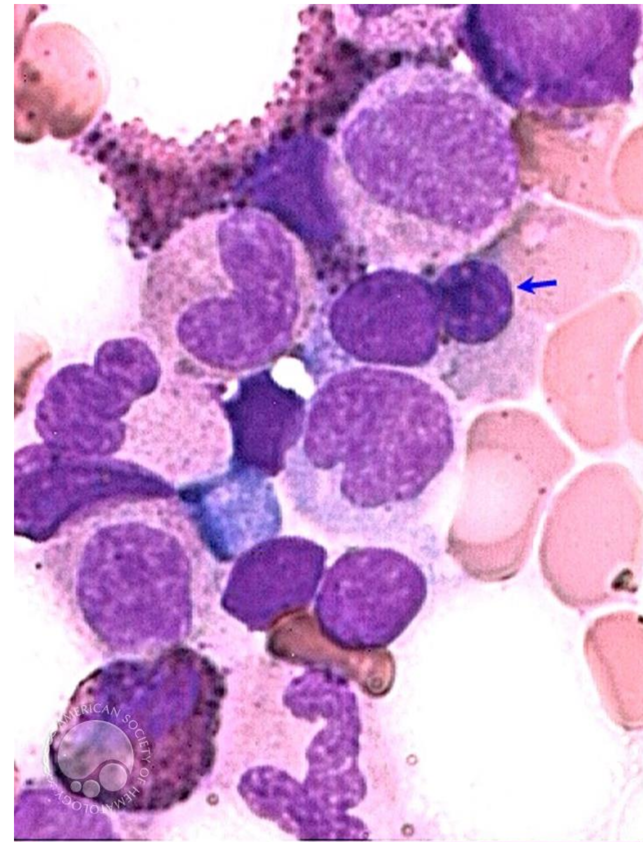
DIFFERENTIATION
CHARACTERISTICS

Biopsia midollare: vetrino



Normal bone marrow



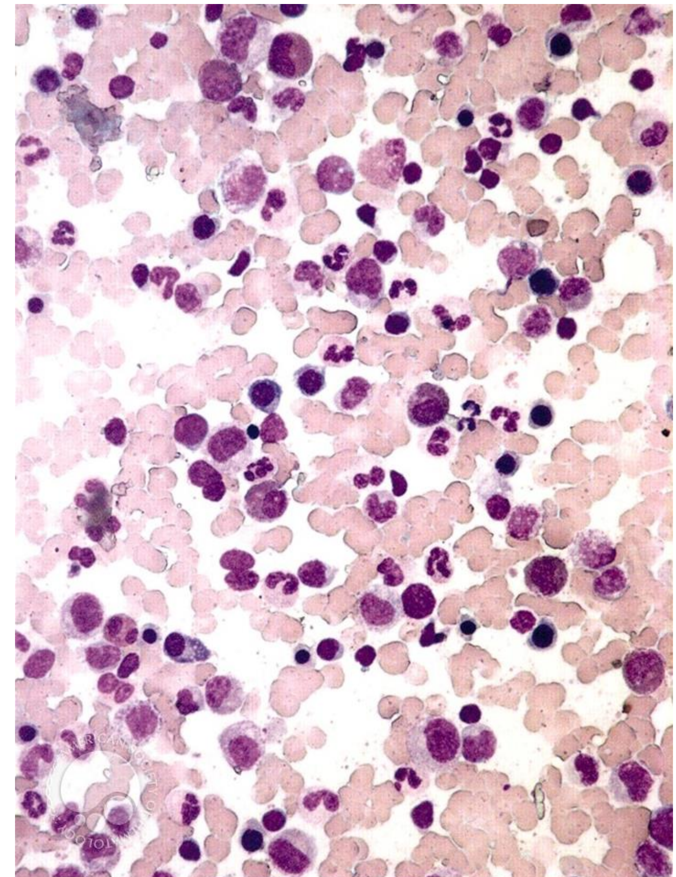


ASH image bank

Mielogramma

.

	%
Cellule reticolari	-
Mieloblasti	2 (0,3-5)
Promielociti	5 (1-8)
Mielociti	
neutrofili	12 (5-19)
eosinofili	1,5 (0,5-3)
basofili	0,3 (0-0,5)
Metamielociti	22 (13-22)
Granulociti	
neutrofili	20 (7-30)
eosinofili	2 (0,5-4)
basofili	0,2 (0-0,7)
Linfociti	10 (3-17)
Monociti	-
Megacariociti	-
Plasmacellule	0,4 (0-2)
Proeritroblasti	4 (1-8)
Eritrobasti	18 (7-32)
basofili	
policromatofili	
ortocromatici	



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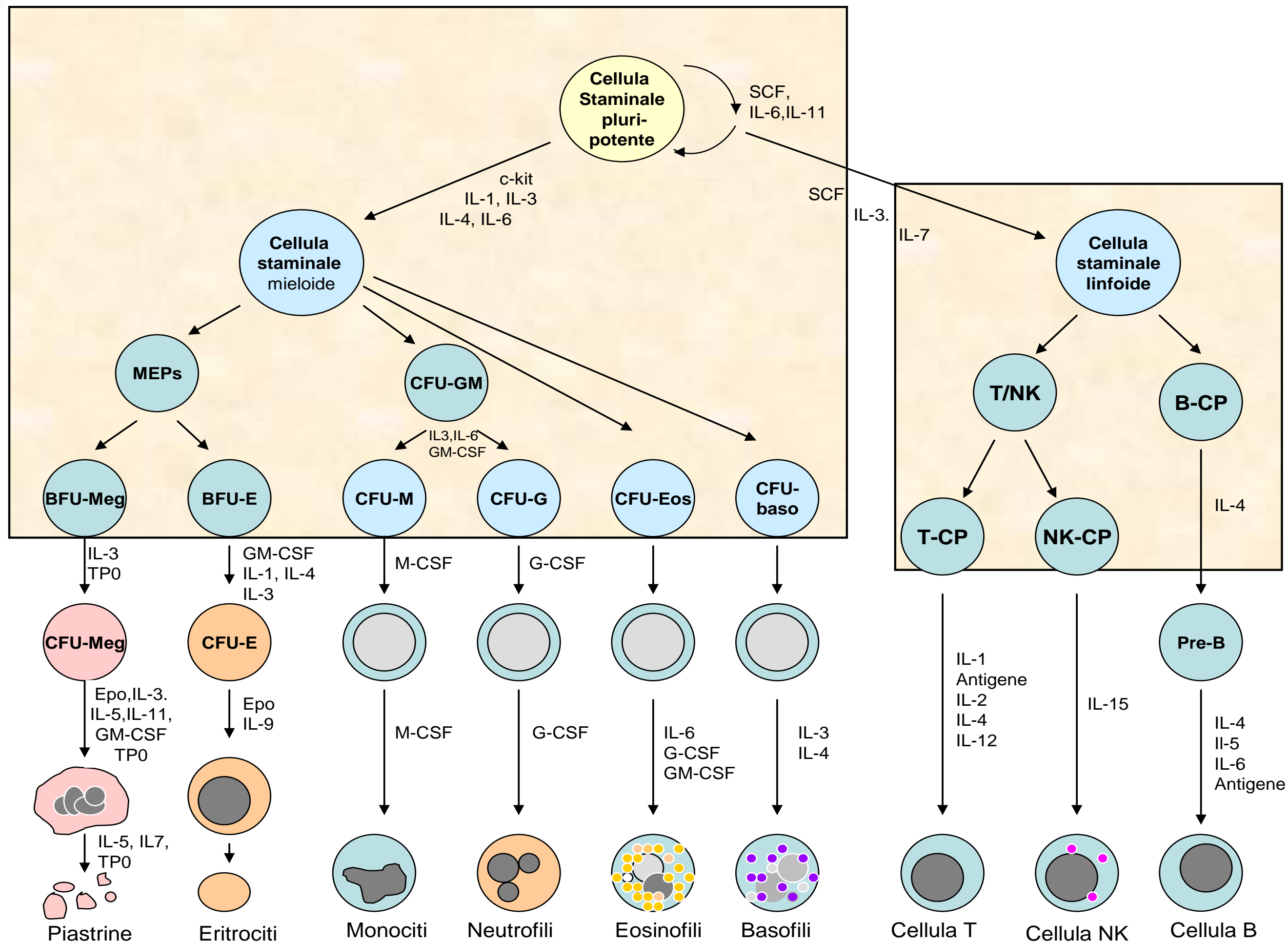
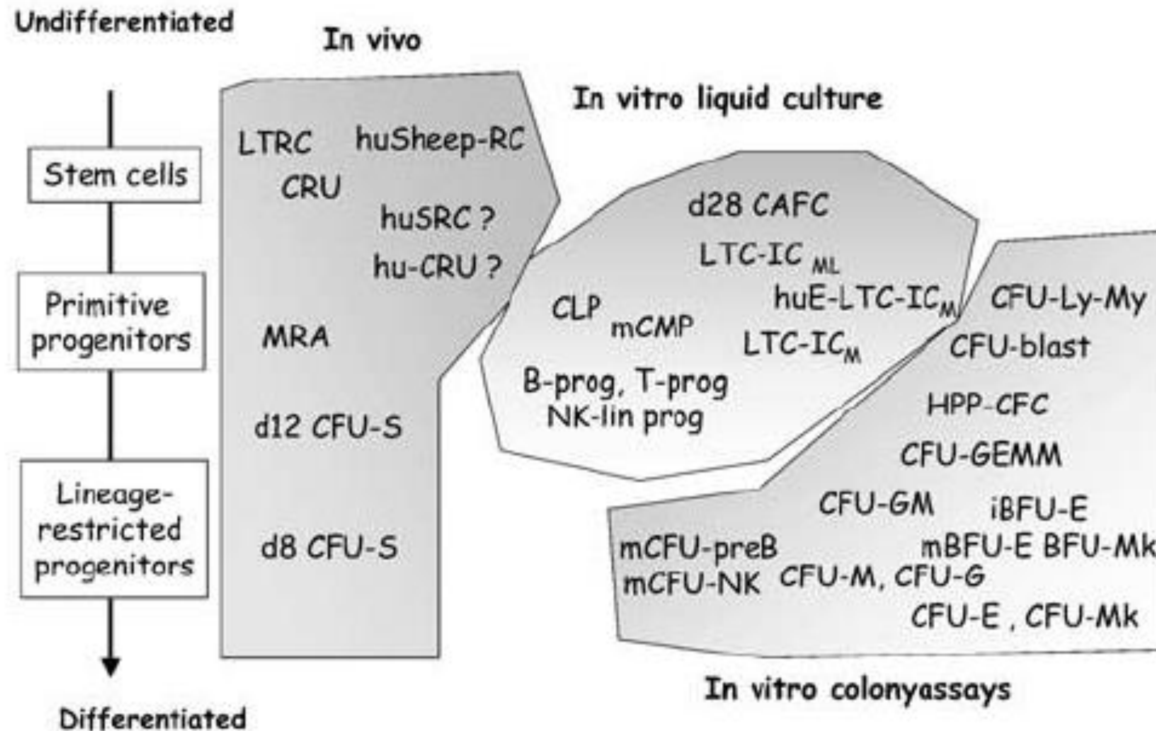
Hemopoiesis



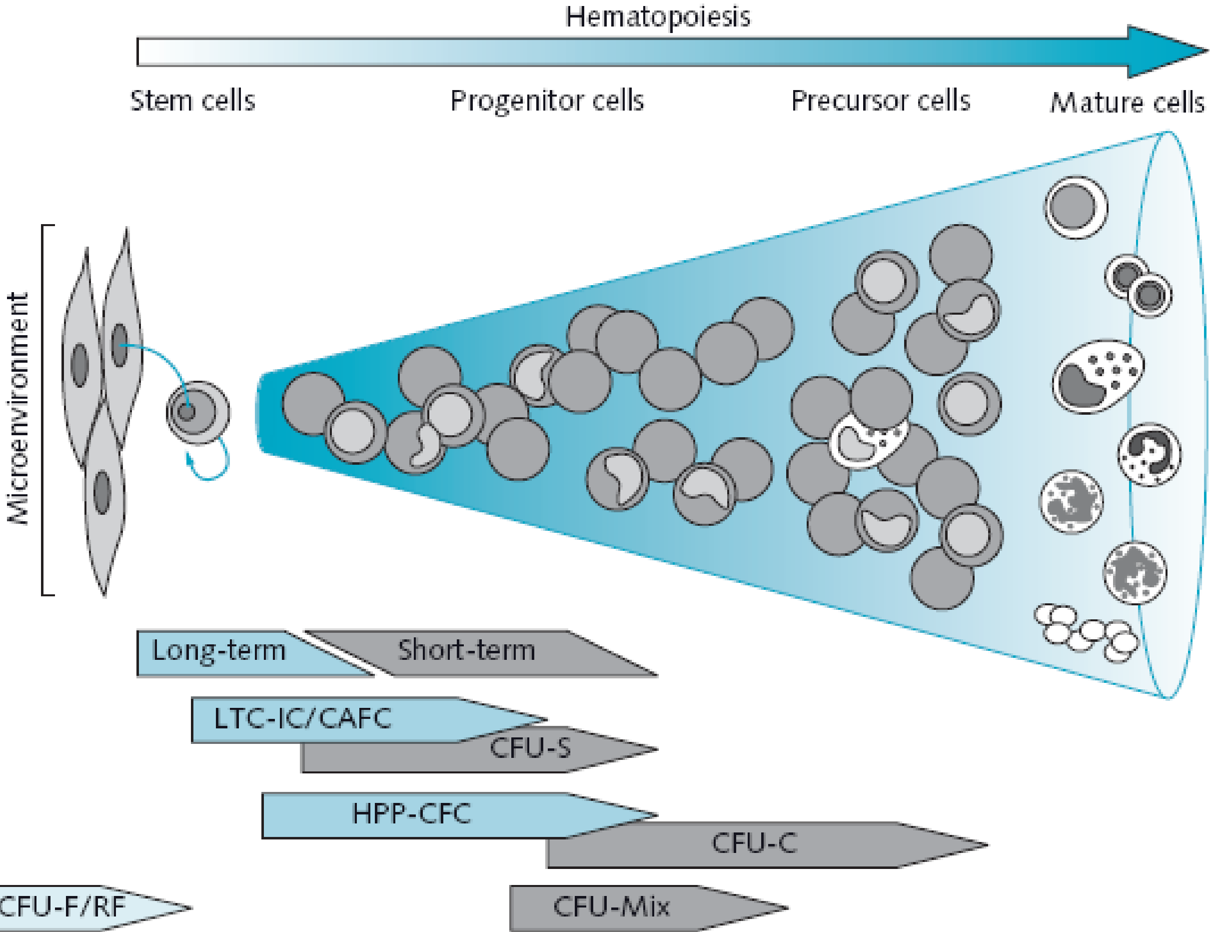
Functional analysis of HSCs

- Functional assays for HSCs **do not actually measure the activity of HSCs** but instead assess more differentiated progeny, such as progenitor and precursor cells.
  - *in vitro* assays measure mature populations,
  - *in vivo* assays detect the activity of primitive cells capable of homing and engrafting in the proper microenvironment to produce functional hematopoietic progeny.

Functional analysis of HSCs



Schematic view of hematopoiesis



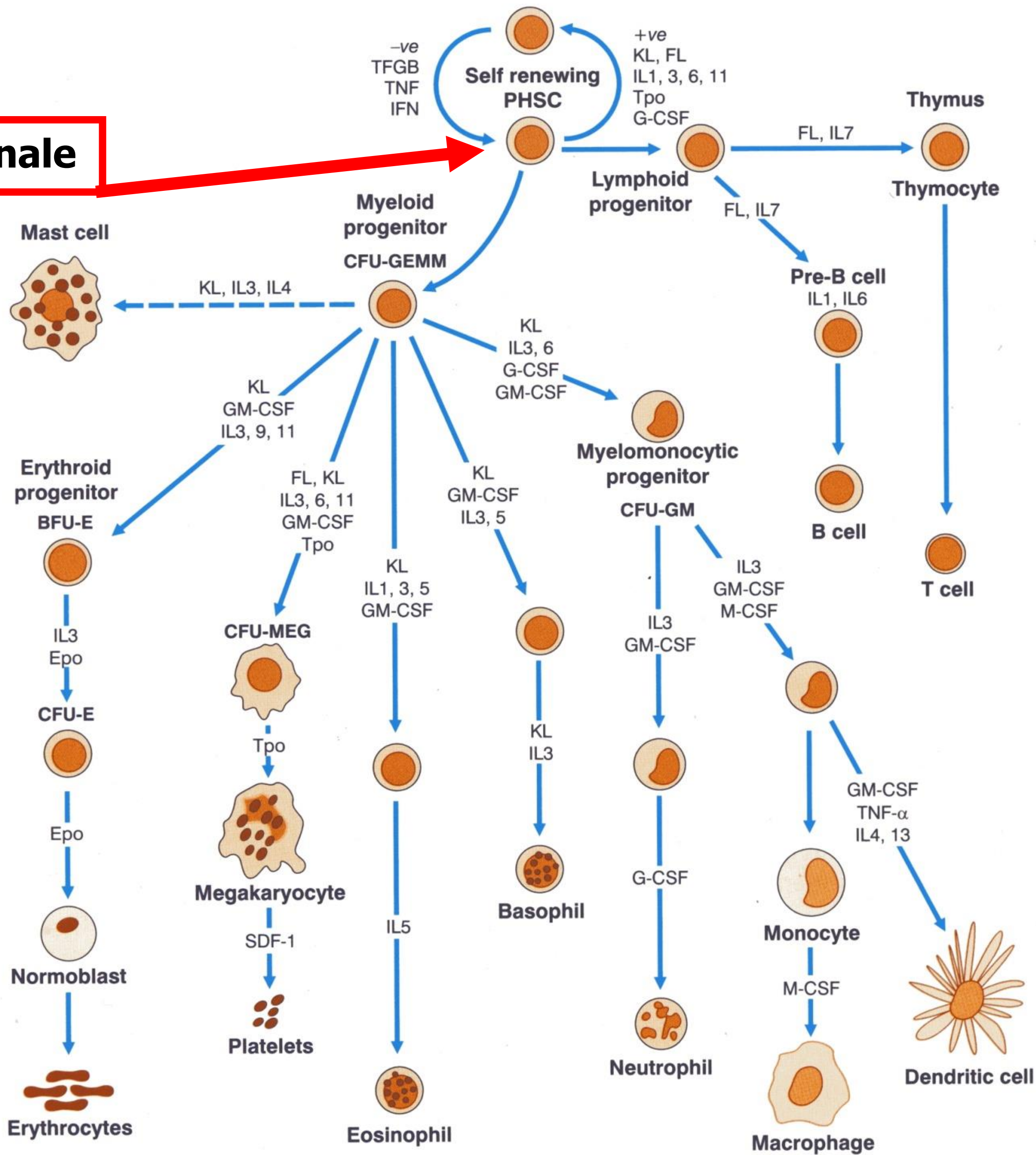
long - term culture – initiating cells (LTC - ICs)
cobblestone area- forming cells (CAFCs)
colony- forming units, spleen (CFU - S)
colony -forming cells (CFCs)

- The **HPP -CFC** possesses a high degree of proliferative and multilineage potential.

  - Formation of HPP - CFC colonies, characterized by size greater than 0.5 mm and multilineage composition, requires the use of multiple cytokines in order to proliferate.

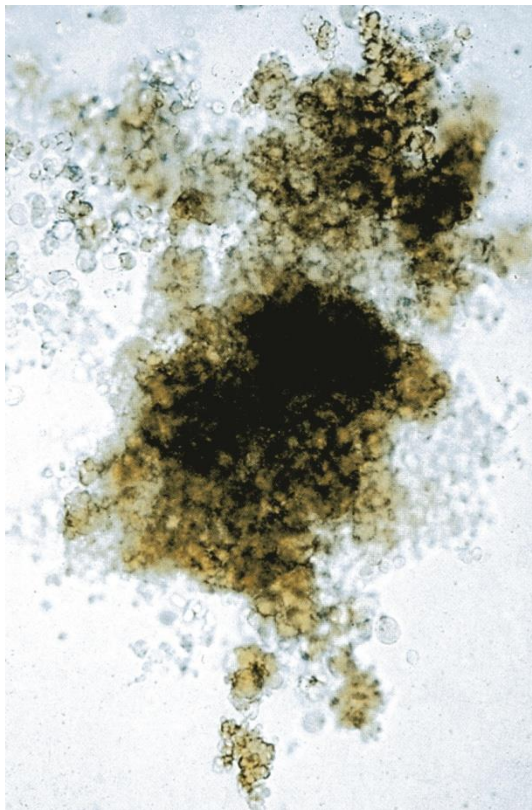
- The **CFU- C** measures hematopoietic progenitor function and is performed by plating cells in semisolid media containing methylcellulose and one or more cytokines.
  - After 5 – 14 days, colonies comprising mature cell populations committed to either myeloid or lymphoid lineages may be observed.
  - While most colonies obtained using this assay are composed of cells of a single lineage, less frequently multipotent progenitors can yield colonies containing multiple lineages.

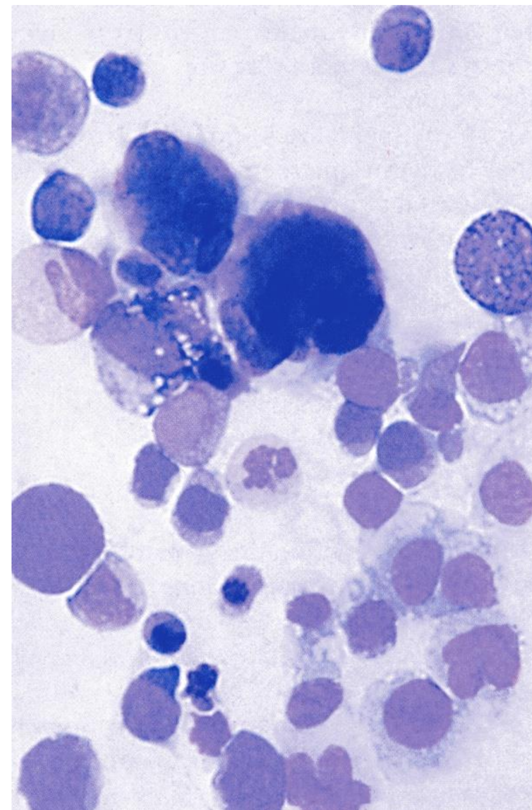
Cellula staminale



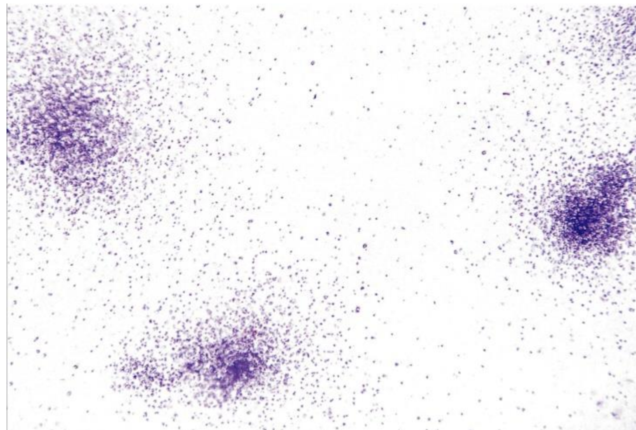
EMOPOIESI

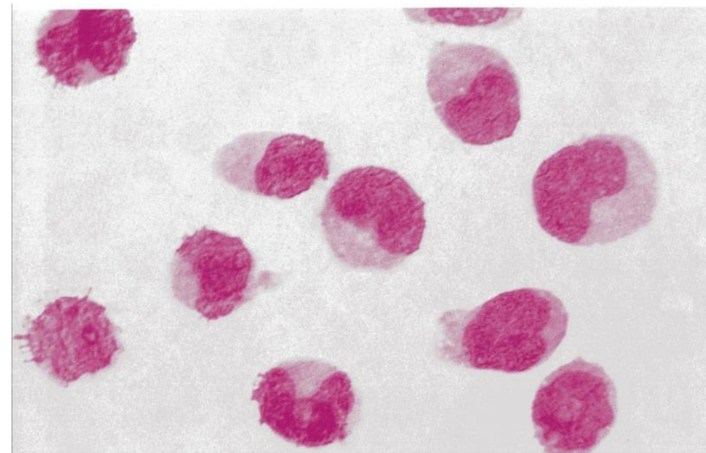
Colture cellulari: CFU-GEMM

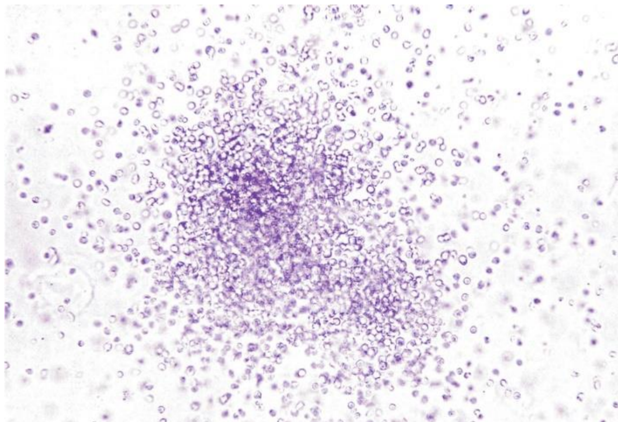


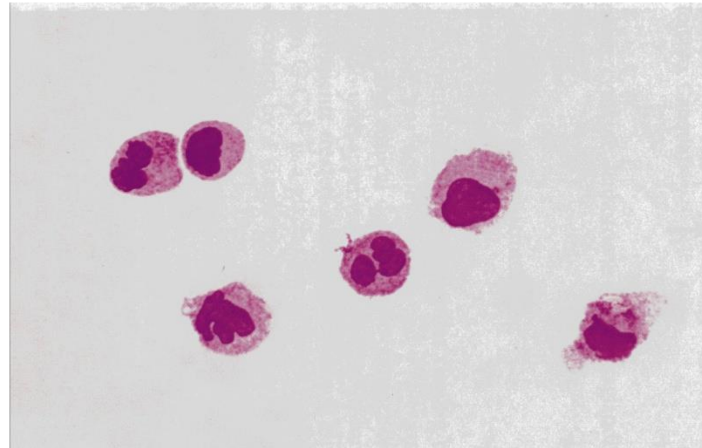


Colture cellulari: CFU-GM

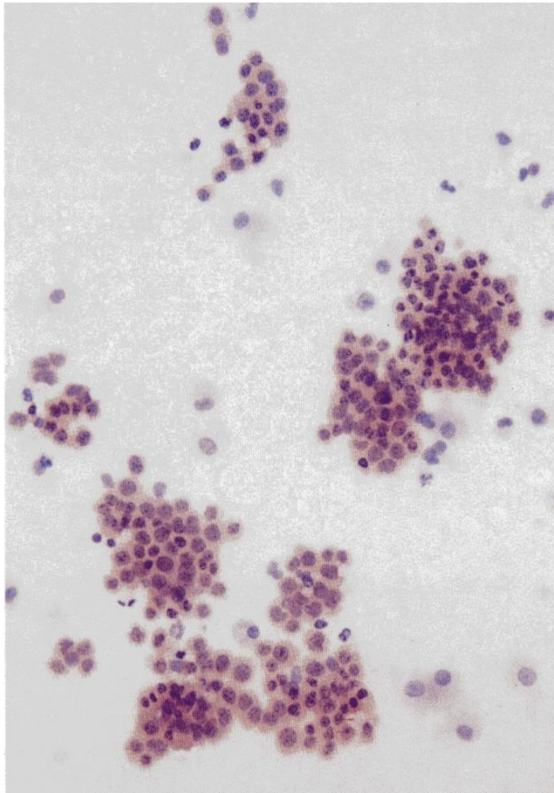


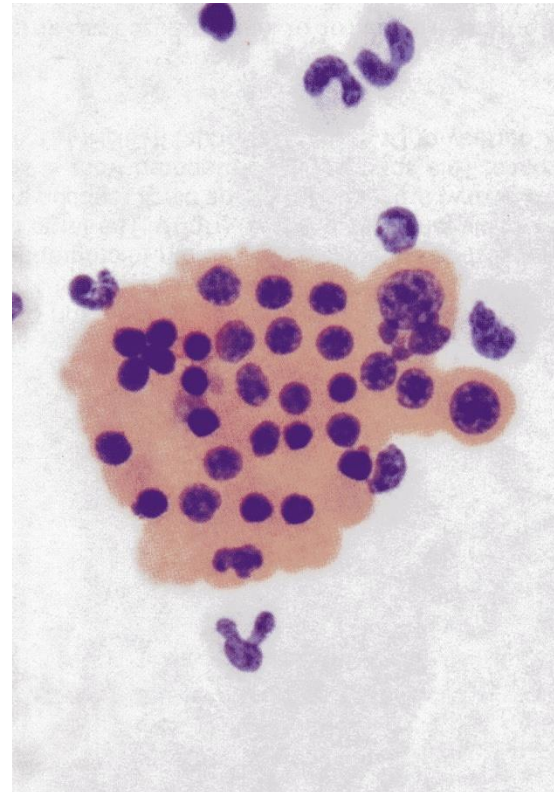




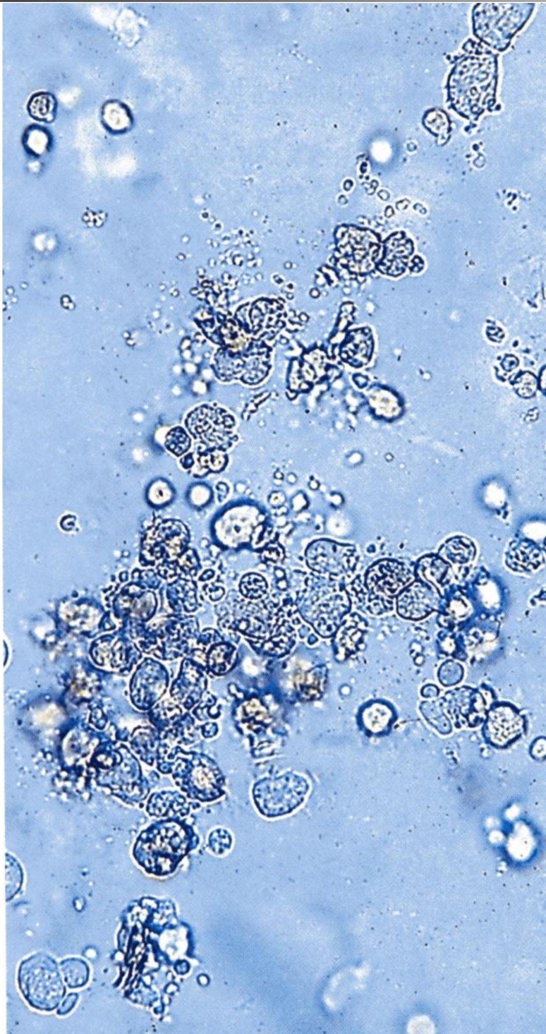


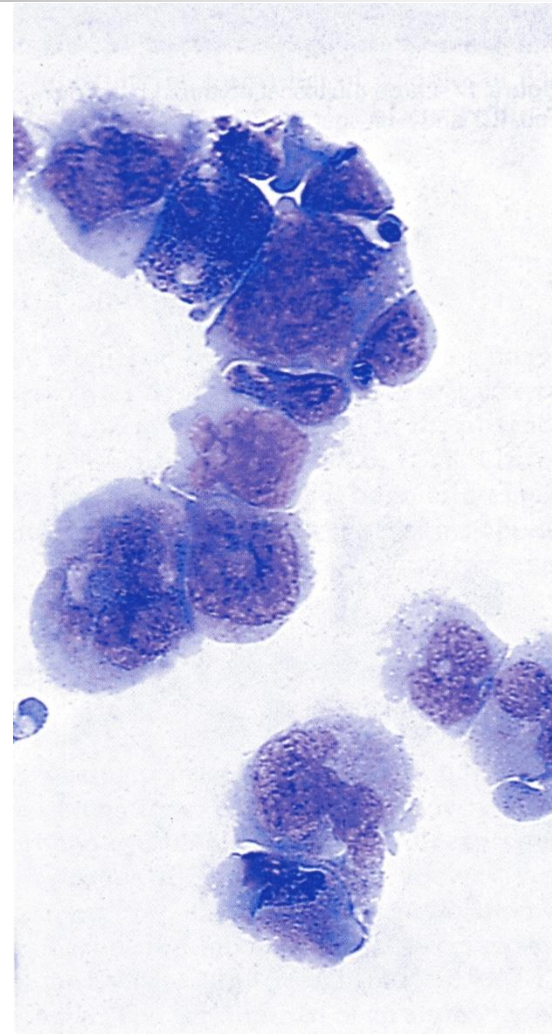
Colture cellulari: CFU-E





Colture cellulari: CFU-Meg



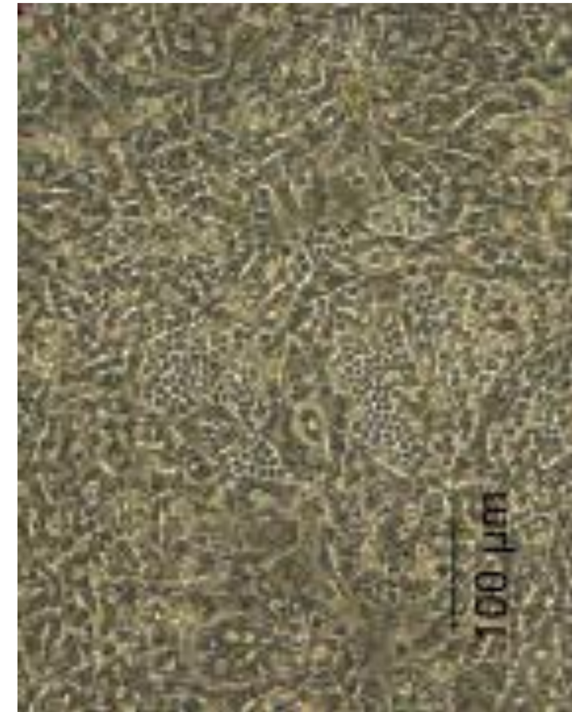


- The **LTC-IC assay** correlates more closely to HSCs.
  - HSC are plated on top of stromal cell lines or irradiated primary BM stroma.
  - Primitive HSCs are able to initiate growth and to generate progeny *in vitro* for up to 12 weeks.
    - Progenitor cells and mature myeloid cells are removed weekly to prevent overgrowth.

- HSCs, characterized by high proliferative and self–renewal capabilities, are able to sustain long-term culture and may be enumerated at the conclusion of the assay.

CAFC assay: cobblestone area-forming cells (CAFCs)

- It is a type of LTC-IC that similarly measures the ability of cells to initiate growth and generate progeny *in vitro* for up to 12 weeks.
  - HC are plated at limiting dilution on top of a monolayer consisting of irradiated BM stroma or a stromal cell line.
  - The growth of colonies consisting of at least 5 small non-refractile cells reminiscent of cobblestones, found underneath the stromal layer, are counted.
  - Such cultures are maintained using weekly half-media changes until up to 5 weeks after seeding.

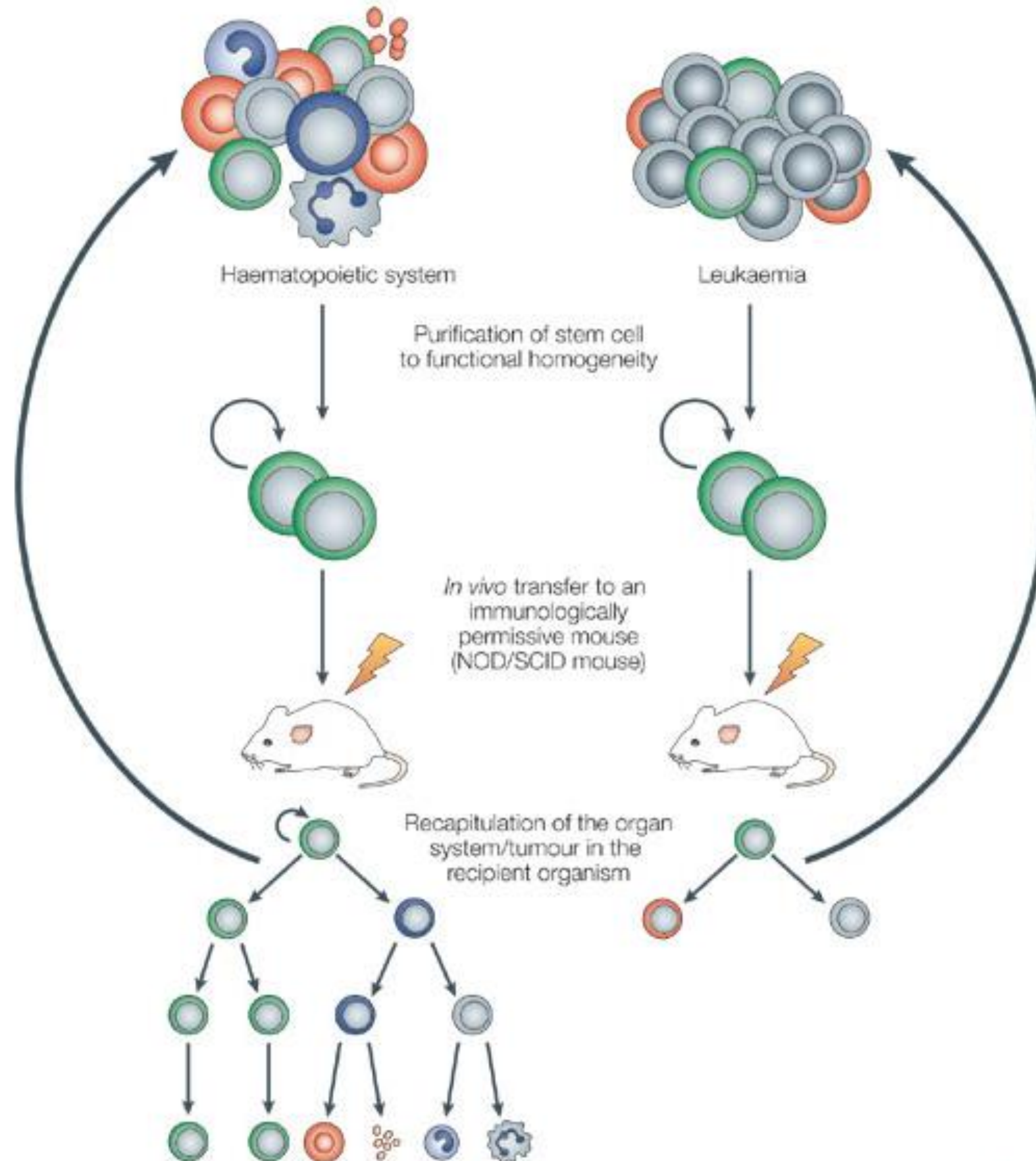


CAFC assay:
cobblestone area- forming cells (CAFCs)

- In this assay,more primitive cells appear later, and day-35 CAFCs represent a close correlate of a cell with *in vivo* long-term multilineage repopulating potential.

- LTC-ICs may be enumerated after day 35 by completely removing the CAFC medium, overlaying methylcellulose and counting the number of colonies produced after 8–10 days.

Functional assays for normal and malignant stem cells



Huntly BJP & Gilliland DG, Nat Rev Cancer 5;311-321:2005.

In vivo *assays:* CFU-S

- In the CFU-S assay, BM or spleen cells are transplanted to irradiated recipients and animals are killed after 8 or 12 days for analysis of spleen colonies, termed CFU-S8 and CFU-S12.
  - Cells that give rise to CFU-S8 are **predominantly unipotential** and produce erythroid colonies.
  - CFU-S12 colonies consist of several types of myeloid cells, including erythrocytes, megakaryocytes, macrophages and granulocytes.
- Cells giving rise to CFU-S12 **represent a more primitive population of multipotent cells** than those that result in CFU-S8.

In vivo *assays*

- The **long-term repopulation assay is a more accurate measure of HSC activity**.
- Whole collections of hematopoietic cells or fractionated subpopulations are transplanted to lethally irradiated syngeneic mice, typically by tail vein injection.
- Recipients are screened for ongoing hematopoiesis 8 – 10 weeks after transplantation.
- By this time, hematopoiesis is firmly established and donor-derived blood is produced by transplanted HSCs.

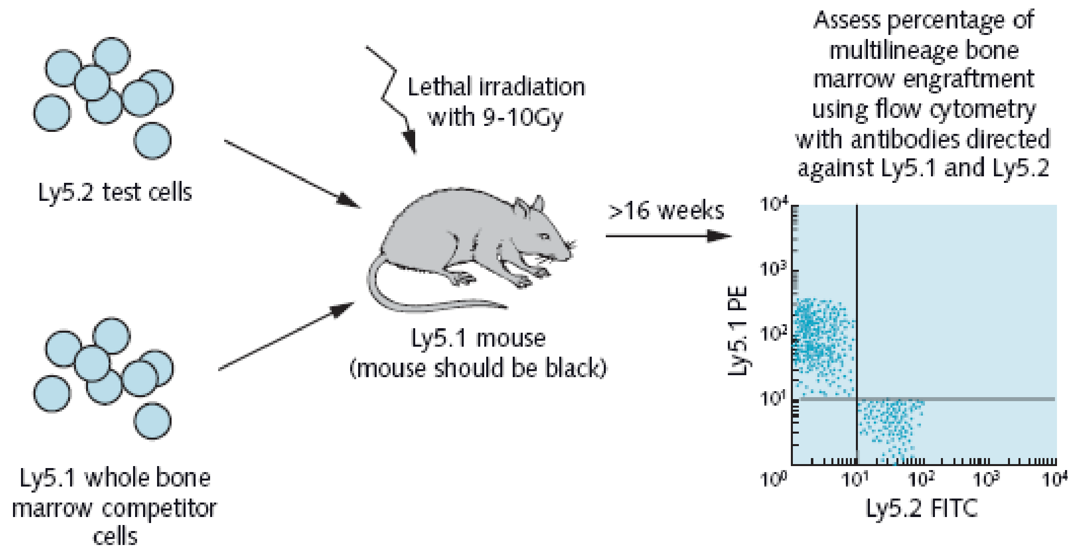
- This assay requires that cells fulfill the two central features of HSCs:
  - multilineage reconstitution, consistent with multipotentiality,
  - indefinite hematopoiesis, indicative of self - renewal.

- Tracking of transplanted cells was originally conducted using radiation - induced chromosomal abnormalities or by retrovirally marking donor cells.

- A major advance in the ability to track transplanted cells has been the development of congenic mice with minor allelic differences in the leukocyte common antigen Ly5, which is expressed on all nucleated blood cells.
  - The C57/BL6 strain contains the Ly5.2 antigen,
  - The BL6/SJL strain contains a separate allele, Ly5.1.

- These syngeneic strains may be transplanted interchangeably.
- Both antibodies are available with distinct fluorescent labels.
- FACS analysis using these antibodies permits measurement of donor–derived reconstitution of the nucleated blood lineages.

Competitive repopulation assay



HSCs are quantified by transplanting limiting-dilution numbers of BM into lethally irradiated recipients.
At 10 – 12 weeks, host PB is assessed to determine whether donor-derived reconstitution has occurred.
Donor cells must constitute at least 1% of the PB to contend that at least one HSC was present in the donor population.
The % of reconstituted animals may be plotted against the number of input cells to determine a limiting-dilution estimate of the frequency of HSCs within the donor population.
In the *competitive repopulation assay*, transplanted HSCs compete with the host's HSCs that survive irradiation - induced death, in addition to host cells transplanted with the graft.

Table 1. MoAbs recognizing stem cell populations[19,20]

Ag	*Other names*	*Differentiated cell expression*	*Stem/progenitor expression*
CD9	P24, DRAP-1, MRP1	Leukocytes, endothelial, epithelial	NSC
CD34	8G12, 581, QBEnd10	Endothelial	HSC, EPC
CD73	Ecto-5′ nucleotidase	Leukocyte subsets, endothelial, epithelial	MSC
CD81	TAPA-1	Lymphocytes, endothelial, epithelial	NSC
CD90	Thy1	Endothelial	MSC, HSC
CD105	Endoglin, SH2	Endothelial, macrophage/ monocyte	MSC with CD73+, CD16+, CD90+ CD29+ CD34−, CD45−
CD117	c-kit, steel, SCF	Breast epithelial, renal tubule, melanocytes	Myeloid, HSC
CD133	AC133, Prominin-1	Endothelium, epithelial	HSC, EPC, NSC
CD135	FMS-like kinase-3 (FLT3) or STK-1, or Flk-2	Macrophage/ monocytes	Human marrow CD34+ and dendritic precursors, murine short-term HSC
CD146	MUC-18, Mel-CAM, MCAM	Activated T cells, endothelial	EPC
CD150	SLAMf1; used with CD244 and CD48	T, B, dendritic, endothelial	Distinguishes HSC from multipotent and B-cell progenitors
Aldefluor			HSC/MSC
Side population			HSC

Abbreviations: EPC = endothelial progenitor cells; HSC = hematopoietic stem cells; NSC = neural stem cells.

Table 3. Flow cytometric markers used to define the most frequently observed stem cell subpopulations[12,26,32,35]

	Cell type	*Lin− subpopulation immunophenotype*
HSC	Hematopoietic stem cell	CD34+/CD38−
CMP	Common myeloid progenitor	CD34+/CD38+/IL3Rα low/CD45RA
GMP	Granulocyte-macrophage progenitor	CD38+/IL3Rα low/ CD45RA+
MEP	Megakaryocyte-erythroid progenitor	CD38+/IL3Rα−/CD45RA−
CLP	Common lymphoid progenitor	CD10+/CD19−
	Pro-B cells	CD10+CD19+
	T and NK progenitors	CD10−/CD7+/CD45RA+
MSC	Mesenchymal stem cell	CD90+/CD105+/ALDH+/ CD45−/CD34−/CD133−/ CD38−/HLA−DR−
STRC-M	Early myeloid short-term repopulating	CD34+/CD38+/CD45−/ ALDH−/CD133−/CD90+
	Late myeloid HSC	CD34+/CD38−/ CD45dim+/ALDH+[a]/ CD133+/CD90+

Abbreviations: ALDH = Aldehyde dehydrogenase; STRC-M = short term repopulating cells-myeloid. [a]Bright positive cells have the highest repopulation capacity compared with dim positive cells.

Beksac M, BMT (2011) 1- 6

Frequency and engraftment potential of HSC subpopulations

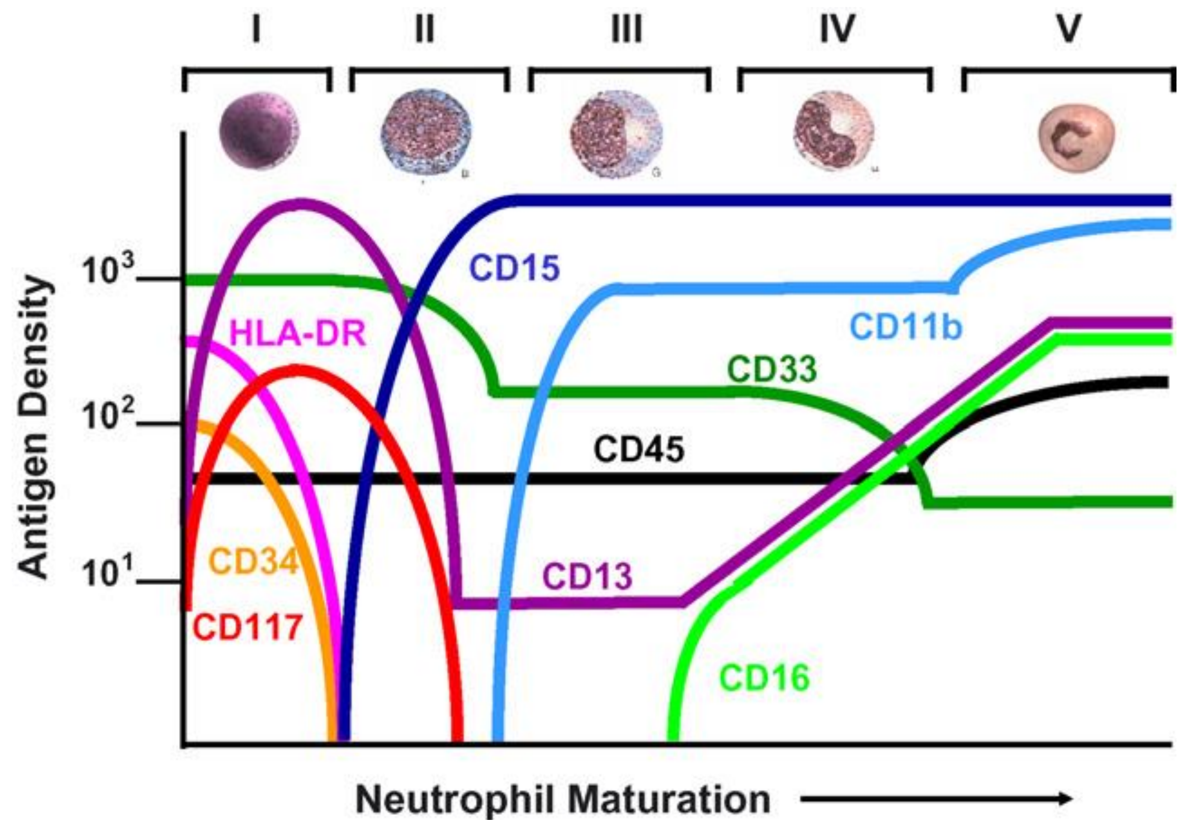
Cell type	*Frequency in*	*Cell dose*	*PNL engraftment*	*Plt engraftment*
Total CD34+[5,7,10]	1/200 (of MNC, BM)	2-8 $\times 10^6$/kg	Yes	Yes (month 3,6,9)
CD34+38–[22-24]	1/40	Unknown	Yes (month 6,9)	Yes
	1/1000 (of MNC, BM)			No
CD34+90+[22,25,26]	1/1000	25-80 $\times 10^4$/kg	Yes	Yes (month 3,6,9,12[a])
			no	
CD34+CD110+[27]				Yes
CD34–/low[24]	1/24000	Unknown	Unknown	Unknown
CD133+[28]	Same as CD34	Same as CD34+	Similar to CD34	Similar to CD34
CD34+133+[22]	Not reported	Not reported	Yes (month 3)	Yes(month 3,6,9)
SSC low/ALDH bright[29-31]	1.2% (BM)	5.4 $\times 10^6$/kg (PNL)	Yes	Yes
		7.22 $\times 10^6$/kg (Plt)	Yes	Yes
			No	No
Lin-CD34+CD38–IL3Ra low	73.20 (17.8-86.2) % (PBSC)	$>4.4 \times 10^6$/kg	Similar to CD34	Similar to CD34
CD45RA- (CMPs)[32]	35.2 (20.4-42.3) % (BM)	(PNL, Plt and erythroid)		
	3.2 (1.3-16.1) % (CB)			

Abbreviations: CMP = common myeloid progenitors; MNC = mononuclear cell; PNL = polymorphonuclear cells; SSC = side light scatter. [a]Also enhanced erythroid engraftment on month 12.

Beksac M, BMT (2011) 1- 6

Antigens and myeloid maturation

I myeloblasts
II promyelocytes,
III myelocytes
IV metamyelocytes /bands
V neutrophils.



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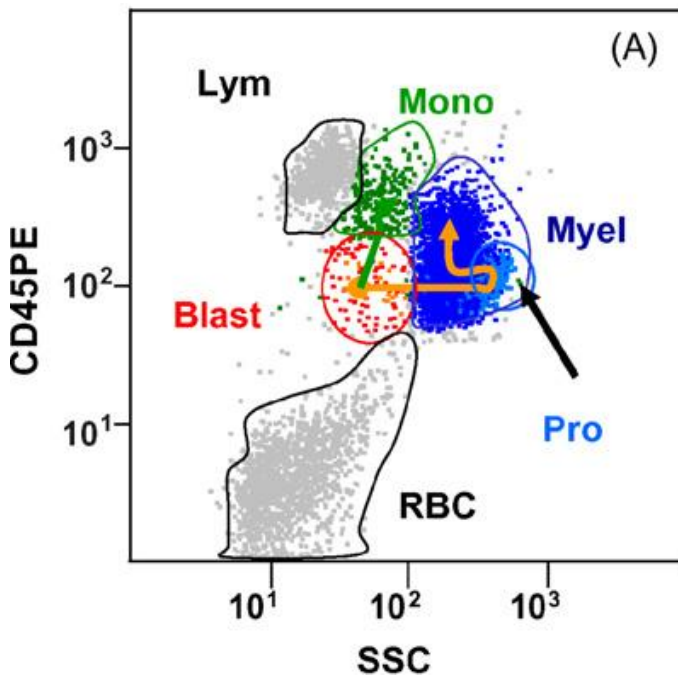
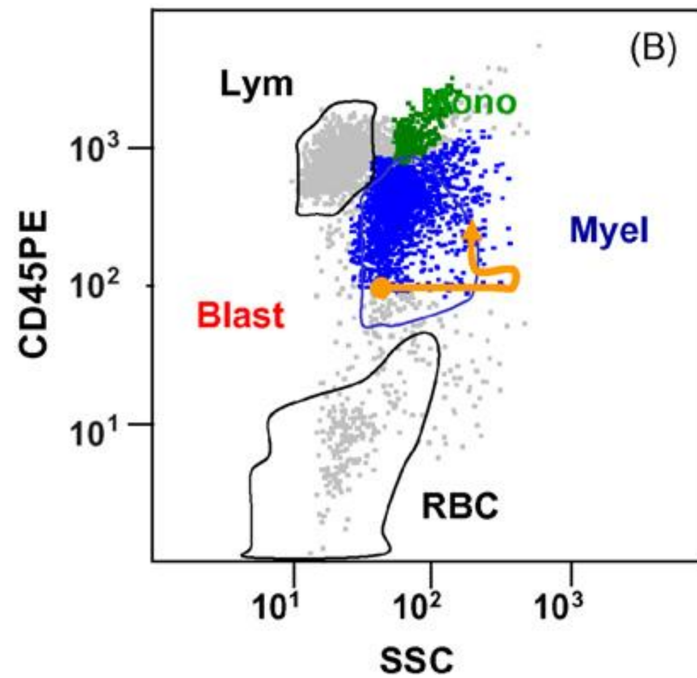
FC CD45 gating



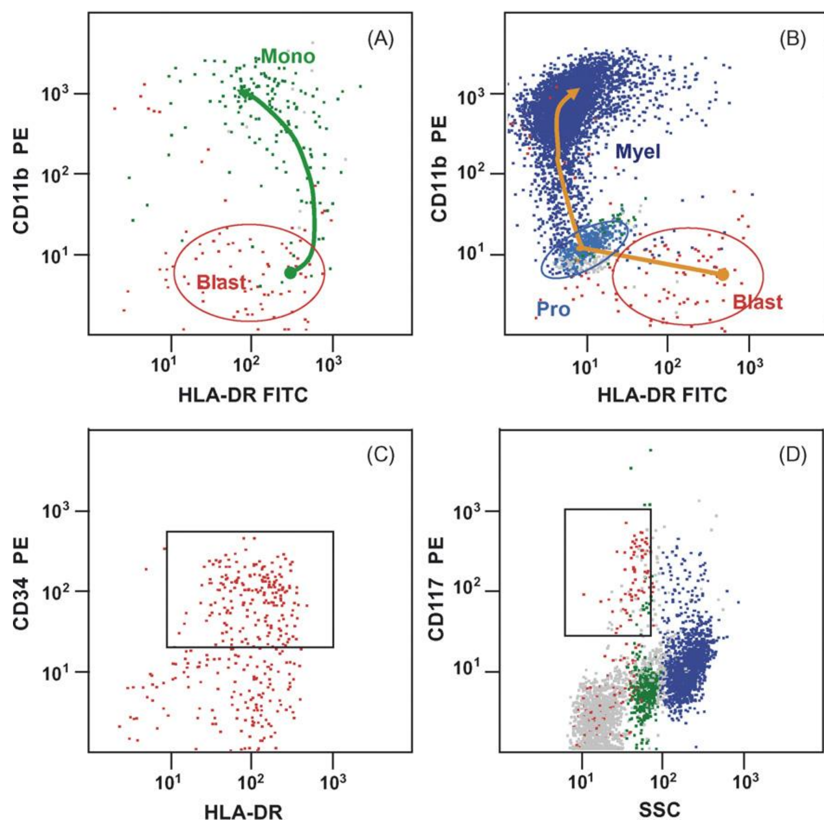
normal



mds

Leukemia Research 32 (2008) 5–17

Normal BM stained with HLA-DR-FITC, CD11b-PE and CD45 PerCP, HLA-DR-FITC, CD34-PE, CD45 PerCP, CD117



immature myeloblasts are red, monocytoid cells are green while maturing myeloid cells are blue.

- **(A)** Cells in the blast and monocyte regions, illustrate the maturation from blast to monocyte noted by the green arrow.
- **(B)** Cells within the blast and myeloid regions illustrate the maturation to neutrophils depicted by the gold arrow.
- **(C)** CD34+, HLA-DR positive cells are only found in the blast region.
- **(D)** CD117 positive cells are identified in both the blast and myeloid region. However, the expression of CD117 on blast cells can be identified based on low SSC.

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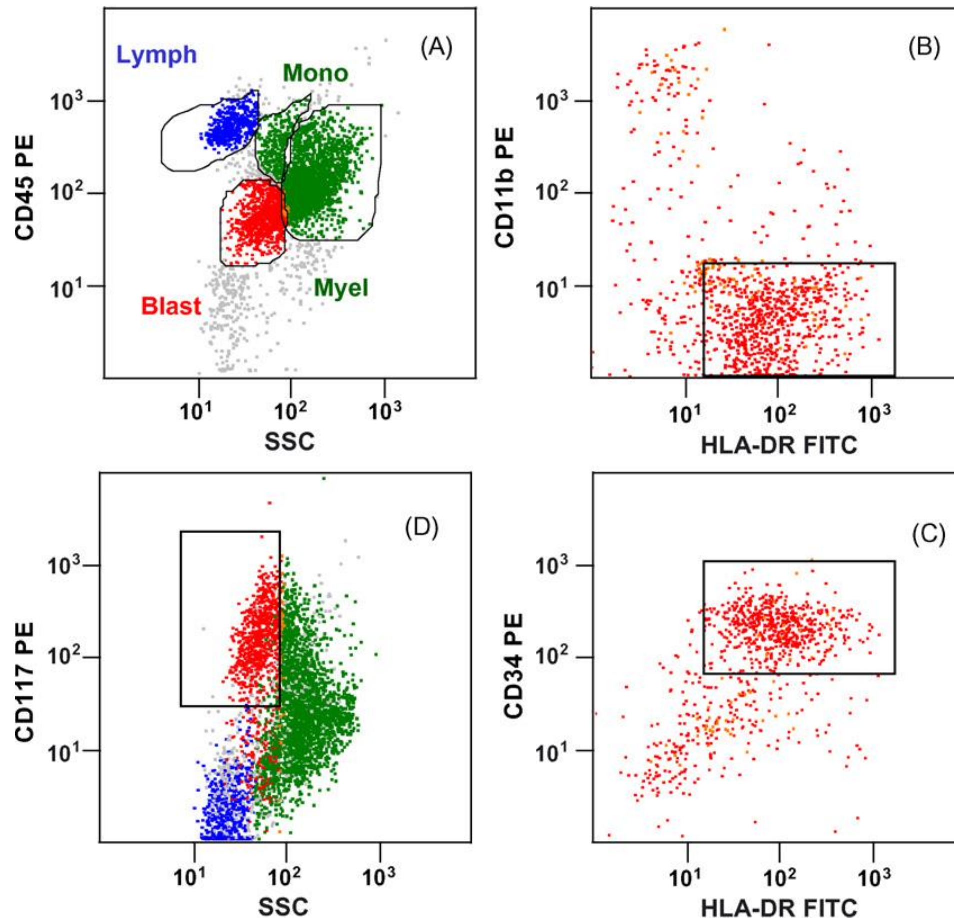
BM from a patient with MDS



(A) CD45 gating identifies the blasts, lymphocytes, maturing monocytes and myeloid cells.

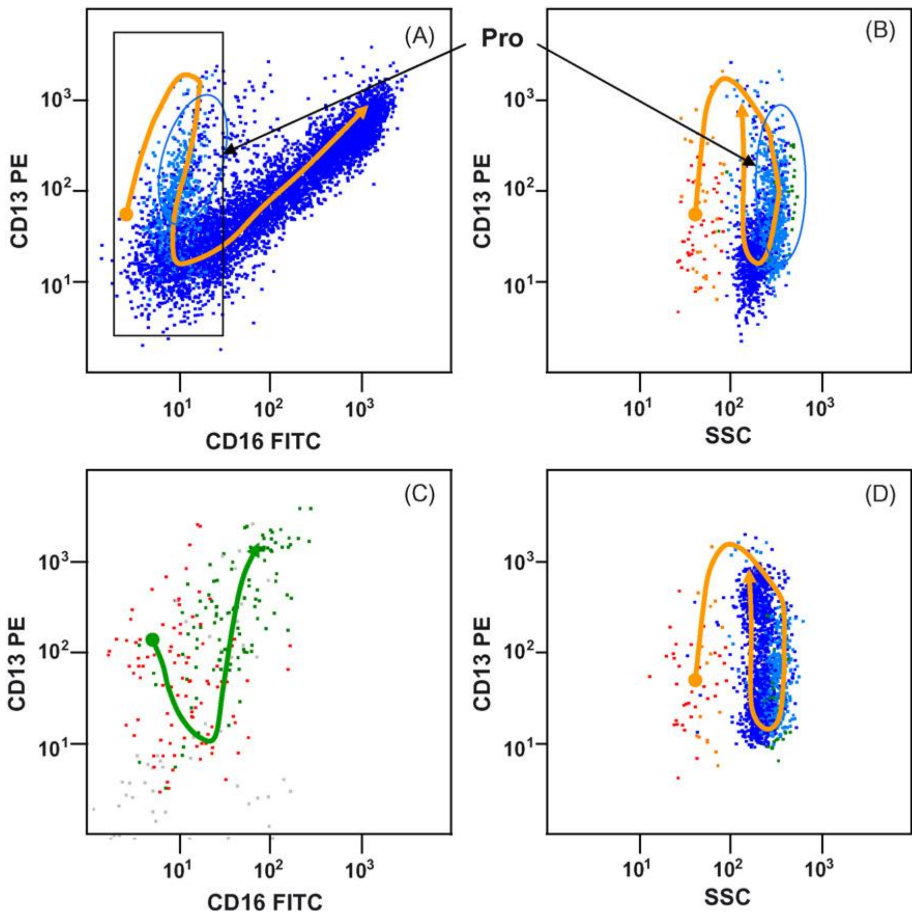
(B) By gating on the blasts the cells which express HLA-DR without expression of CD11b can be identified (13.9%).

(C) Cells expressing CD34 and HLA-DR within the blast region can also be quantified (13.3%).

(D) Cells expressing CD117 within the blast gate also give an identical proportion of myeloblasts (14.1%). The percents were calculated per non-erythroid cell (i.e., per CD45 positive event).

Leukemia Research 32 (2008) 5–17

Normal CD13, CD16, and SSC patterns.



Leukemia Research 32 (2008) 5–17

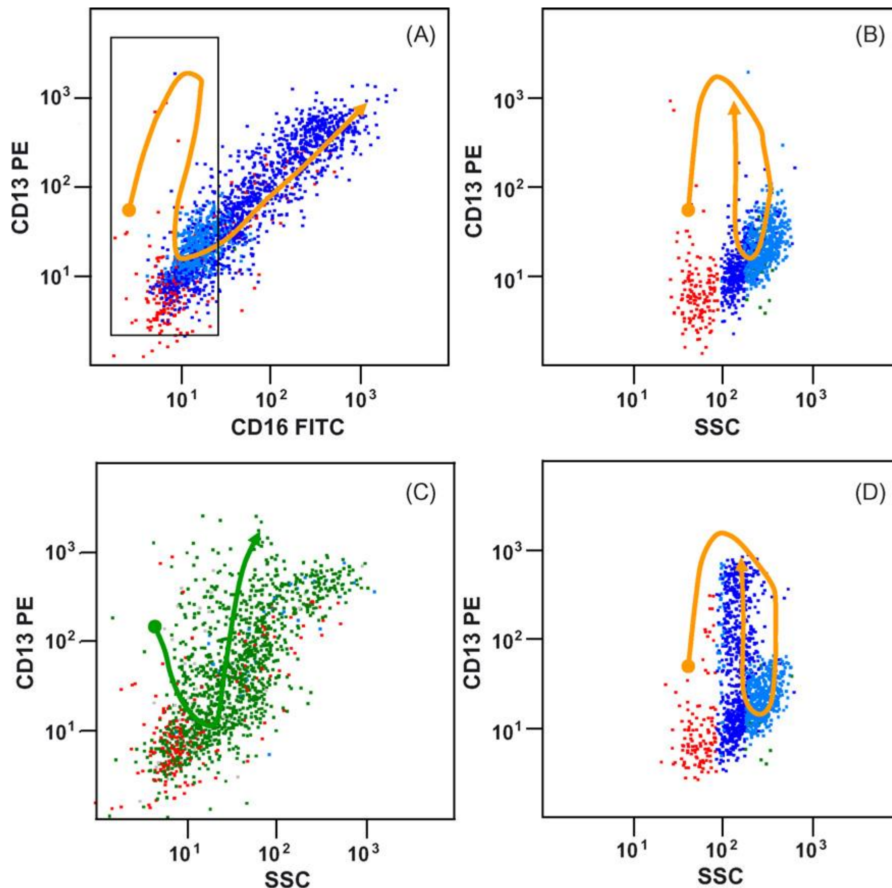
(A) Gold arrows follow the intensities of CD13 and CD16 paired antigens from myeloblasts to neutrophils in the classic sickle shape.

(B) The relationship between CD13 and SSC on early myeloid maturation is shown by gating on CD16 negative maturing myeloid cells in the CD13/CD16 histogram (A). Myeloblasts gain CD13 as they mature to promyelocytes, then precipitously lose CD13 as they become myelocytes.

(C) Myeloblasts lose CD13 to become monocytes (both gated by CD45/SSC, then re-express this molecule.

(D) The relationship between CD13 and SSC on all myeloid cells (including both CD16 negative and positive maturing myeloid cells) illustrates the limitation of displaying the data in only 2 dimensions.

CD13 and CD16



Leukemia Research 32 (2008) 5–17

Abnormal patterns of maturation for CD13/CD16 in the MDS marrow. The gold lines illustrate the maturation expected for normal myeloid cells.

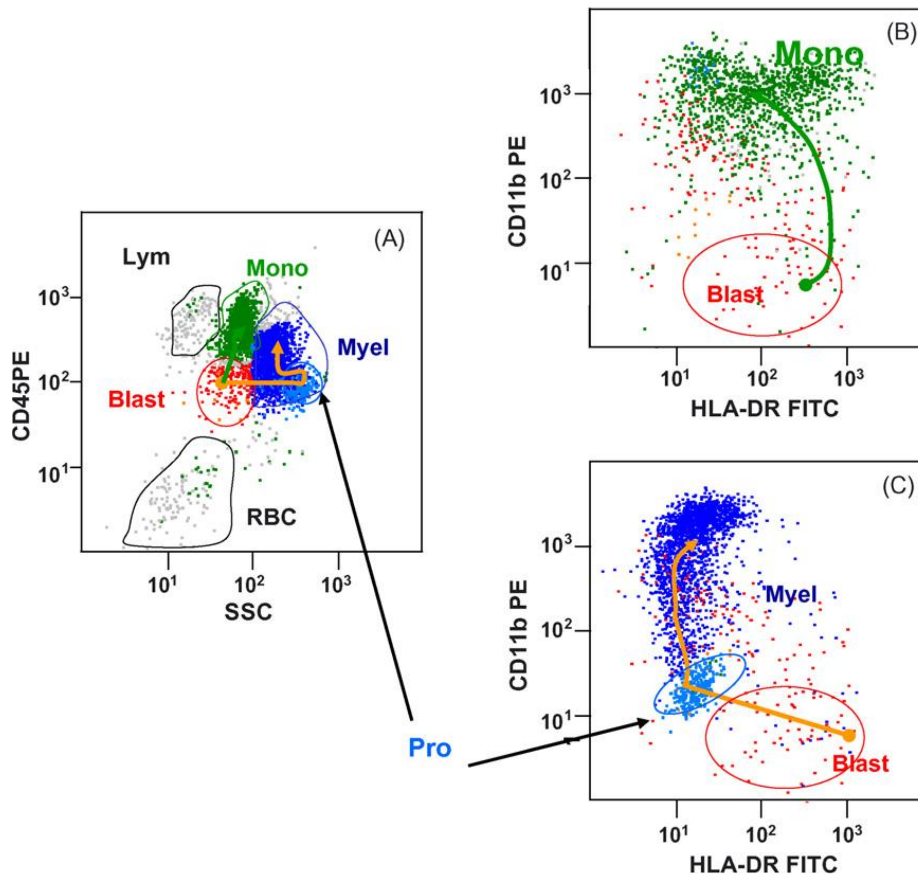
(A) CD13 vs. CD16 expression on the myeloblasts (red) and maturing myeloid cells (blue) demonstrate a lack of CD13 on the most immature myeloid cells.

(B) Lack of expression of CD13 on myeloblasts and promyelocytes is also demonstrated by gating on CD16 negative cells.

(C) Maturation of myeloblasts to monocytes appears relatively normal.

(D) Relationship between CD13 and SSC on all developing myeloid cells appears normal in this projection because the lack of CD13 on the immature cells is hidden by the more mature cells.

HLA-DR and CD11b: MDS patients



Marrow aspirate from MDS patient.

(A) Different lineages identified by CD45 vs. SSC gating.

(B) Monocytes are relatively increased, with a significant proportion lacking expression of HLA-DR.

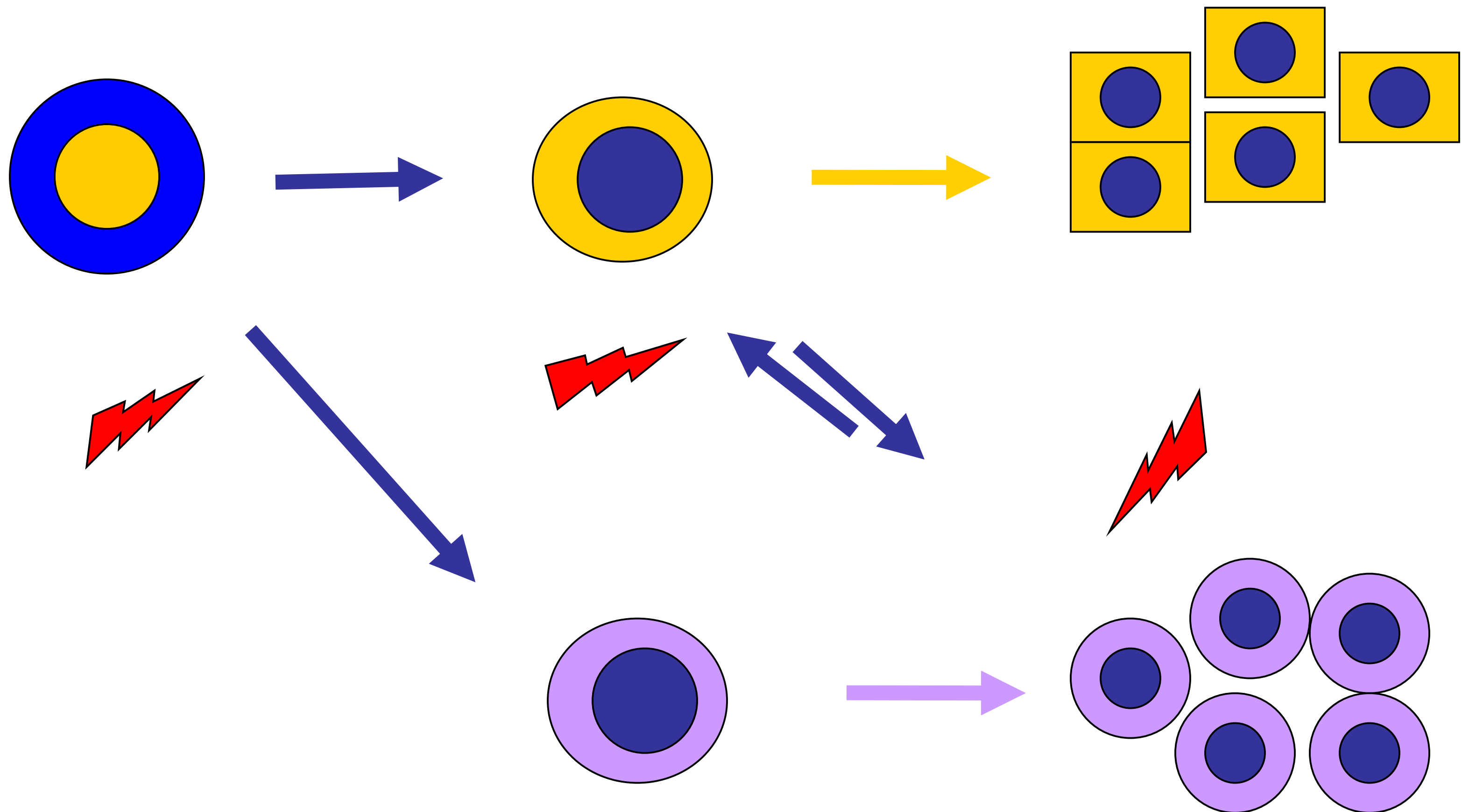
(C) Normal maturation of maturing myeloid cells based on CD11b and HLA-DR.

Leukemia Research 32 (2008) 5–17

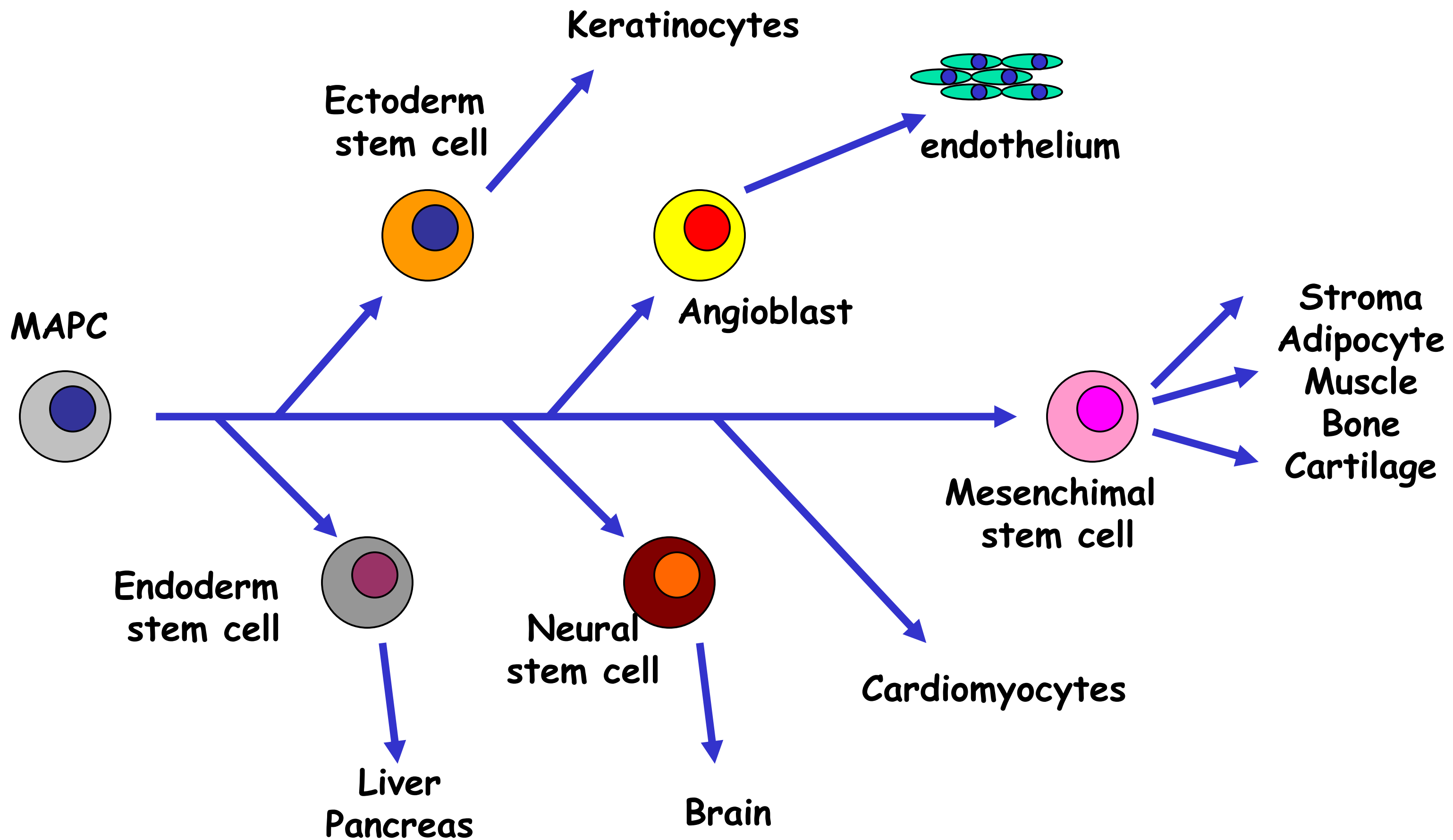
Plasticità delle cellule staminali

- Capacità delle cellule staminali di uno specifico tessuto od organo
  1. di acquisire caratteristiche fenotipiche e funzionali di un differente tessuto od organo
  2. di crossare in alcuni casi verso linee somatiche di diversa derivazione embrionale

Stem cell plasticity

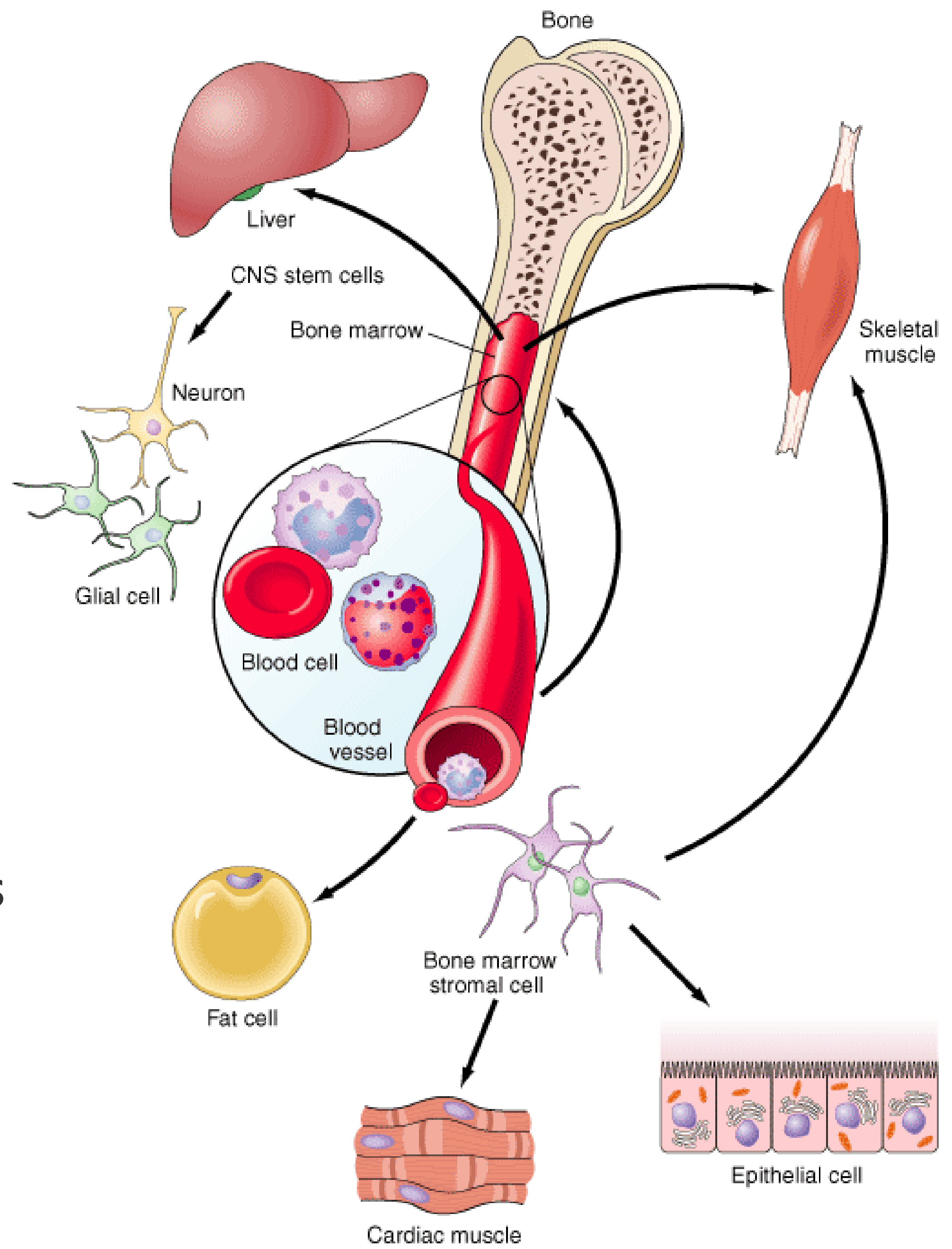


Multipotent adult progenitor cell (MAPC)



Moore JCI 2002;109:313

Plasticity of adult stem cells



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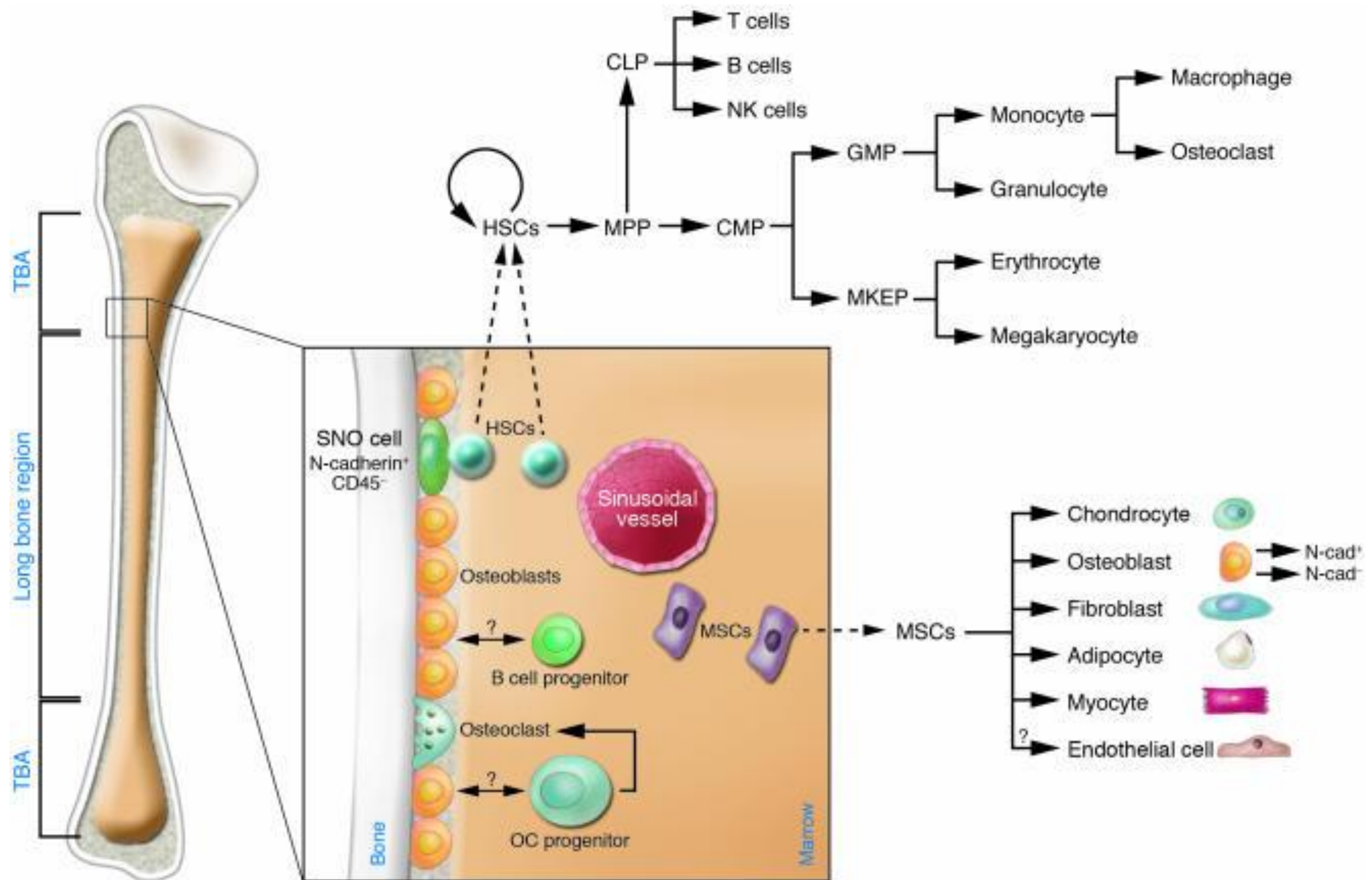
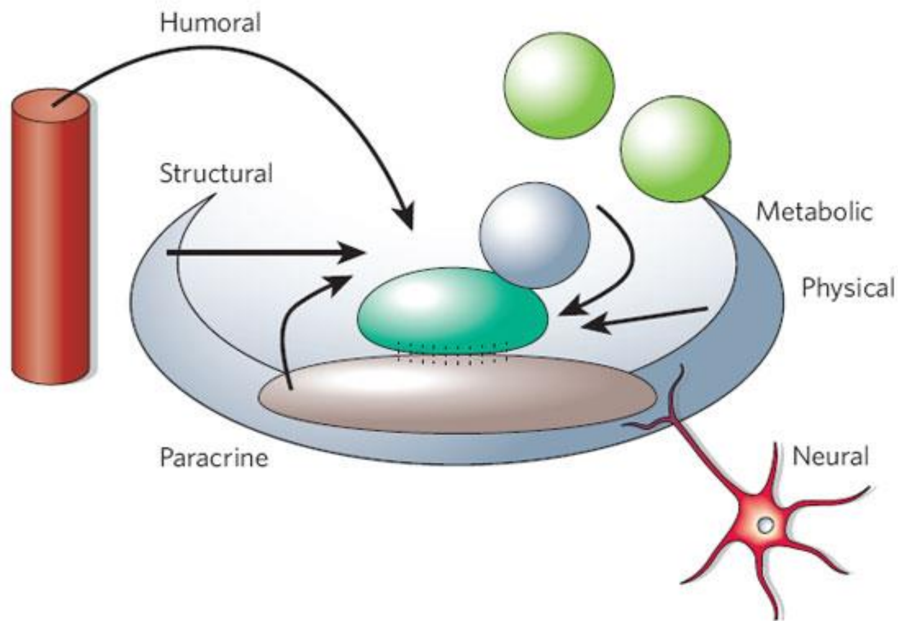
Stem cell niche

Hematopoiesis of bone cells and marrow stromal cells



J Clin Invest. 116:1195–1201;2006

Inputs feeding back on stem-cell function in the niche



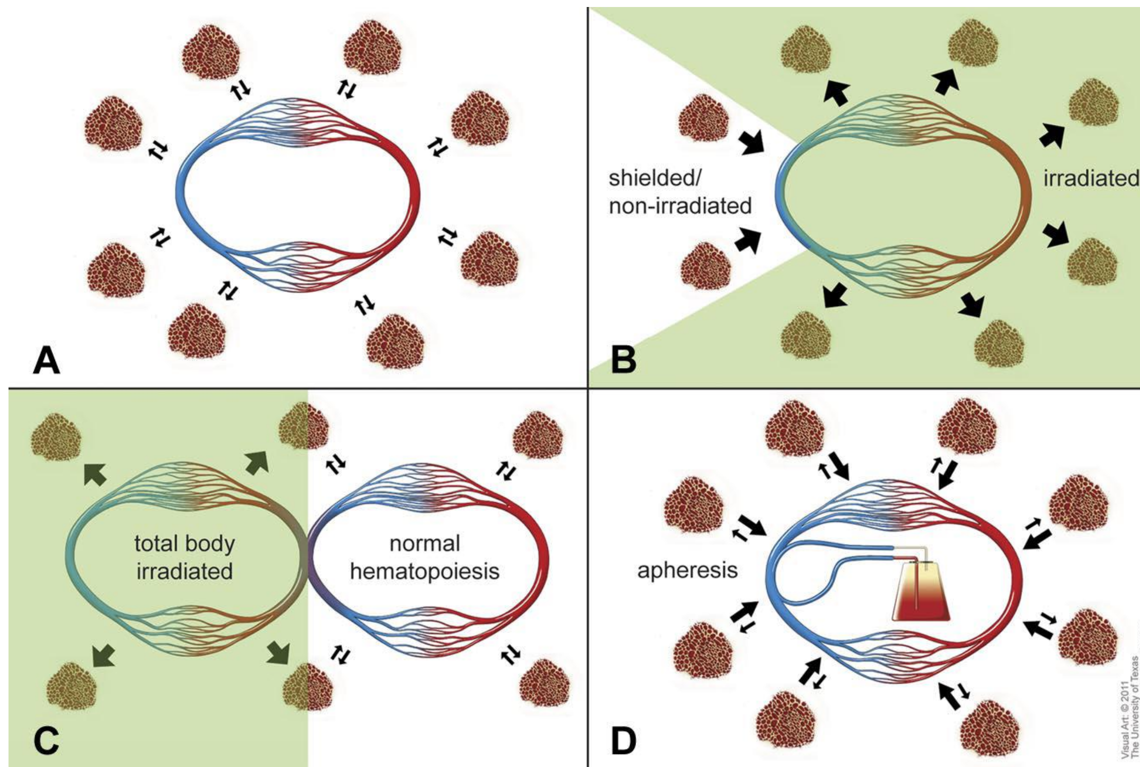
- Elements of the environment that participate in regulating the system of a SC in its tissue state include
  1. the constraints of the architectural space,
  2. physical engagement of the cell membrane with tethering molecules on neighbouring cells or surfaces,
  3. signalling interactions at the interface of stem cells and niche or descendent cells,
  4. paracrine and endocrine signals from local or distant sources,
  5. neural input and
  6. metabolic products of tissue activity

David T. Scadden, Nature 2006;441, 1075-79

Trafficking of primitive hematopoietic cells

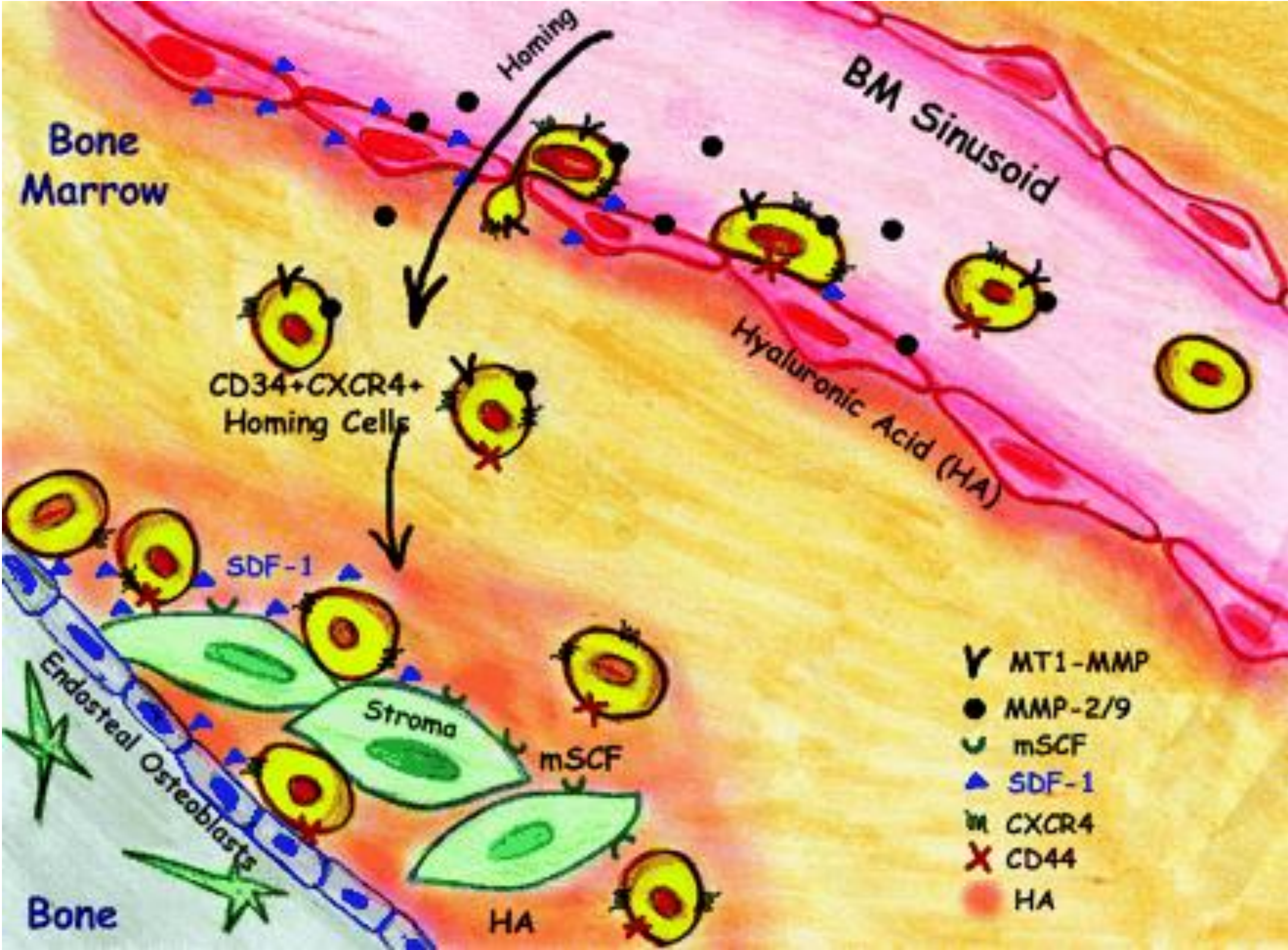
- Trafficking of HSCs can be divided into the components of homing, retention and engraftment.
  - **Homing** describes the tendency of cells to arrive at a particular environment,
  - **Retention** is their ability to remain in such an environment after arrival.
  - **Engraftment** reflects the ability of cells to divide and form functional progeny in a given microenvironment.

Stem cell migration between BM and PB.

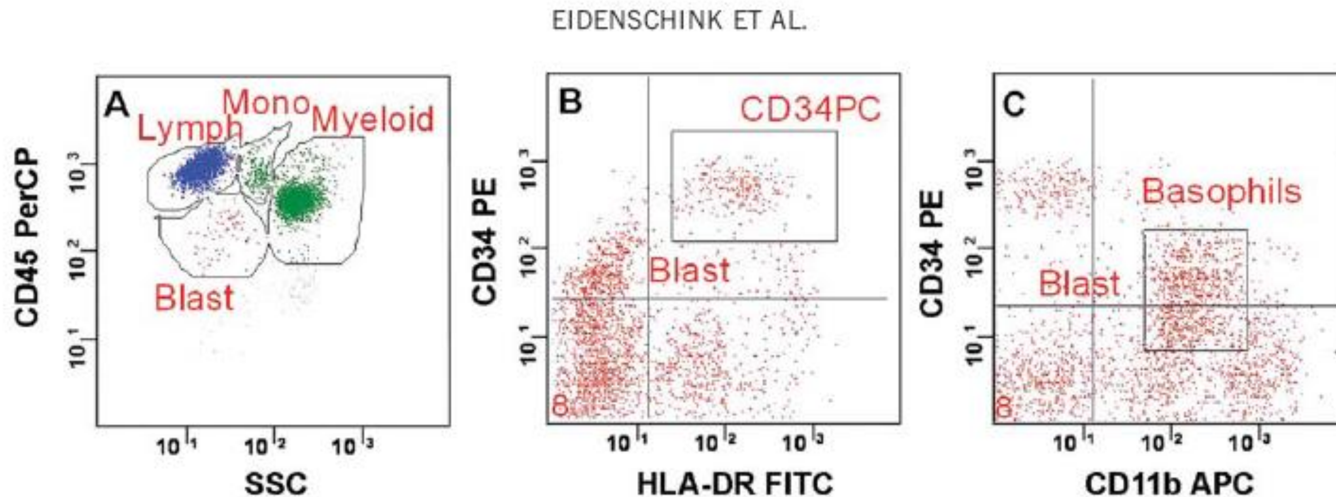


Blood. 2011;117(24):6411-6416

Stem cell homing to the endosteum



Basal Levels of CD34 Positive Cells in Peripheral Blood



Gating strategy used to identify CD34 positive progenitor cells. Progenitor Cells are identified in the Blast gate by CD45/SSC (A). Cells that are positive for CD34 and HLA-DR (B) excluding the cells that are positive for CD11b (C) are used for the total CD34 count in a specimen.

Cytometry Part B (Clinical Cytometry) 82B:18–25 (2012)

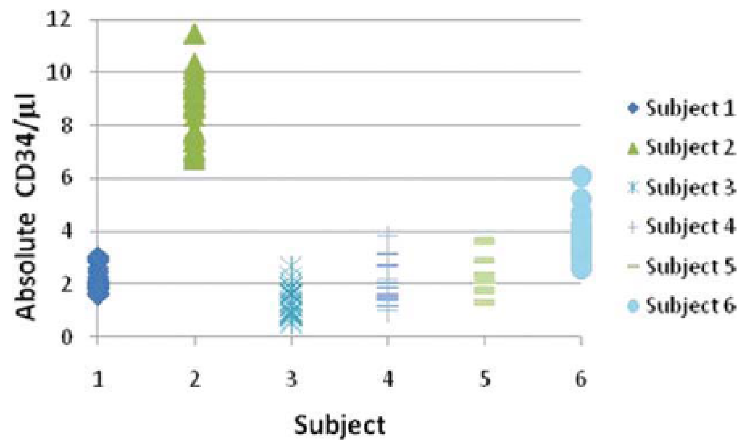


Fig. 2. Absolute CD34/µl values for individual subjects assayed 26 times in the 6 day study. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

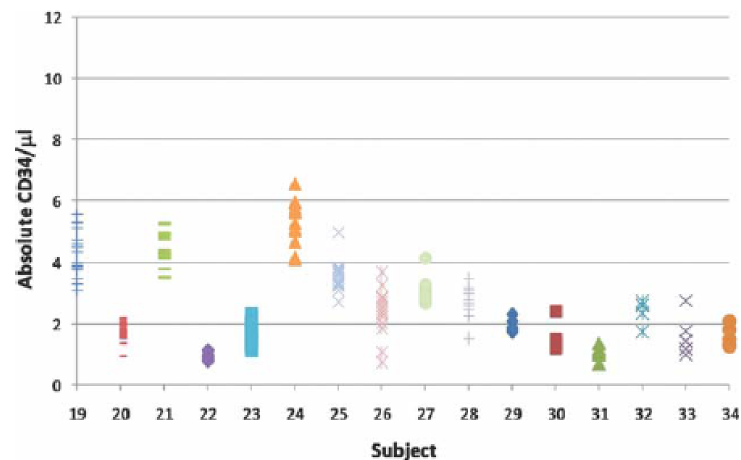


Fig. 4. Absolute CD34/µl values for individual subjects assayed 14 separate times during a 10 week study (subjects 19–28) and absolute CD34/µl values for individual subjects assayed six different times over an 18 month period (subjects 29–34).

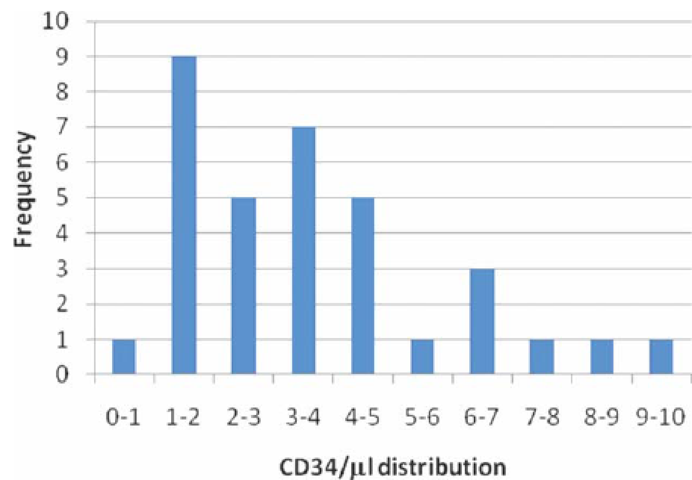


Fig. 5. A Normal range distribution of CD34 µl absolute amounts for subjects in all three studies.

Cytometry Part B (Clinical Cytometry) 82B:18–25 (2012)

- Mobilization is the iatrogenic augmentation of HSC recirculation that occurs at low levels in steady state.

***Blood*. 2011;118(17):4530-4540**

G-CSF–based mobilization protocols

- G-CSF w or w/o myelosuppressive chemotherapy is been the most commonly used mobilization protocols.
- When used alone G-CSF is given at 10 μg/kg/day sc with apheresis beginning on the 5th day until the yield target is reached.
- Combining G-CSF with chemotherapy achieves the twin aims of mobilization and antitumor activity and has been shown to result in a higher CD34 cell yield than G-CSF alone.

***Blood*. 2011;118(17):4530-4540**

G-CSF–based mobilization protocols

- Regimens such as CHOP, high-dose cytarabine, and cisplatin or CTX 1-5 g/m2 have been used with G-CSF that starts 1-3 days after CTX.
- Apheresis is usually started when PB CD34 cells are > 20 μl.
- The benefit of higher mobilization yield with higher doses of chemotherapy needs to be balanced against more red cell and platelet transfusions, and, frequently, hospitalization for febrile neutropenia, and it is more justified when significant antitumor effects exist.
- G-CSF 5 g/kg/day is used for combined G-CSF/chemotherapy mobilization.

Blood. 2011;118(17):4530-4540

Mobilization and growth factors

- The pegylated form of G-CSF (**pegfilgrastim**) showed similar kinetics of mobilization as filgrastim.
- **Lenograstim** is a glycosylated form of G-CSF, whereas filgrastim is nonglycosylated.
- **Biosimilar G-CSF**.

Blood. 2011;118(17):4530-4540

- HSC mobilization occurs during the recovery phase after CTX.
- A marked reduction of
  - osteoblasts,
  - osteoblast-supportive endosteal macrophages (osteomacs)
  - CXCL12 expression results in the disruption of the CXCL12 gradient.
- The synergism with G-CSF may result from
  - a more complete down-regulation of CXCL12
  - enhanced number of BM HSCs because of the induction of HSC self-renewal in response to chemotherapy.

***Blood*. 2011;118(17):4530-4540**

- Plerixafor a **CXCR4 antagonist**, reduces the binding and chemotaxis of HSCs to the BM stroma.
- It is used at 0.24 mg/Kg subcutaneously the evening before the scheduled apheresis because it generates peak CD34 levels 6-9 hours after administration.
- It synergizes with G-CSF and chemotherapy.

***Blood*. 2011;118(17):4530-4540**

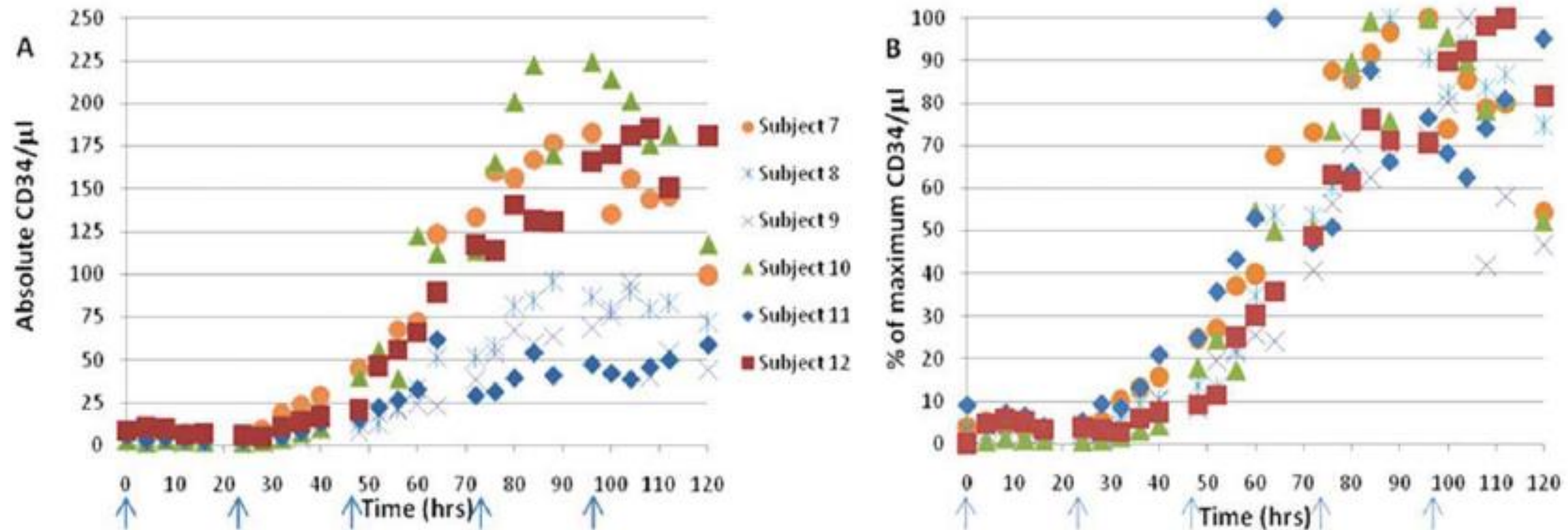
CXCR4 antagonists: Plerixafor

- The chemotactic interaction between SDF-1 and its receptor CXCR4 is critical to HSC retention within the BM.
- CXCR4 blockade effectively mobilizes HSCs.
- Alone, plerixafor rapidly mobilizes HSCs, peaking at 6-9 hours.
  - Plerixafor directly binds to CXCR4 and inhibits chemotactic signaling in cells.
  - Plerixafor also promotes the release of SDF-1 from CXCR4 osteoblastic and endothelial cells into the circulation.
  - It therefore seems to counter the chemotactic SDF-1 gradient between the BM stroma and the circulation, favoring the egress of HSCs from the BM.

- Because of their different and complementary mechanisms of action, plerixafor shows marked synergism with G-CSF (30% of patients failing G-CSF mobilization still fail to mobilize).

***Blood*. 2011;118(17):4530-4540**

Absolute CD34 counts in response to G-CSF treatment



Arrows indicate time points when G-CSF was administered.

Cytometry Part B (Clinical Cytometry) 82B:18–25 (2012)

Risk factors, mechanisms, and strategies to optimize collection in predicted poor mobilizer patients

Risk factor	Postulated mechanism	Mobilization strategy
Low steady-state platelet counts and PB $CD34^+$ level	Reflects overall HSC reserve	Regimen promoting HSC proliferation, eg, SCF, cyclophosphamide
Low steady-state TNF-α level	May reflect niche dysfunction, including the macrophage response to G-CSF	Regimen bypassing the macrophage-dependent pathways, eg, plerixafor-containing regimen
Increasing age	Reduced HSC reserve because of the following:	Regimen promoting HSC proliferation, eg, SCF, cyclophosphamide
	Age-related HSC senescence	Add risk-adapted plerixafor to augment niche response to G-CSF
	Age-related loss or dysfunction of HSC niche	Bisphosphonate treatment continued throughout collection PTH of interest in experimental models
	Age-related bone loss or altered bone metabolism	
Underlying disease	Paraneoplastic niche dysfunction	Aim to clear BM of disease before collection
	Loss of niche to mass effect of tumor	
Prior extensive radiotherapy (RT) to red marrow	Direct HSC toxicity	Rainy day collection before extensive RT when possible
	Toxicity to HSC niche	Risk-adapted plerixafor
		Regimen promoting HSC proliferation, eg, SCF, cyclophosphamide
Prior chemotherapy		
Melphalan	Direct HSC toxicity	Avoid melphalan until autologous cells collected
Fludarabine	Direct HSC toxicity, niche damage	Collect HSCs early, after < 4 cycles of fludarabine
Intensive chemotherapy (eg, hyper-CVAD)	Dose-dense cycles may cause niche damage, and HSCs forced into cell cycle may not engraft as well	Use SCF or preemptive risk-adapted plerixafor for fludarabine-exposed and heavily pretreated patients
Prior lenalidomide	Possible effects on HSC motility	Collect HSC early, after < 4 cycles of treatment
	Possible dysregulated HSC niche because of antiangiogenic effects	Temporarily withhold lenalidomide during collection.

Blood. 2011;118(17):4530-4540

constitutive poor mobilizers

- Up to 5% of healthy donors fail to mobilize with conventional regimens, and some patients with no obvious risk factors will also.
- The mechanistic understanding of these constitutive poor mobilizers” is complex and incomplete.

***Blood*. 2011;118(17):4530-4540**

G-CSF 10ug/kg D1-4
Or other conventional mobilizing regimen

Morning PBCD34 count on expected Day 1 apheresis

PBCD34 count above institutional threshold for commencing apheresis

Same day apheresis

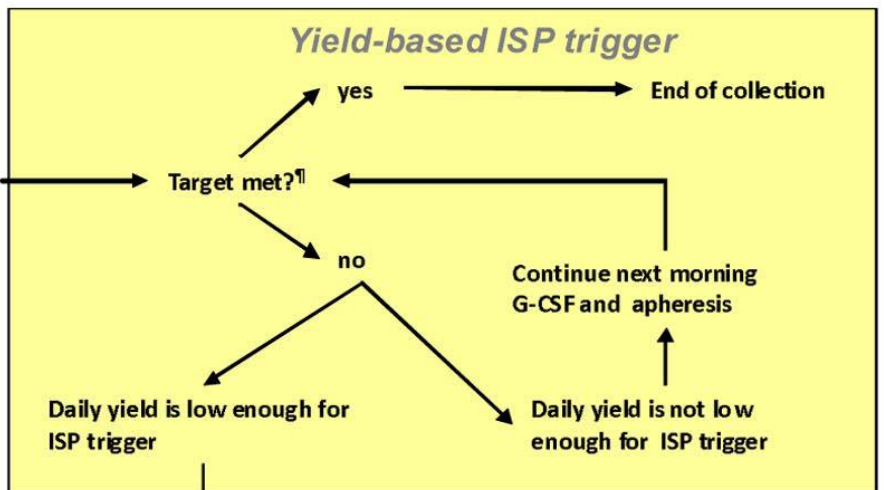
PBCD34 count inadequate to commence apheresis but not meeting ISP trigger

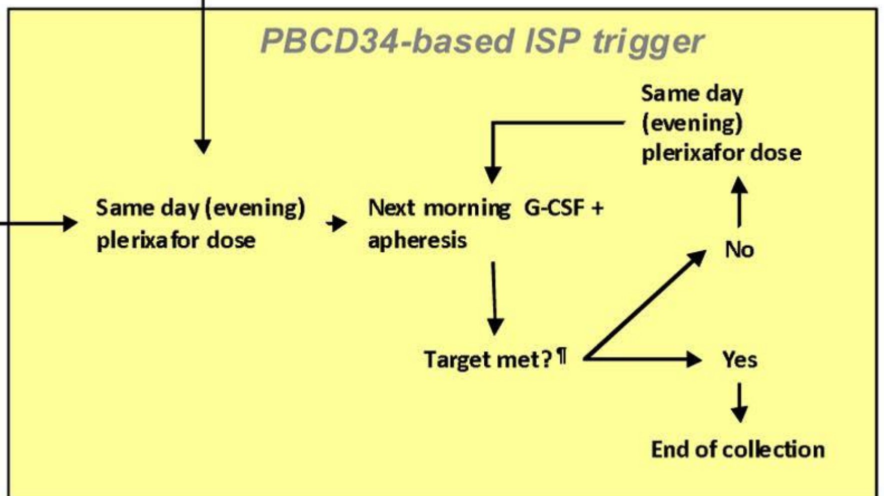
PBCD34 count following day (expected D2 apheresis):

PBCD34 count inadequate to commence apheresis

PBCD34 count adequate to commence apheresis

PBCD34 count meets ISP trigger*





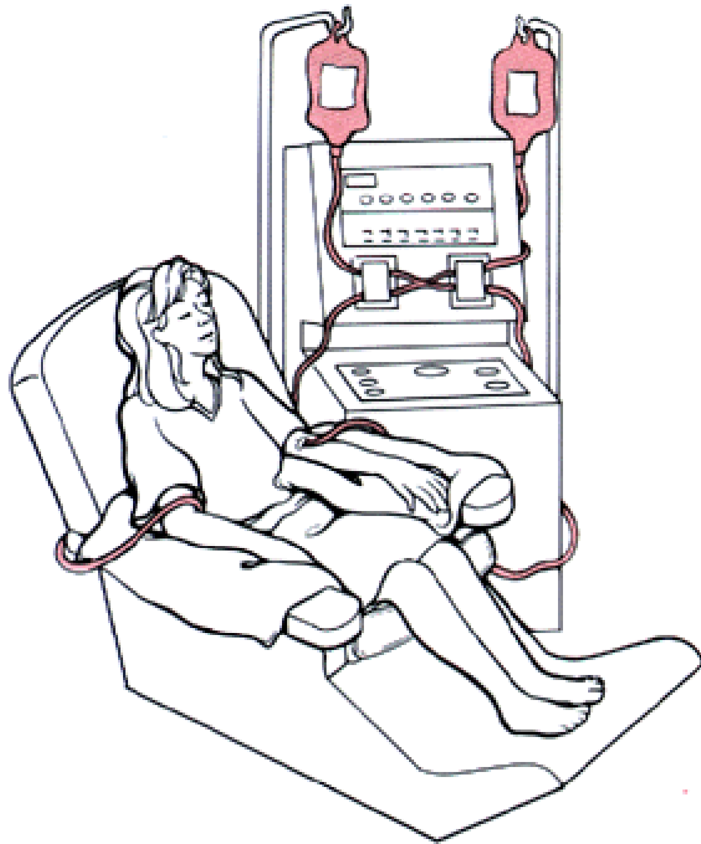
*Immediate Salvage plerixafor (ISP) trigger defined as any one of:

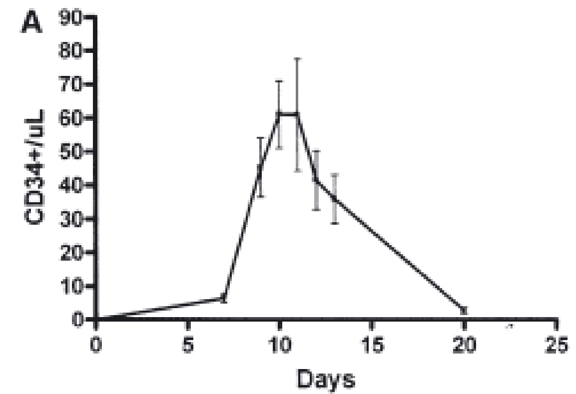
- PBCD34 on expected D1 apheresis below defined threshold for likely successful collection
- First apheresis yield below defined threshold for likely successful collection (Ideally based on institutional data using local collection techniques)
- PBCD34 inadequate for apheresis on both D1 and D2 apheresis

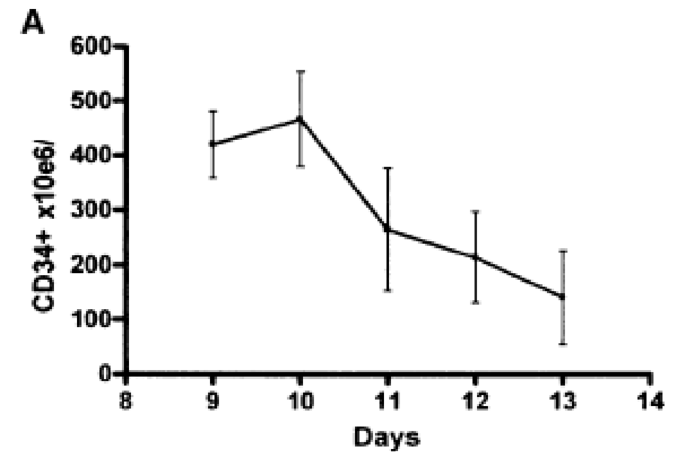
¶Parameters for mobilization futility need to be defined: eg if target not met after 4-5 days' apheresis, define as a failed mobilization and consider remobilization strategies

***Blood*. 2011;118(17):4530-4540**

CD34+ cell mobilization: chemio + G-CSF



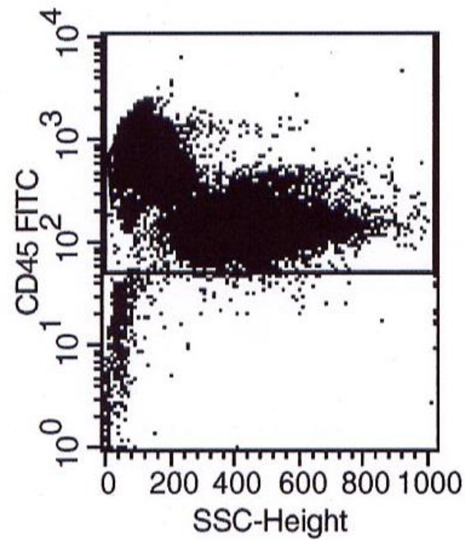


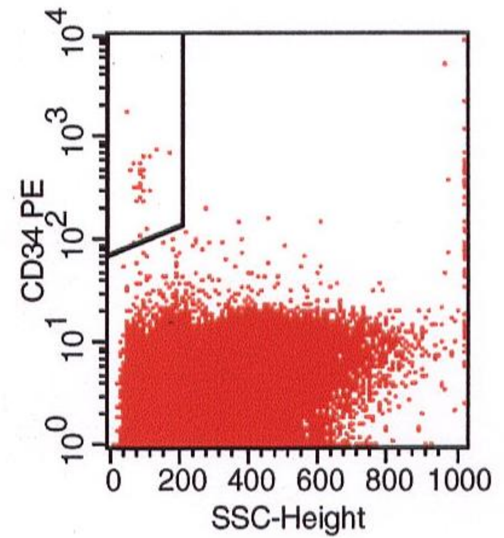


Mauro et al EJH 78 (374–380) a 2007

Conteggio cellule CD34+

- CD34
- CD45
- CD38
- CD117 (C-KIT)
- CD90 (Thy-1)
- HLA-DR
- Etc.





Enumeration of CD34+ Hematopoietic Stem and Progenitor Cells

- The Basic Protocol is a modified version of the protocol developed by Sutherland et al. (1994) that was subsequently incorporated into a set of clinical guidelines for the International Society for Hematotherapy and Graft Engineering (ISHAGE; Sutherland et al., 1996).
  - The counterstaining of CD34 by the CD45 MAb, allowing the identification of leukocytes (CD45+)
  - The verification of “true” CD34+ cells as being dim for CD45 fluorescence and having low side scatter (CD45dim, SSlow).
- CD34+ cell enumeration can be applied to the PB, apheresis products, bone marrow, and cord blood.

Enumeration of CD34+ Hematopoietic Stem and Progenitor Cells

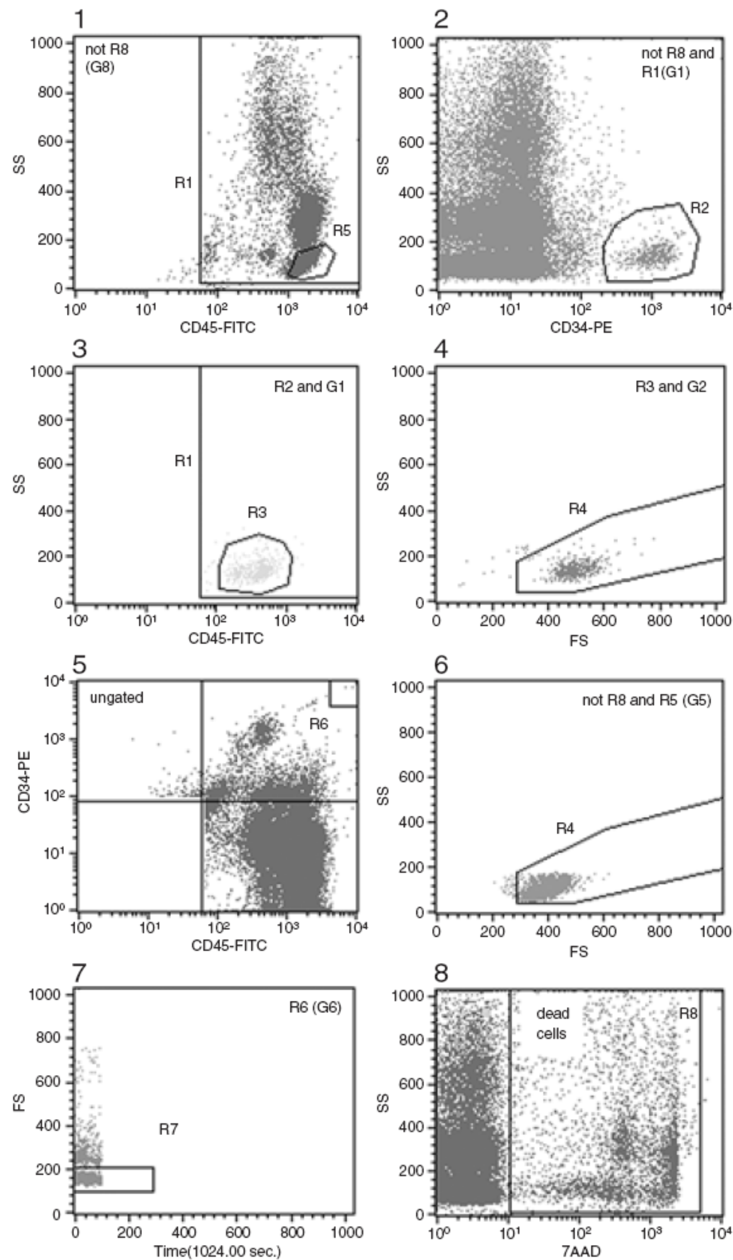
- The addition of predefined numbers of counting beads to the cell suspension yields the concentration of CD34+ cells per unit of sample volume (i.e., the absolute CD34+ cell count) from a single flow-cytometric assessment (**single-platform technique**).

- Dead cells can be excluded from analysis using the DNA stain 7-aminoactinomycin D (7-AAD).
  - 7-AAD is excited at 488 nm and has maximum emission at 660 nm.
  - The dye cannot be used in single-laser systems with other fluorochromes that emit at >600 nm, such as PerCP or PE-Cy5.

Table 6.4.1 Summary of Instrument-Specific Logical Gates Using Dead Cell Exclusion

Histogram	Beckman-Coulter XL	BD Biosciences FACS
1	Gated on J	G8 = not R8
2	Gated on AJ	G1 = (not R8) and R1
3	Gated on ABJ	G2 = R2 and G1
4	Gated on ABCJ	G3 = R3 and G2
5	Ungated	Ungated
6	Gated on EJ	G5 = (not R8) and R5
7	Gated on H	G6 = R6
8	Ungated	Ungated

Current Protocols in Cytometry **(2003) 6.4.1-6.4.23**



File: PBSC080499.008
Gate: G1
Total Events: 50253

Gate	Events	% Gated
G1	41643	100.00
G2	491	1.18
G3	472	1.13
G4	456	1.10
G5	10782	25.89
G6	4584	11.01
G7	4079	9.80
G8	41643	100.00
G9	37059	88.99

Stored PBSC
(24 hr old)

Abs viable
CD34+ cells

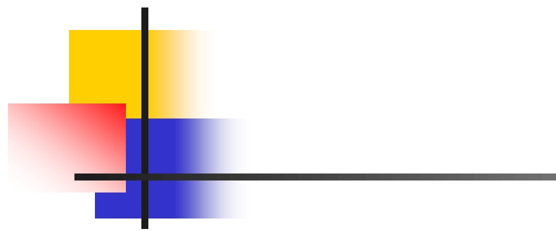
$= \frac{G4}{G7} \times \text{bead conc}$

$= \frac{456}{4079} \times 1046$

$= 117/\mu l$

Example of the Basic Protocol combined with optional dead-cell exclusion for a BD Biosciences FACScan flow cytometer. Data were acquired and analyzed using CellQuest version 3.3 software. Data are shown from apheresis Collection. Sample contained 11% dead cells (i.e., 7-AAD+ gated in R8) and 117 viable CD34+ cells/µl. In this example, 456 viable CD34+ cells were identified in gate 4 and 4079 singlet beads were enumerated in gate 7. The assayed bead concentration was 1046/µl.

Current Protocols in Cytometry **(2003) 6.4.1-6.4.23**



Enumeration of viable CD34+ cells according to ISHAGE protocol

Cytometry Part B (Clinical Cytometry) 82B:9–17 (2012)

External quality assessment

Table 3
Multivariate Analysis of Factors with Systematic Effects (bias) on Absolute CD34⁺ Cell Count Results

Variable (overall *P*)	Category	*P*-value
Gating strategy (<0.01)	Milan	<0.01
	Bender	n.s.
	ISHAGE	n.s.
	SIHON	n.s.
	ProCOUNT™	n.s.
	Stem-KIT™	<0.01
Laboratory (<0.01)	n.a.	
Labeling CD34 mAb (0.01)	FITC	<0.01
	PE	n.s.
Flow cytometer (0.02)	FACScan™	n.s.
	FACScalibur™	0.03
	FACStar™	n.s.
	Epics XL™	n.s.
	Cytoron™	0.04
Sample preparation (0.03)	LW	n.s.
	LNW	0.05
	NLNW	n.s.
	MNC	n.s.

Only categorical variables with significant effects are shown. n.a., not applicable; n.s., not significant.

Table 4
Multivariate Analysis of Factors Influencing Variability of Absolute CD34⁺ Cell Count Results

Variable (overall *P*)	Category	*P*-value
Platform methodology (<0.01)	Single platform	n.s.
	Dual platform	0.01
Laboratory (<0.01)	n.a.	

Only categorical variables with significant effects are shown. n.a., not applicable; n.s., not significant.

the variability in results of CD34+ cell enumeration has declined with time; in particular, after a practical workshop in which participants were trained to use the "single platform ISHAGE protocol."

Between-laboratory variation in CD34+ cell enumeration can be reduced by standardization of methodologies between centres

Cytometry Part B (Clinical Cytometry) 72B:178–188 (2007)

Recovery of viable CD34+ cells from cryopreserved hemopoietic progenitor cell products

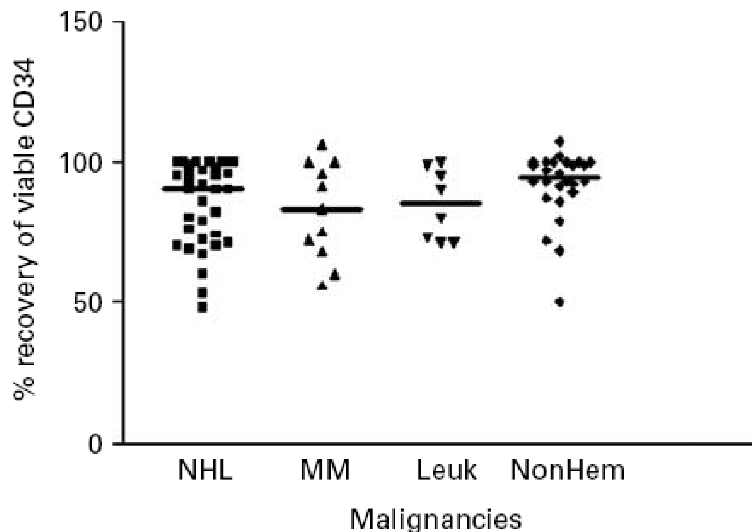


Figure 4 Comparison of the median recovery of viable $CD34^+$ cells within four groups of malignancies. NHL: median recovery of viable $CD34^+$ cells 90% (range 48–100%, $n = 34$). MM: 83% (range 56–106%, $n = 11$). Acute leukaemias (Leuk): 85% (range 71–100%, $n = 7$). Non-hematological malignancies (Non-Hem): 94.5% (range 50–107%, $n = 18$). There was no significant difference in the recovery of viable $CD34^+$ cells for all groups $P > 0.17$.

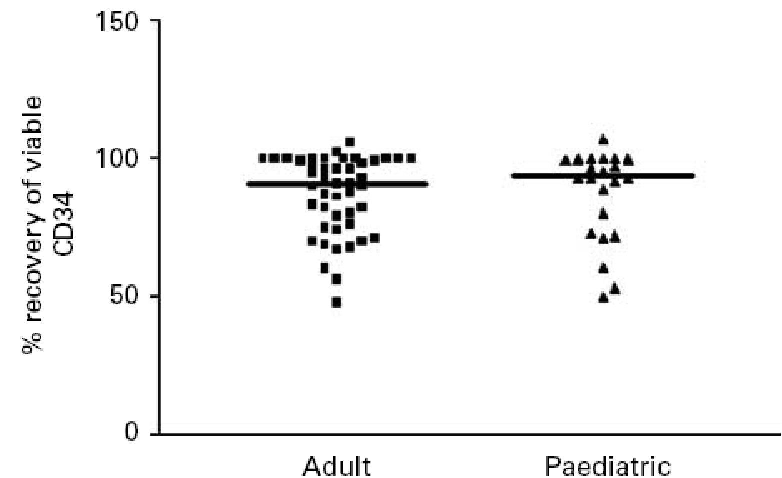


Figure 5 Comparison of the median recovery of viable $CD34^+$ cells between adult and pediatric HPC products. Adult HPC recovered a median 91% viable $CD34^+$ cells (range 48–106%, $n = 51$), compared to 94% (range 50–107%, $n = 28$) for pediatric HPC products, $P = 1.06$).

Bone Marrow Transplantation (2005) 36, 199–204.

TRAPIANTO DI CELLULE STAMINALI EMOPOIETICHE (I)

- Definizione
  - Prelievo di cellule staminali da midollo, sangue periferico o cordonale, ad un donatore od al paziente, e reinfusione delle stesse dopo trattamento radio-chemioterapico mieloablativo

Nobel prize for stem-cell transplantation

Photograph of the Seattle team after announcement of the Nobel Prize in Medicine, which was awarded to E. D. Thomas in 1990. From left to right: Paul Neiman, Alexander Fefer, E. Donnall Thomas, C. Dean Buckner and Rainer Storb.

***Marie-Térèse Little & Rainer Storb Nature Reviews Cancer* 2, 231-238 (2002)**

TRAPIANTO DI CELLULE STAMINALI EMOPOIETICHE (II)

- Sorgente delle cellule staminali emopoietiche
  - Midollo osseo:
  - Sangue periferico:
  - Sangue cordonale: al momento del parto

haematopoietic stem-cell transplantation

Autologous stem cell transplant

patient

Stem cell mobilization

chemotherapy

Stem cell collection

Stem cell infusion

Chemotherapy
radiotherapy

patient

chemotherapy

Chemotherapy
radiotherapy

donor

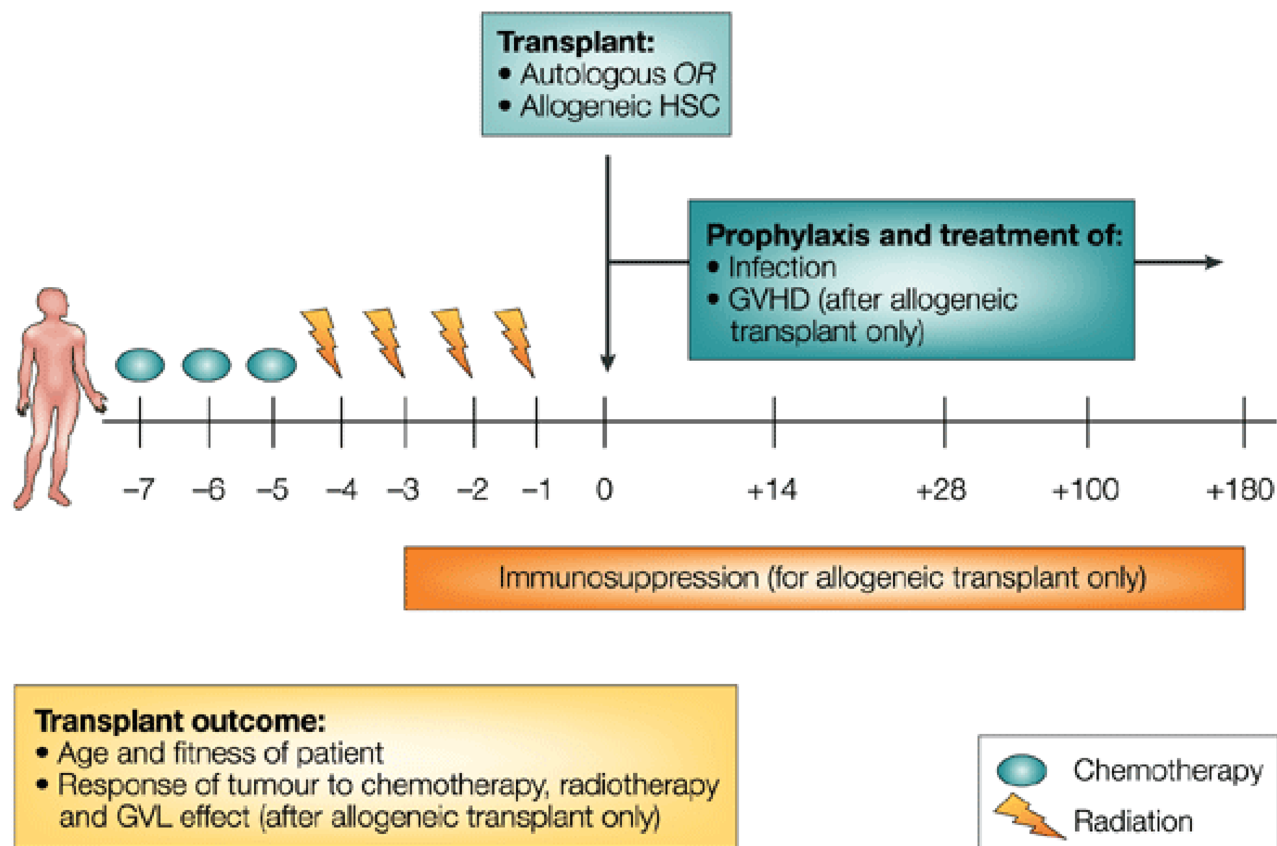
Stem cell mobilization

Stem cell collection

Stem cell infusion

allogeneic stem cell transplant

Treatment regimen and factors influencing the outcome of haematopoietic stem-cell transplantation.



Juliet N. Barker & John E. Wagner Nature Reviews Cancer **3**, 526-532 (2003);

Diseases Commonly Treated with Hematopoietic Stem-Cell Transplantation

Copelan EA. NEJM 354;1813-26:2006

Table 1. Diseases Commonly Treated with Hematopoietic Stem-Cell Transplantation.

Autologous transplantation*

Cancers
- Multiple myeloma
- Non-Hodgkin's lymphoma
- Hodgkin's disease
- Acute myeloid leukemia
- Neuroblastoma
- Ovarian cancer
- Germ-cell tumors

Other diseases
- Autoimmune disorders
- Amyloidosis

Allogeneic transplantation†

Cancers
- Acute myeloid leukemia
- Acute lymphoblastic leukemia
- Chronic myeloid leukemia
- Myelodysplastic syndromes
- Myeloproliferative disorders
- Non-Hodgkin's lymphoma
- Hodgkin's disease
- Chronic lymphocytic leukemia
- Multiple myeloma
- Juvenile chronic myeloid leukemia

Other diseases
- Aplastic anemia
- Paroxysmal nocturnal hemoglobinuria
- Fanconi's anemia
- Blackfan–Diamond anemia
- Thalassemia major
- Sickle cell anemia
- Severe combined immunodeficiency
- Wiskott–Aldrich syndrome
- Inborn errors of metabolism

* More than 30,000 autologous transplantations are performed annually worldwide, two thirds for multiple myeloma or non-Hodgkin's lymphoma.

† More than 15,000 allogeneic transplantations are performed annually worldwide, nearly half for acute leukemias. The vast majority are performed to treat lymphoid and hematologic cancers.

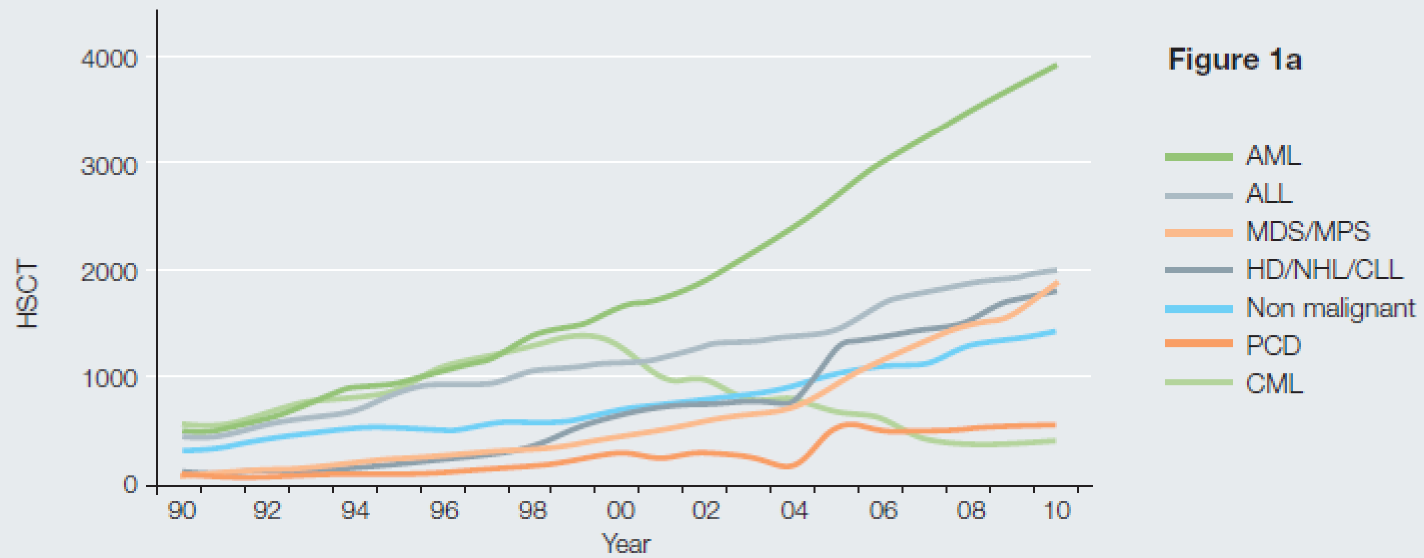
Outcomes of Hematopoietic Stem-Cell Transplantation in Selected Diseases

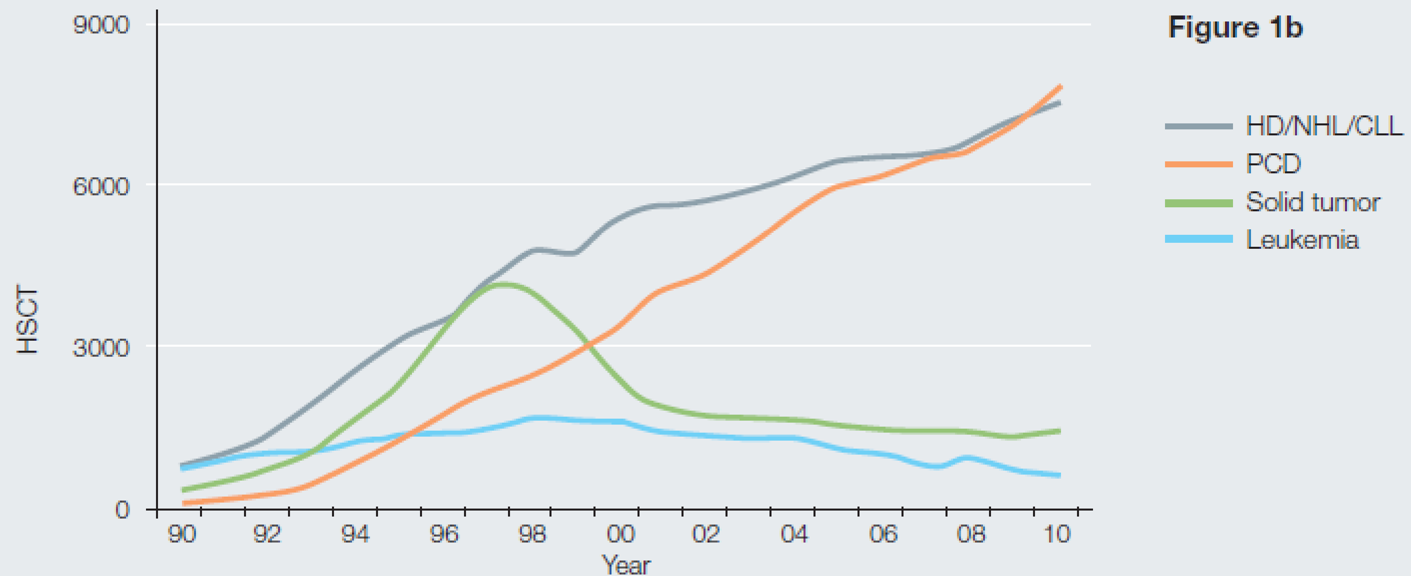
Table 2. Outcomes of Hematopoietic Stem-Cell Transplantation in Selected Diseases.*

Disease	Most Common Preparative Regimen	100-Day Mortality Rate	5-Yr Event-free Survival
		percent	
Autologous transplantation			
Diffuse large-cell non-Hodgkin's lymphoma	Carmustine, cyclophosphamide, and etoposide		
First chemotherapy-sensitive relapse		3–5	45–50
Second chemotherapy-sensitive relapse		5–8	30–35
Refractory		10–20	5–10
Allogeneic transplantation†			
Acute myeloid leukemia	Cyclophosphamide and total-body irradiation		
First complete remission		7–10	55–65
Second complete remission		10–20	30–40
Refractory		30–40	15–20
Chronic myeloid leukemia	Busulfan and cyclophosphamide		
Chronic phase <1 yr after diagnosis		5–10	70–80
Chronic phase >1 yr after diagnosis		10–15	50–60
Accelerated		15–20	30–35
Blastic		35–45	5–15

* The estimated ranges of data are based on recent reports.
† This category refers to the transplantation of hematopoietic stem cells from an HLA-identical sibling donor.

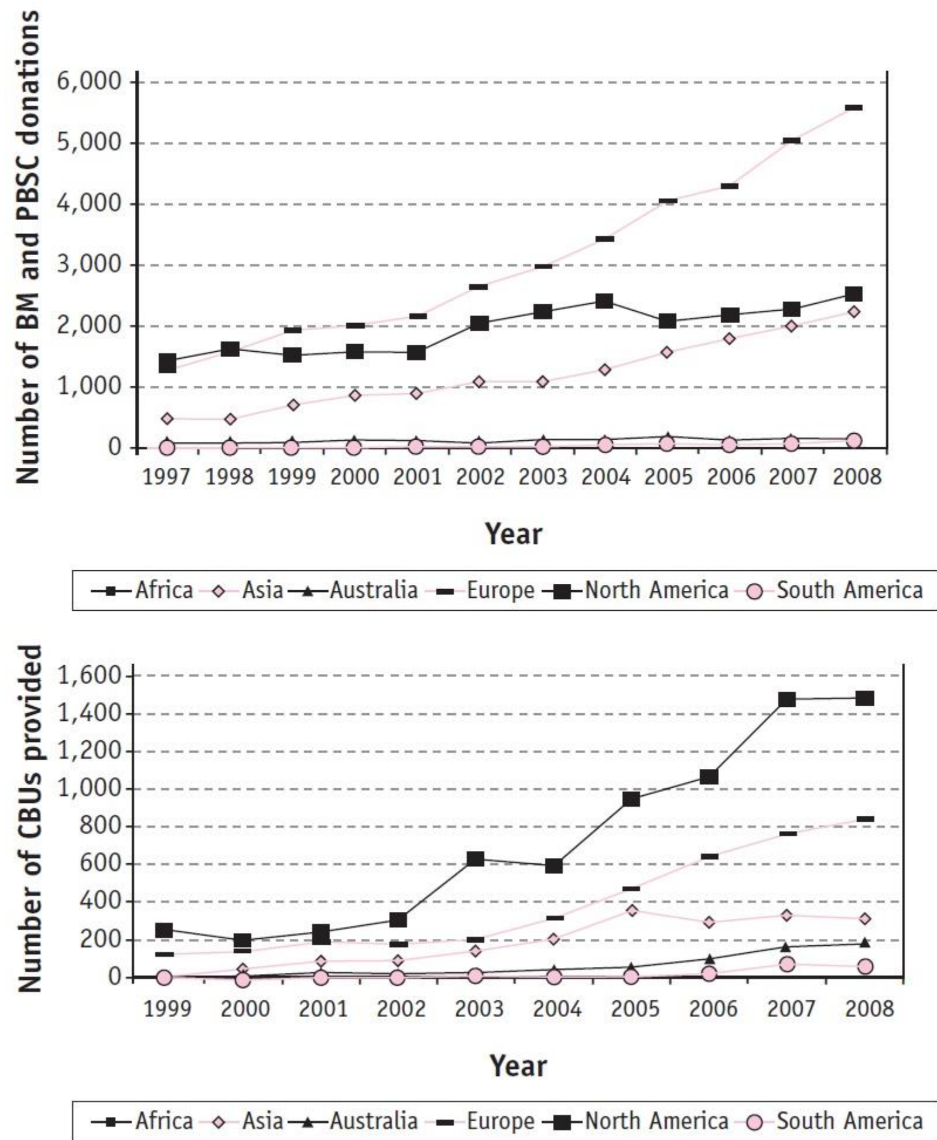
Copelan EA. NEJM 354;1813-26:2006





AML: Acute Myeloid Leukaemia, ALL: Acute Lymphoblastic Leukaemia MDS/MPS: Myelodysplastic syndromes/ Myeloproliferative Neoplasm, HD/NHL/CLL: Hodgkin's disease, Non Hodgkin's Lymphoma, Chronic Lymphatic Leukaemia, PCD: Plasma Cell Disorders, CML: Chronic Myeloid Leukaemia

Figure 1: Trends from 1990 to 2010 in allogeneic (fig1a) and autologous (Fig 1b) transplants separately for different disease categories



WMDA data

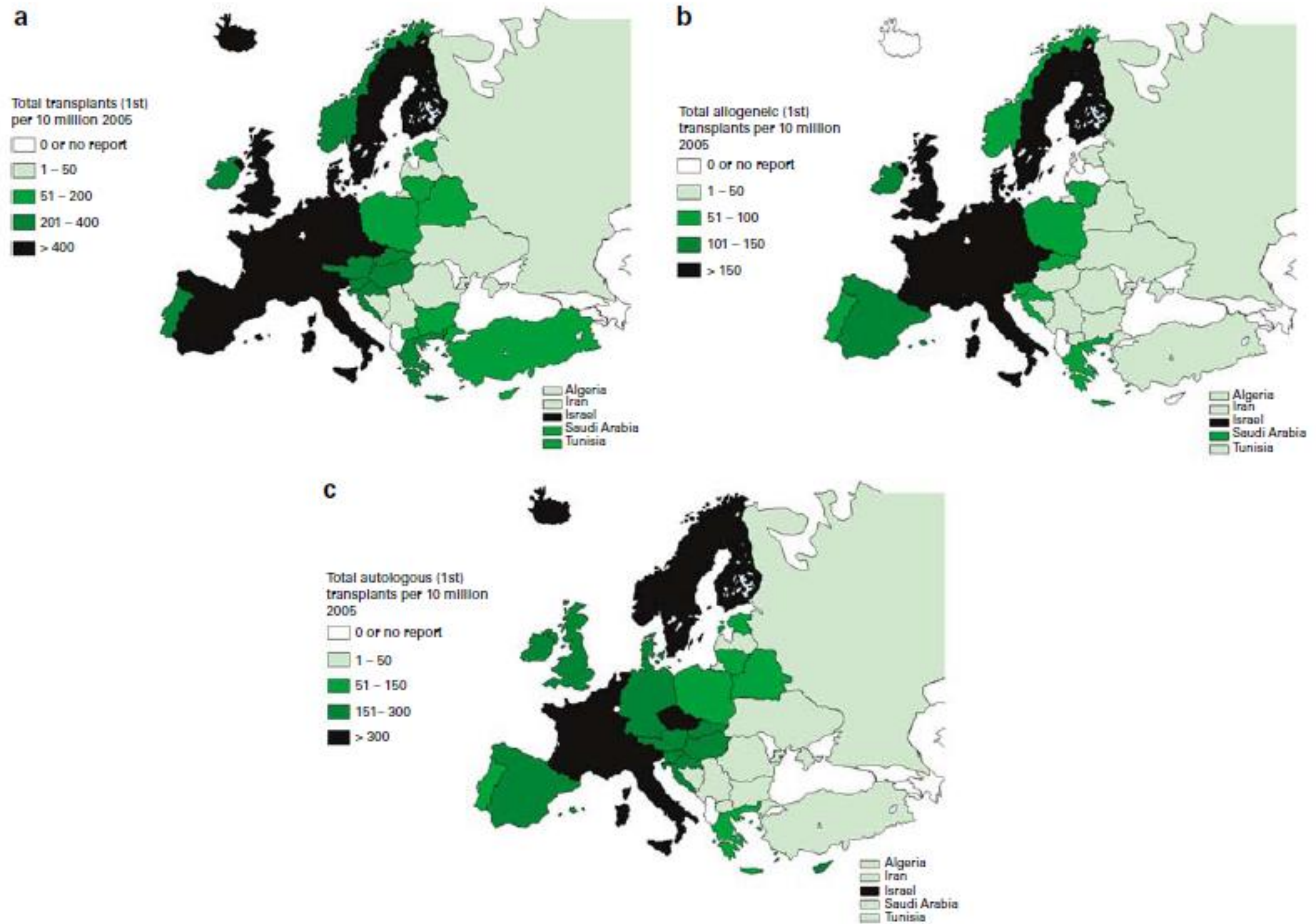


Figure 1 Transplant rates (number of HSCT per 10 million inhabitants) in European countries in 2005. (**a**) All HSCT combined. (**b**) Allogeneic HSCT only. (**c**) Autologous HSCT only.

Bone Marrow Transplantation (2007) 39, 71–87

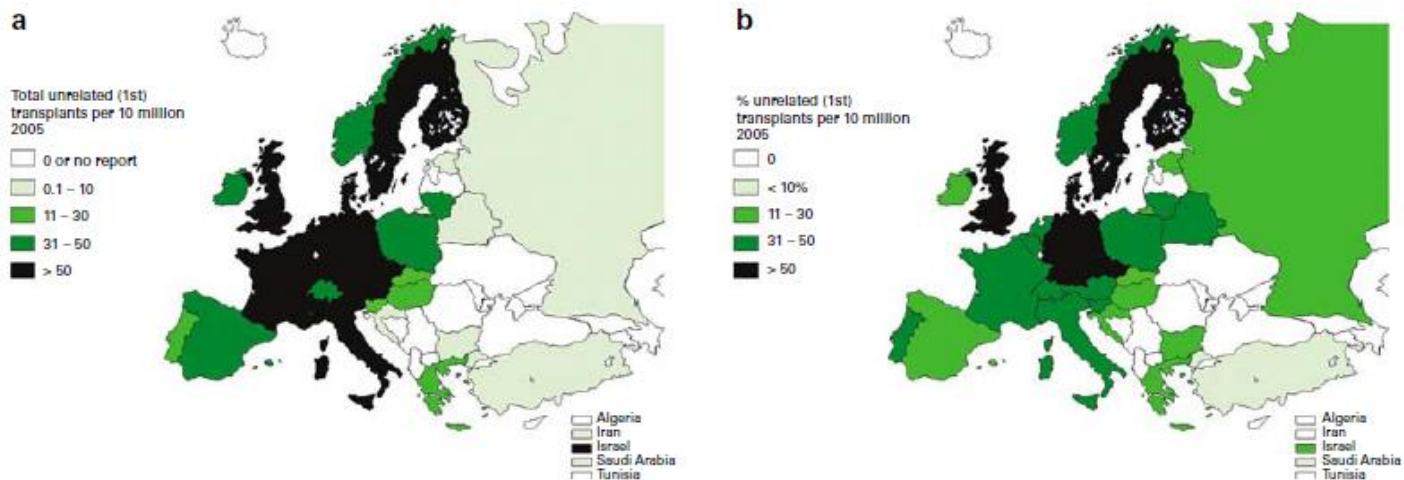


Figure 2 Unrelated HSCT in Europe. (**a**) Transplant rates (number of HSCT per 10 million inhabitants) in participating European countries in 2005. (**b**) Proportion of unrelated HSCT among allogeneic HSCT. Shades reflect percentage.

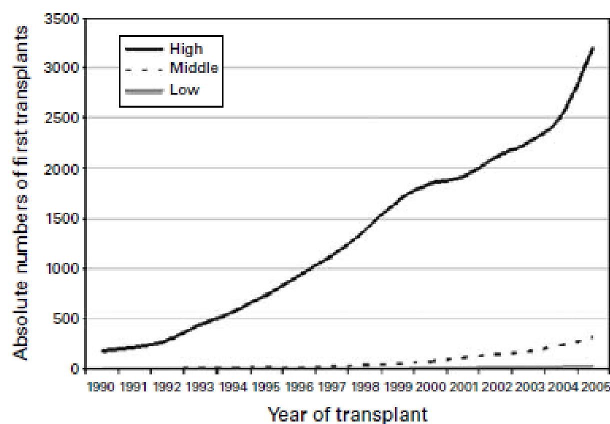


Figure 3 Evolution of unrelated HSCT in Europe from 1990 to 2005. Curves reflect absolute transplant numbers according to World Bank category (see text) of the participating countries.

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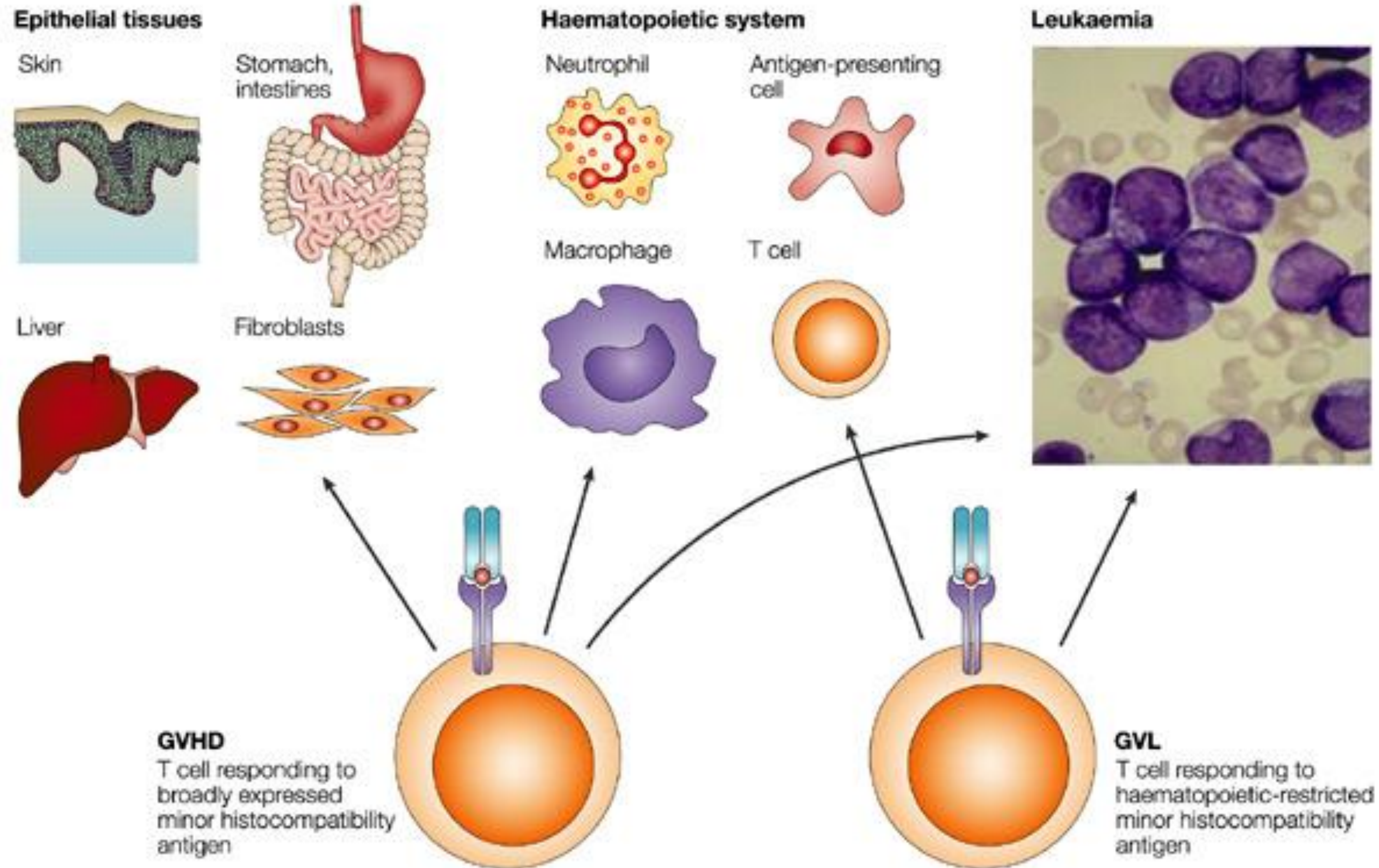
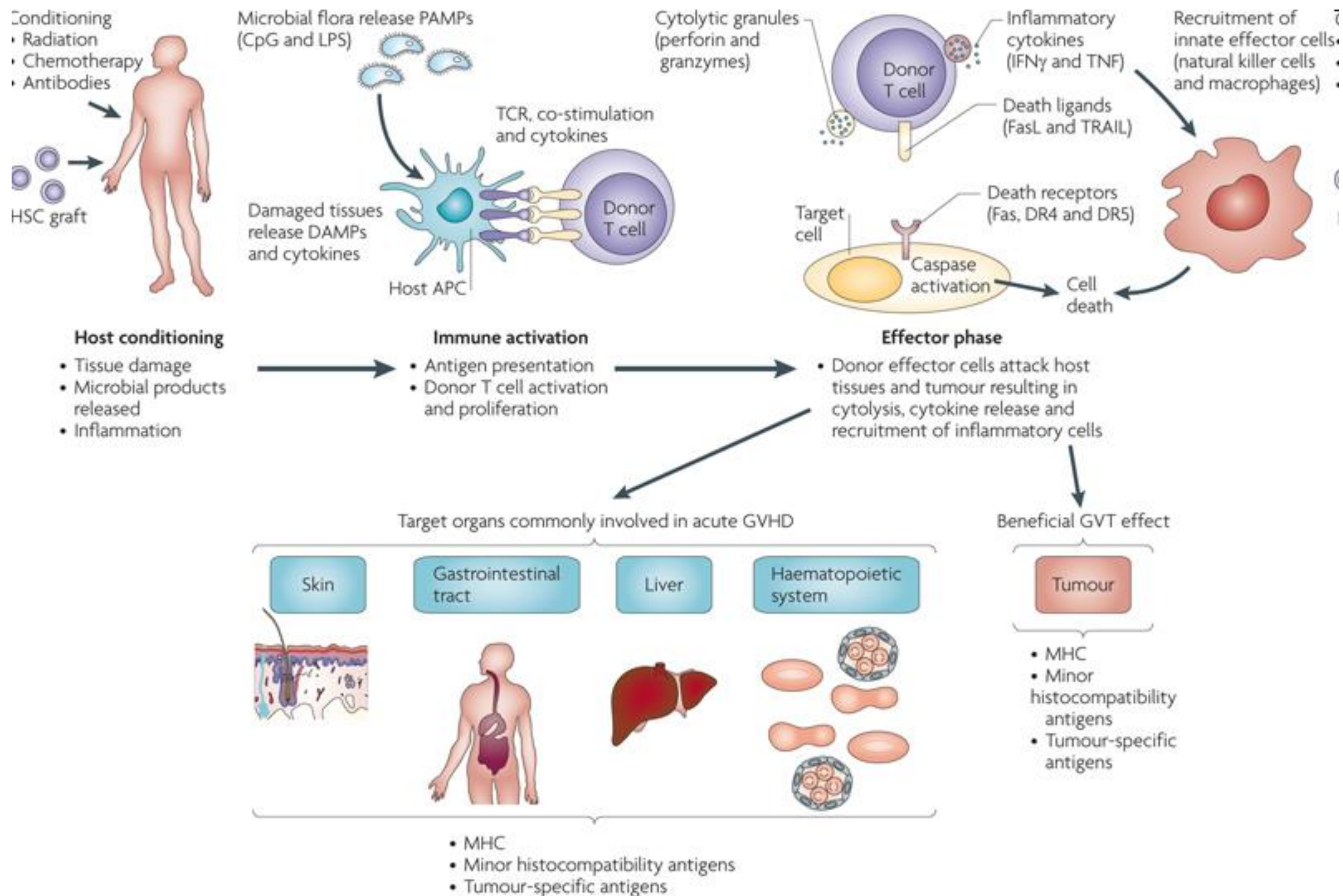
GVL and GVHD



Marie Bleakley & Stanley R. Riddell Nature Reviews Cancer **4**, 371-380 (2004);

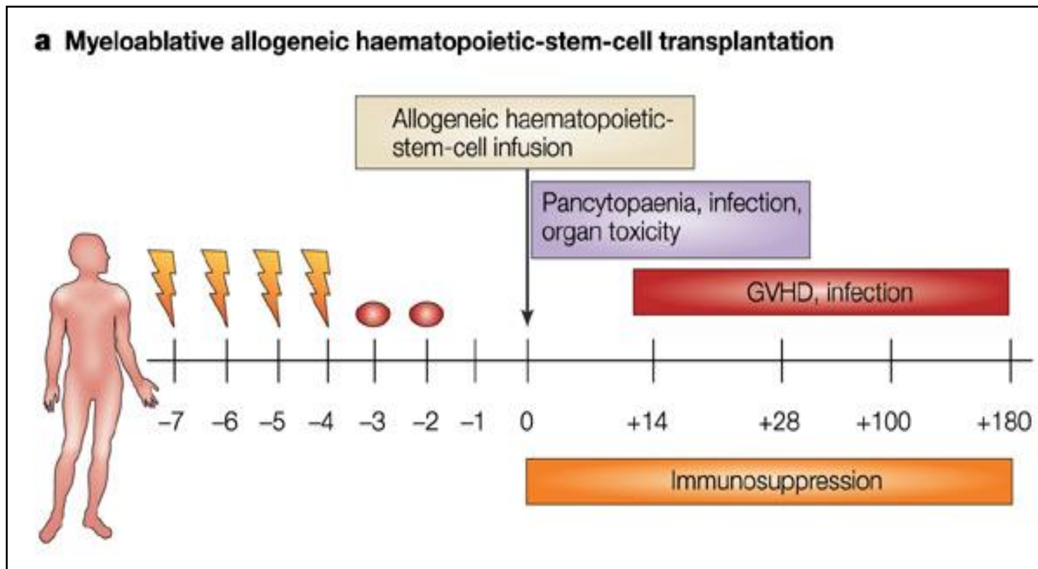


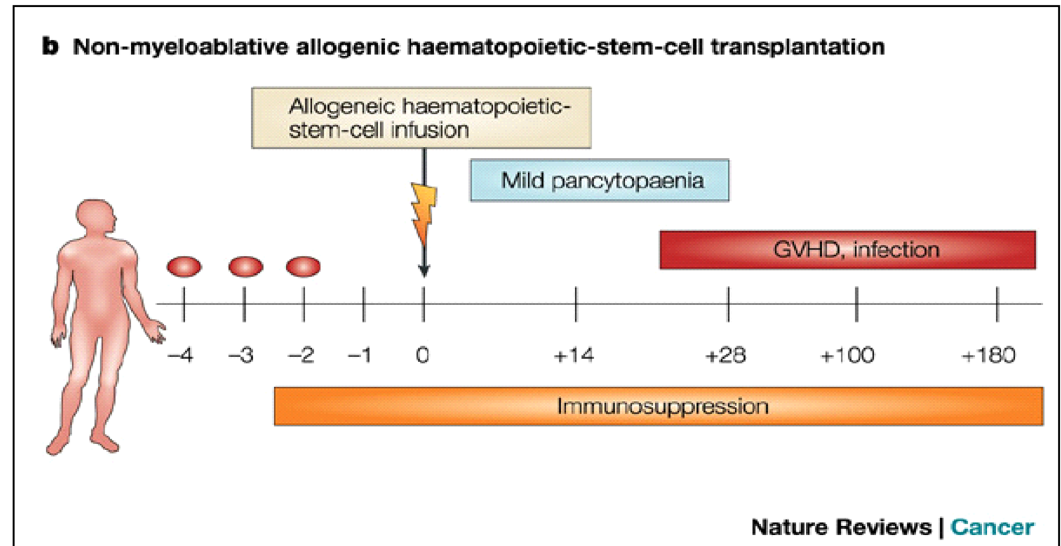
The 3 phases of acute GVHD and graft-versus-tumour (GVT) effects.

First, the host conditioning stage, in which microbial products are released and tissue damage and inflammation occur. Antigen presentation then leads to donor T cell activation and proliferation.

In the effector phase, after immune system activation, donor effector immune cells attack both host tissues (leading to GVHD) and the tumour (the beneficial GVT effect). APC, antigen-presenting cell; DAMP, damage-associated molecular pattern; DR, death receptor; PAMP, pathogen-associated molecular pattern; TRAIL, TNF-related apoptosis-inducing ligand.

Myeloablative and non myeloablative stem-cell transplant





Marie Bleakley & Stanley R. Riddell Nature Reviews Cancer **4**, 371-380 (2004);

Fasi del trapianto di midollo allogenico

1. scelta del donatore

a) Gemello omozigote

Attecchimento ++++
GVHD ----
GVL ---
Probabilità recidiva +++

b) Fratello HLA-identico

Attecchimento +++-
GVHD ++--
GVL ++/--
Probabilità recidiva ++/--

c) Donatore non famigliare HLA-identico banca

Attecchimento ++/--
GVHD +++/-
GVL +++/-
Probabilità recidiva +/---

d) Donatore famigliare aploidentico

Attecchimento +/---
GVHD ++++
GVL ++++
Probabilità recidiva ----

d) Sangue cordone ombelicale banca

Attecchimento ----
GVHD ++/--
GVL ++/--
Probabilità recidiva ++/--

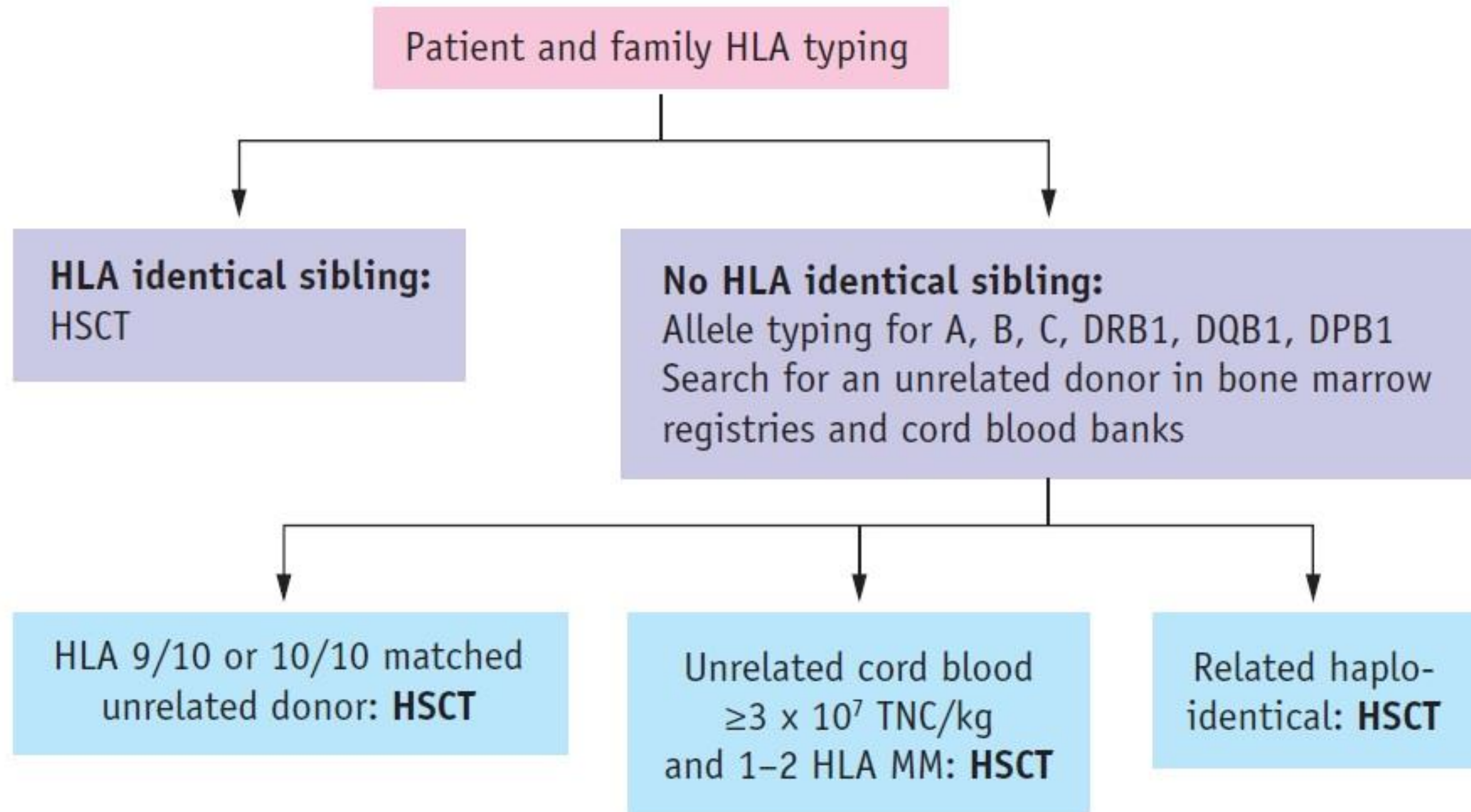


Figure 4: Algorithm of donor search
Patient and family HLA typing
HLA identical sibling:
HSCT
No HLA identical sibling:
Allele typing for A, B, C, DRB1, DQB1, DPB1
Search for an unrelated donor in bone marrow registries and cord blood banks
HLA 9/10 or 10/10 matched unrelated donor: HSCT
Unrelated cord blood ≥3 x 10⁷ TNC/kg and 1–2 HLA MM: HSCT
Related haplo-identical: HSCT
- If transplant urgent: prefer cord blood or related haploidentical transplant
- If not enough cells in a single cord blood unit: consider double cord
- Consider other factors: indication of the transplant, ABO, CMV, donor sex
- Expertise of the centres is very important for donor selection for HLA mismatched transplants

Manipulating hematopoietic stem cells for clinical use

Isolating stem cells for manipulation

Table 1. MoAbs recognizing stem cell populations[19,20]

Ag	*Other names*	*Differentiated cell expression*	*Stem/progenitor expression*
CD9	P24, DRAP-1, MRP1	Leukocytes, endothelial, epithelial	NSC
CD34	8G12, 581, QBEnd10	Endothelial	HSC, EPC
CD73	Ecto-5′ nucleotidase	Leukocyte subsets, endothelial, epithelial	MSC
CD81	TAPA-1	Lymphocytes, endothelial, epithelial	NSC
CD90	Thy1	Endothelial	MSC, HSC
CD105	Endoglin, SH2	Endothelial, macrophage/ monocyte	MSC with CD73+, CD16+, CD90+ CD29+ CD34−, CD45-
CD117	c-kit, steel, SCF	Breast epithelial, renal tubule, melanocytes	Myeloid, HSC
CD133	AC133, Prominin-1	Endothelium, epithelial	HSC, EPC, NSC
CD135	FMS-like kinase-3 (FLT3) or STK-1, or Flk-2	Macrophage/ monocytes	Human marrow CD34+ and dendritic precursors, murine short-term HSC
CD146	MUC-18, Mel-CAM, MCAM	Activated T cells, endothelial	EPC
CD150	SLAMf1; used with CD244 and CD48	T, B, dendritic, endothelial	Distinguishes HSC from multipotent and B-cell progenitors
Aldefluor			HSC/MSC
Side population			HSC

Abbreviations: EPC = endothelial progenitor cells; HSC = hematopoietic stem cells; NSC = neural stem cells.

Table 3. Flow cytometric markers used to define the most frequently observed stem cell subpopulations[12,26,32,35]

	Cell type	*Lin − subpopulation immunophenotype*
HSC	Hematopoietic stem cell	CD34+/CD38−
CMP	Common myeloid progenitor	CD34+/CD38+/IL3Rα low/CD45RA
GMP	Granulocyte-macrophage progenitor	CD38+/IL3Rα low/ CD45RA+
MEP	Megakaryocyte-erythroid progenitor	CD38+/IL3Rα−/CD45RA−
CLP	Common lymphoid progenitor	CD10+/CD19−
	Pro-B cells	CD10+CD19+
	T and NK progenitors	CD10−/CD7+/CD45RA+
MSC	Mesenchymal stem cell	CD90+/CD105+/ALDH+/ CD45−/CD34−/CD133−/ CD38−/HLA−DR−
STRC-M	Early myeloid short-term repopulating	CD34+/CD38+/CD45−/ ALDH−/CD133−/CD90+
	Late myeloid HSC	CD34+/CD38−/ CD45dim+/ALDH+[a]/ CD133+/CD90+

Abbreviations: ALDH = Aldehyde dehydrogenase; STRC-M = short term repopulating cells-myeloid. [a]Bright positive cells have the highest repopulation capacity compared with dim positive cells.

Beksac M, BMT (2011) 1- 6

Frequency and engraftment potential of HSC subpopulations

Cell type	*Frequency in*	*Cell dose*	*PNL engraftment*	*Plt engraftment*
Total CD34+[5,7,10]	1/200 (of MNC, BM)	$2-8 \times 10^6$/kg	Yes	Yes (month 3,6,9)
CD34+38–[22-24]	1/40	Unknown	Yes (month 6,9)	Yes
	1/1000 (of MNC, BM)			No
CD34+90+[22,25,26]	1/1000	$25-80 \times 10^4$/kg	Yes	Yes (month 3,6,9,12[a])
			no	
CD34+CD110+[27]				Yes
CD34–/low[24]	1/24000	Unknown	Unknown	Unknown
CD133+[28]	Same as CD34	Same as CD34+	Similar to CD34	Similar to CD34
CD34+133+[22]	Not reported	Not reported	Yes (month 3)	Yes(month 3,6,9)
SSC low/ALDH bright[29-31]	1.2% (BM)	5.4×10^6/kg (PNL)	Yes	Yes
		7.22×10^6/kg (Plt)	Yes	Yes
			No	No
Lin-CD34+CD38–IL3Ra low CD45RA- (CMPs)[32]	73.20 (17.8-86.2) % (PBSC) 35.2 (20.4-42.3) % (BM) 3.2 (1.3-16.1) % (CB)	$> 4.4 \times 10^6$/kg (PNL, Plt and erythroid)	Similar to CD34	Similar to CD34

Abbreviations: CMP = common myeloid progenitors; MNC = mononuclear cell; PNL = polymorphonuclear cells; SSC = side light scatter. [a]Also enhanced erythroid engraftment on month 12.

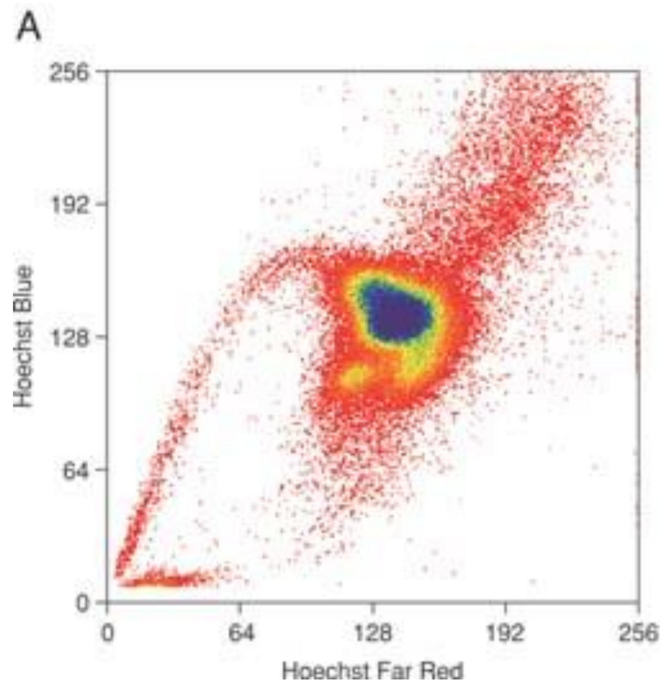
Beksac M, BMT (2011) 1- 6

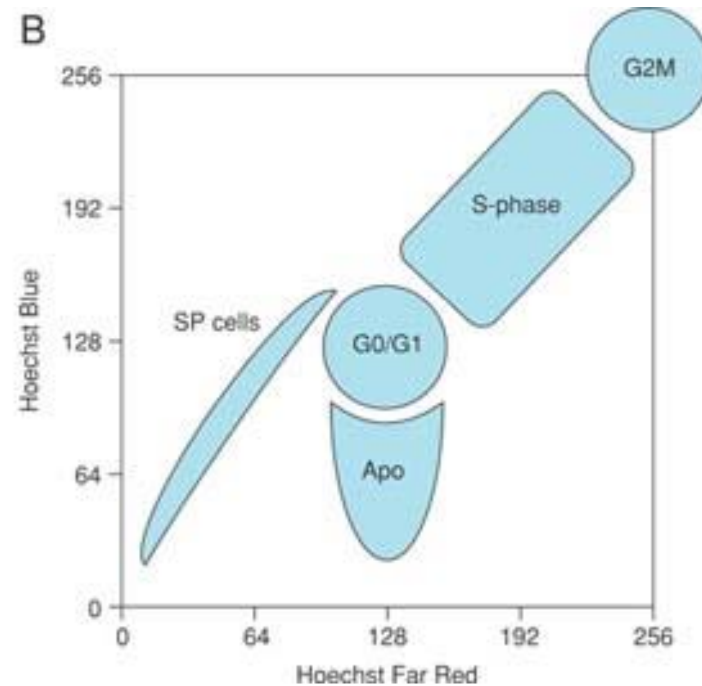
Supravital stains

- Since HSCs are inherently quiescent, spend most of their time in inactive portions of the cell cycle and are resistant to toxins, exclusion of dyes has been used as a method of isolation.
- The DNA dye Hoechst 33342 was first used to separate HSC.
  - Cells with low-intensity staining were enriched for high proliferative potential (HPP)-CFC and day-12 CFU-S.

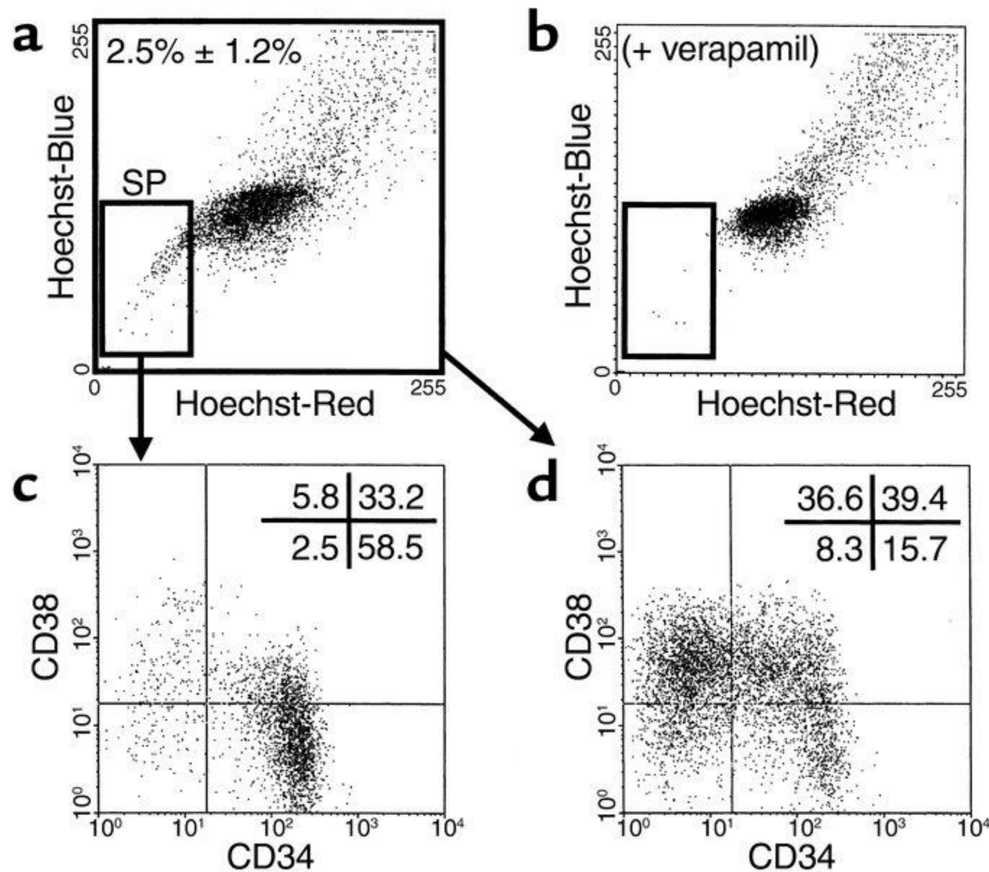
- The red and blue emissions from this dye have been used to define a small subset of BM cells (the side population - SP).
  - SP cells have extremely low fluorescence emission, resulting from efflux of Hoechst 33342 by multidrug resistance pumps that are highly expressed on HSCs.
  - SP cells constitute approximately 0.1% of the BM and are highly enriched in reconstitution potential.

Supravital stains





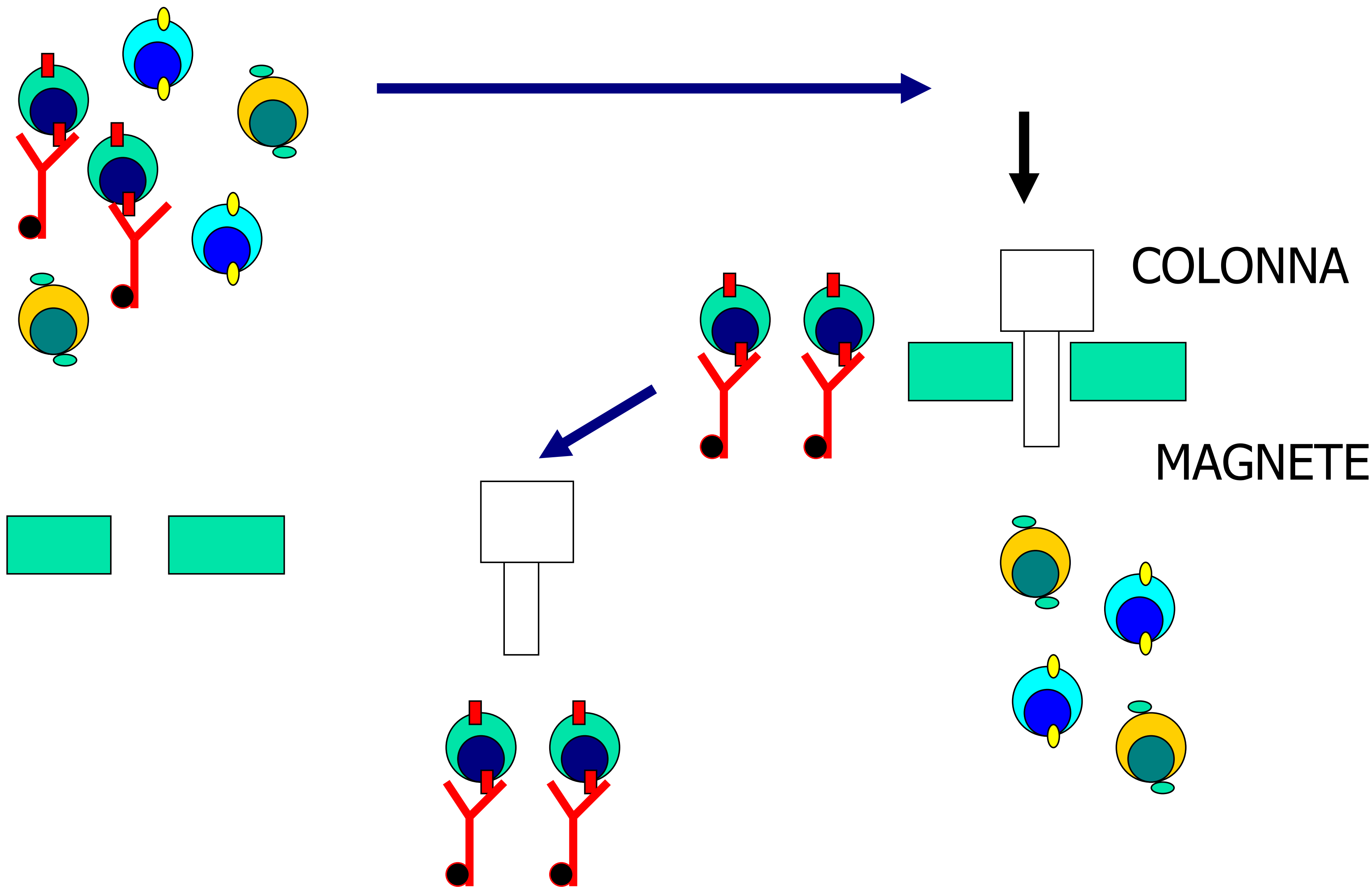
Supravital stains



Methods of isolation of HSCs

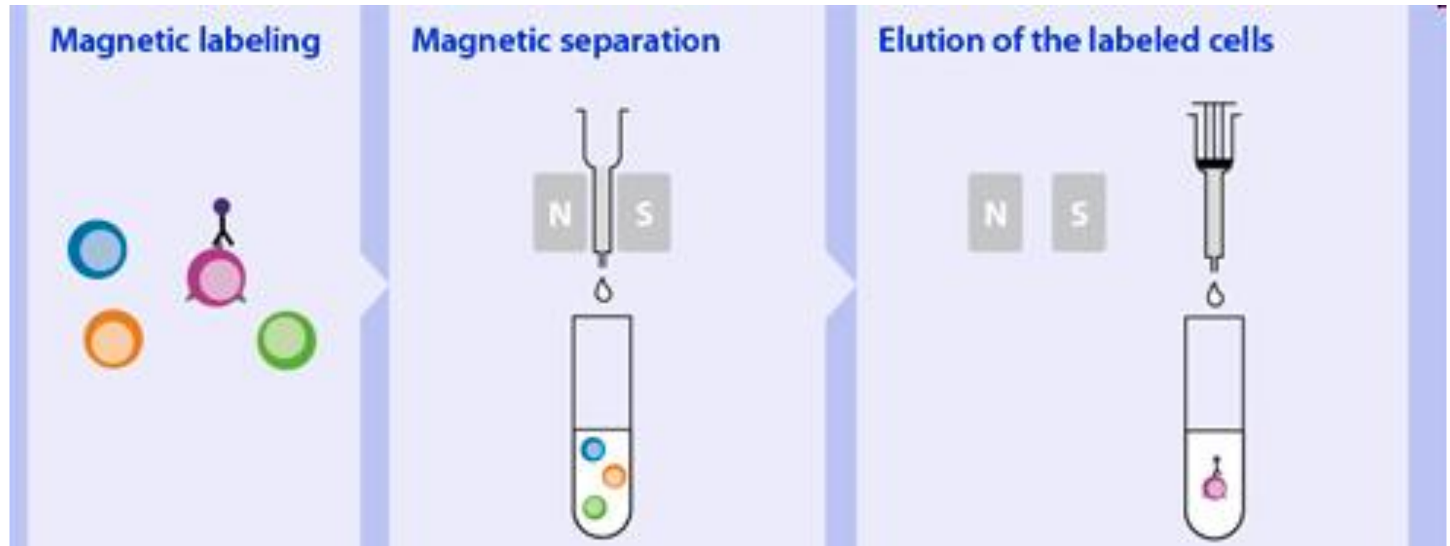
Magnetic beads

SEPARAZIONE IMMUNOMAGNETICA



MACS® Microbeads Technology

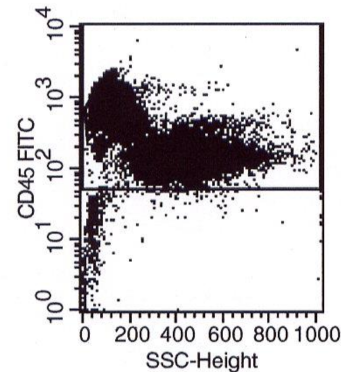
Basic principle

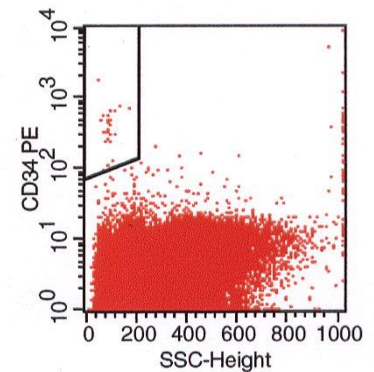


Cells in a single-cell suspension are magnetically labeled with MACS® MicroBeads.
The sample is applied to a MACS Column placed in a MACS Separator.
The unlabeled cells pass through while the magnetically labeled cells are retained within the column.
The flow-through can be collected as the unlabeled cell fraction.
After a short washing step, the column is removed from the separator, and the magnetically labeled cells are eluted from the column.

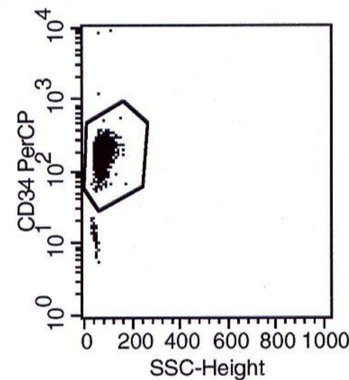
Separazione immunomagnetica di cellule staminali CD34+

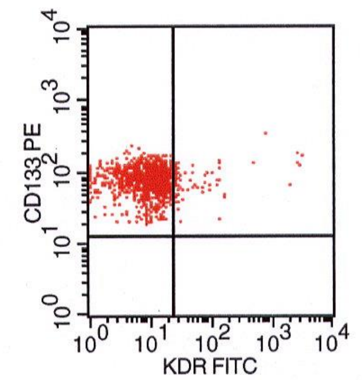
Prima della separazione





Dopo la separazione





Labeling methods: direct

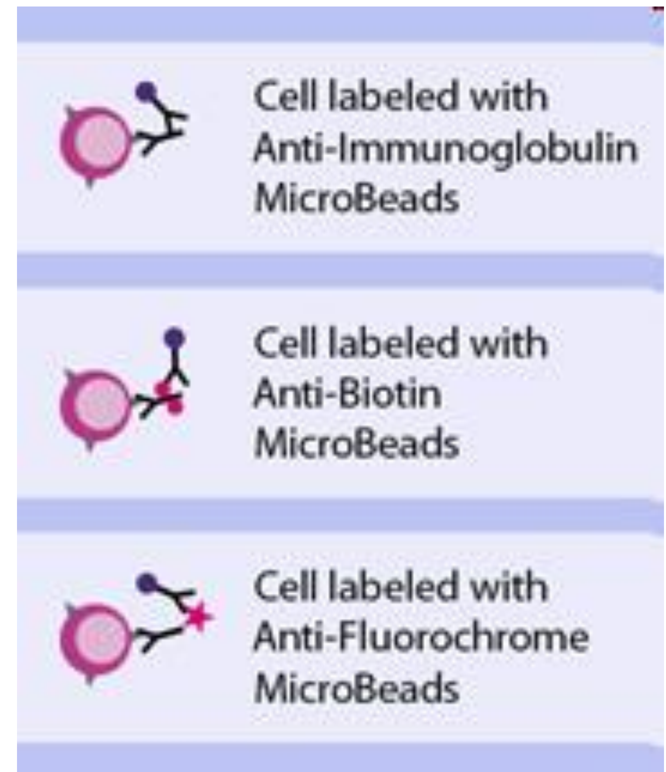
MACS
Miltenyi Biotec

- **Direct labeling with MicroBeads is the fastest way of magnetic labeling.**
  - **MACS MicroBeads specifically bind to antigens on the cell surface.**
  - **Only one incubation step is required.**
  - **Direct magnetic labeling requires a minimal number of washing steps and therefore minimizes cell loss.**

Labeling methods: indirect

MACS Miltenyi Biotec

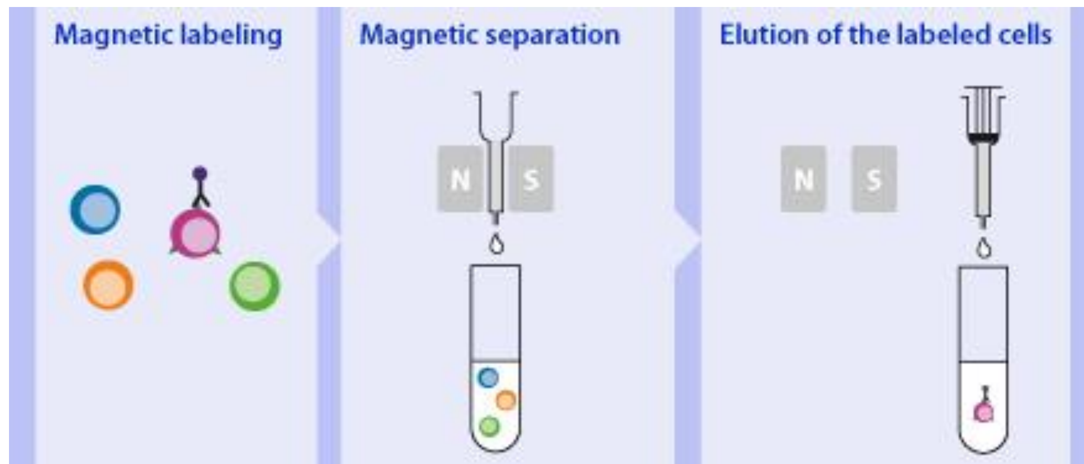
- Indirect magnetic labeling is based on a two-step procedure.
- In a first step, the cells are labeled with a primary antibody directed against a cell surface marker.
- In a second step, the cells are magnetically labeled with MicroBeads, which either bind to the primary antibody or to a molecule that is conjugated to the primary antibody.
- The primary antibody can either be unconjugated, biotinylated, or fluorochrome-conjugated.
- Accordingly, magnetic labeling is achieved with Anti-Ig MicroBeads, Anti-Biotin MicroBeads, or Anti-Fluorochrome MicroBeads.
- Indirect labeling can also be performed by using a cocktail of primary antibodies to concurrently label a number of unwanted cell types



Separation strategies: positive selection

Miltenyi Biotec

1. Positive selection means that a particular target cell type is magnetically labeled. During separation, the magnetically labeled cells are retained within the column. Unlabeled cells flow through. After a washing step, the column is removed from the magnetic field of the separator, and the target cells are eluted from the column.
2. Positive selection can be performed by direct or indirect magnetic labeling.



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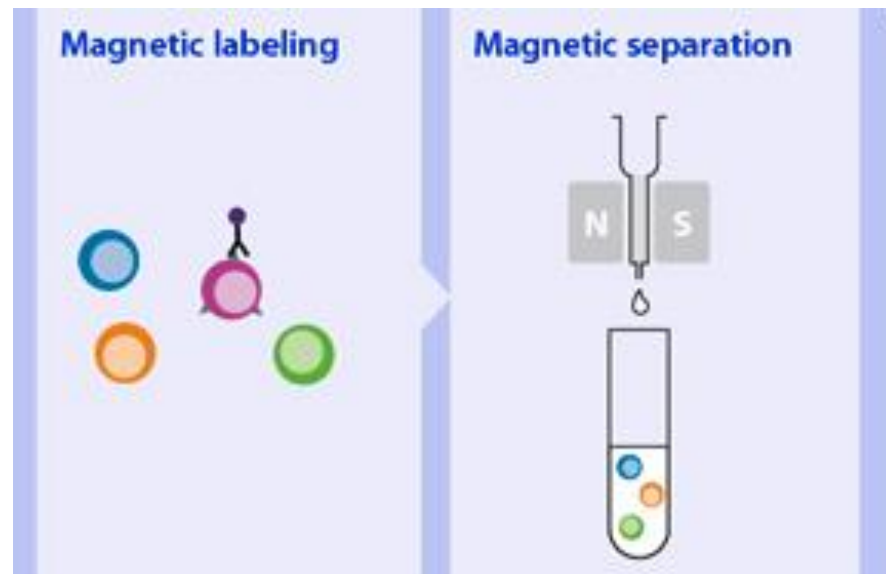
Separation strategies: depletion

Removal of an unwanted cell type

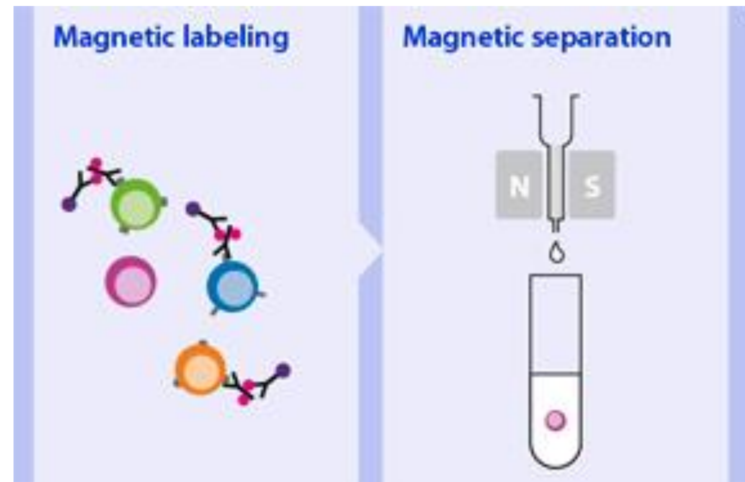
To remove a certain cell type from a mixture of cells, the unwanted cell type is magnetically labeled. During separation, the unlabeled target cells are collected in the flow-through fraction.

The unwanted cell type is retained within the column.



Separation strategies: untouched isolation

- **To isolate a particular target cell type in an unlabeled, i.e., untouched form, non-target cells are magnetically labeled and depleted. During separation, the unlabeled target cell type is collected in the flow-through fraction.**
- **The mixture of magnetically labeled non-target cells is retained within the column.**



Sequential sorting

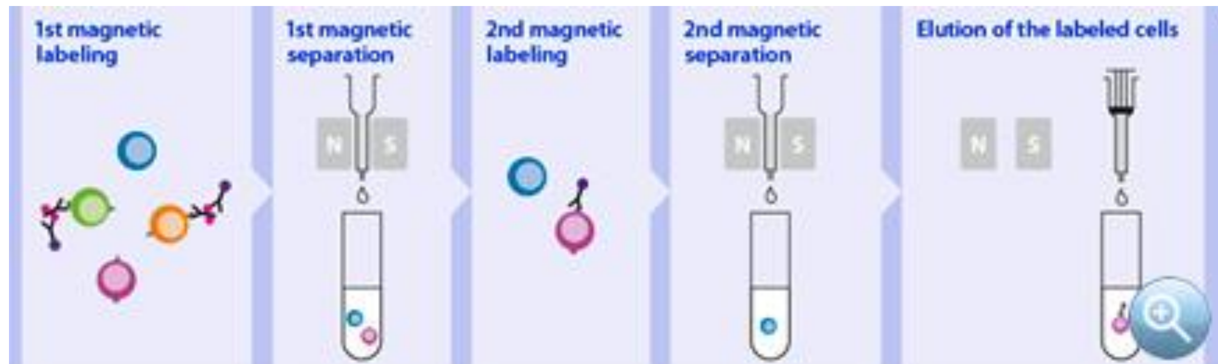
MACS
Miltenyi Biotec

- **Isolation of a cell subset by using more than one marker** A combination of two subsequent separations is applied to isolate cell subsets that can be distinguished from other cell types through their expression of two different markers.
- This includes cell types for which a specific marker has not been defined.

Sequential sorting

MACS
Miltenyi Biotec

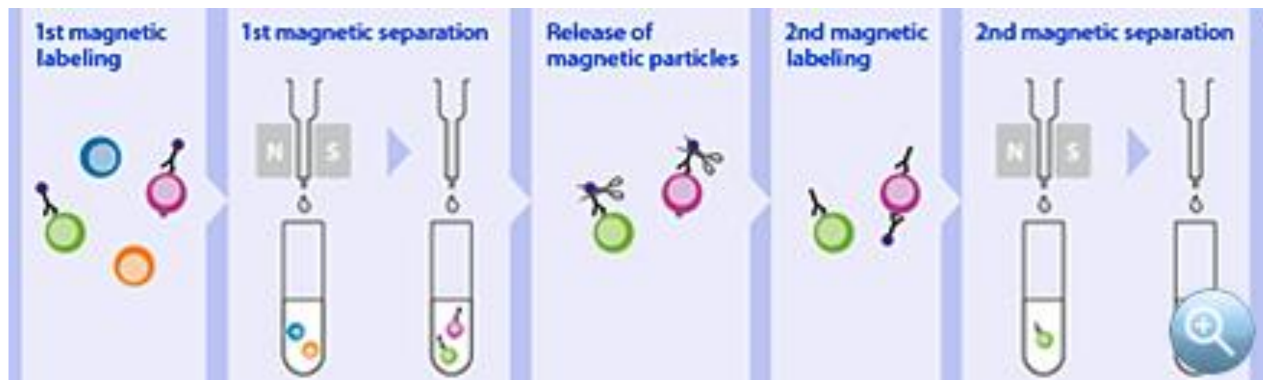
- **Depletion followed by positive selection:**
  - This separation strategy is useful, if undesired cells and target cells have one marker in common. In this case, the target cells cannot be isolated in a single positive selection step using this marker.
  - Undesired cells expressing this marker are magnetically labeled via antigens distinct from the common marker, and depleted.
  - The cells appearing in the flow-through fraction during this separation are subsequently labeled with MACS MicroBeads that bind to the common marker.
  - Target cells are then isolated by positive selection.



Sequential sorting

MACS
Miltenyi Biotec

- **Two subsequent positive selections:** Multiparameter sorting with MACS MultiSort MicroBeads is based on 2 sequential positive selections according to 2 different markers.
- MACS MultiSort MicroBeads specific for the first marker allow the first positive selection.
- After the separation, the cells are incubated with the MultiSort Release Reagent, which enzymatically removes the MultiSort MicroBeads from the cells.
- In the next step, the target cells are magnetically labeled with MACS MicroBeads directed against the second marker and again subjected to positive selection.



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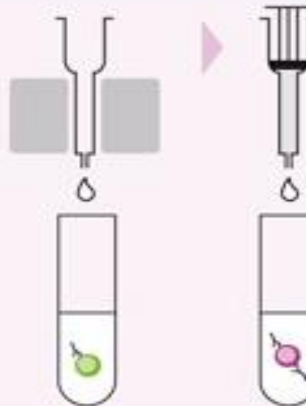
Cells are magnetically labeled with CD34 MultiSort MicroBeads.

CD34⁺ cells are isolated by positive selection. Magnetically labeled CD34⁺ cells are retained in MACS® Column placed in a MACS Separator. CD34⁺ cells are eluted after removal of the column from the separator.

Release of the magnetic particles.

Labeling with MACS MicroBeads according to a second marker.

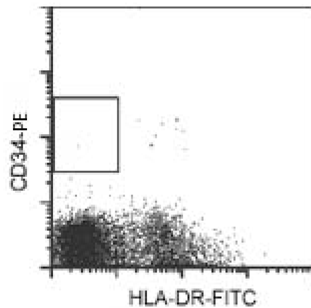


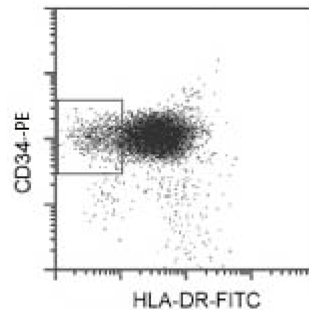
Target cells are positively selected a second time.

CD34+ HLA-DR– cells isolated from human PBMCs using the CD34 MultiSort Kit.

Positive selection of CD34+ cells from PBMCs was followed by the depletion of CD34+HLA-DR+ cells using HLA-DR-FITC and Anti-FITC MicroBeads resulting in a population of CD34+HLA-DR– cells

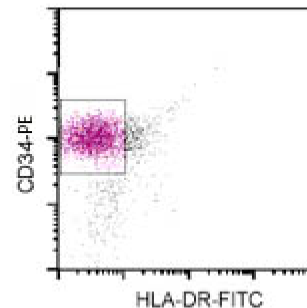
A: PBMCs before separation

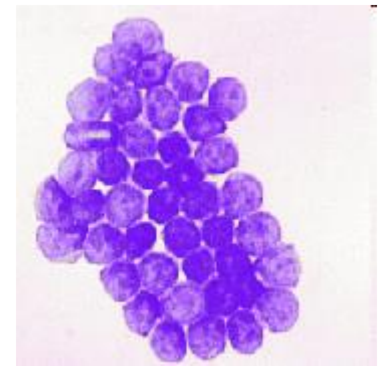




B: CD34+ cells

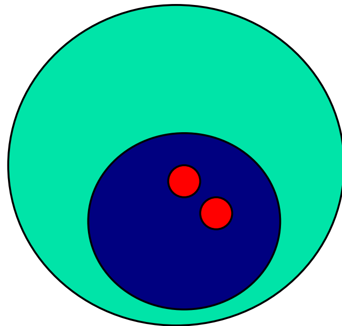
C: CD34+HLA-DR- cells

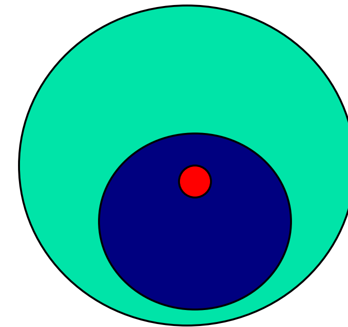




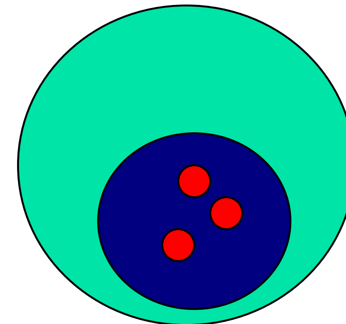
CD34+ lin- cells isolated from peripheral blood using CD34 MultiSort Kit, May-Grünwald-Giemsa stained.

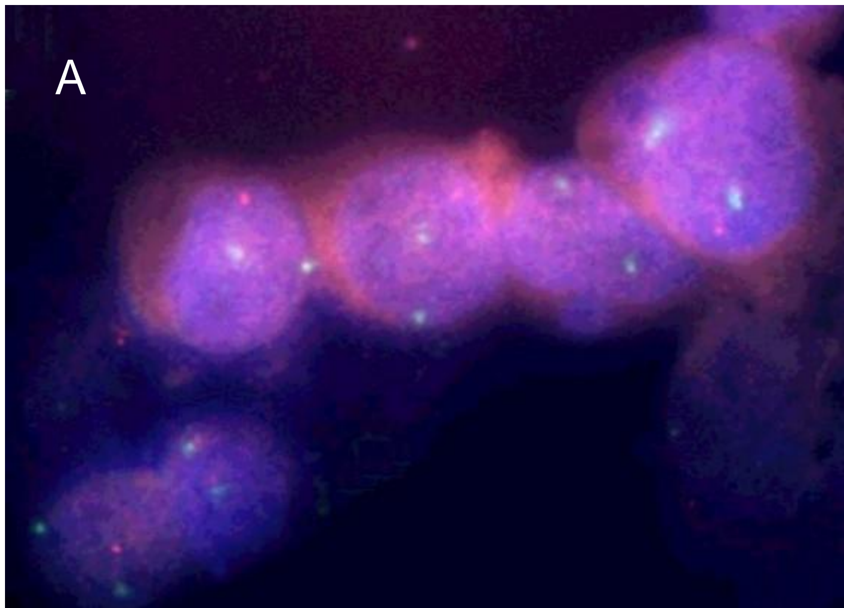
normale

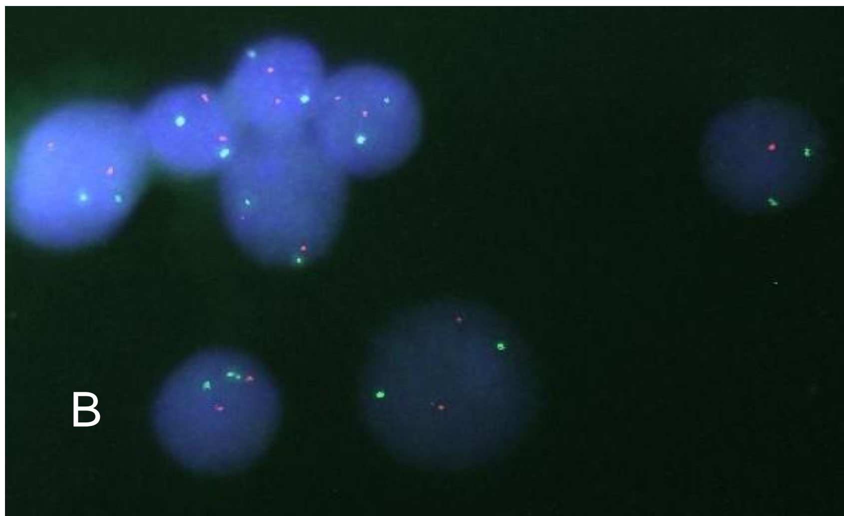




patologica





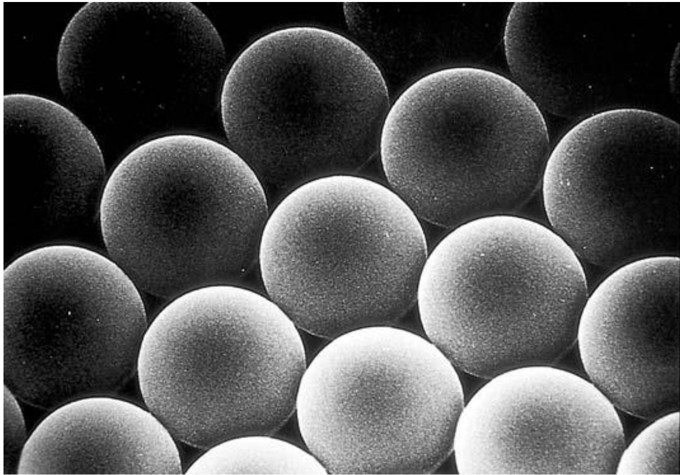


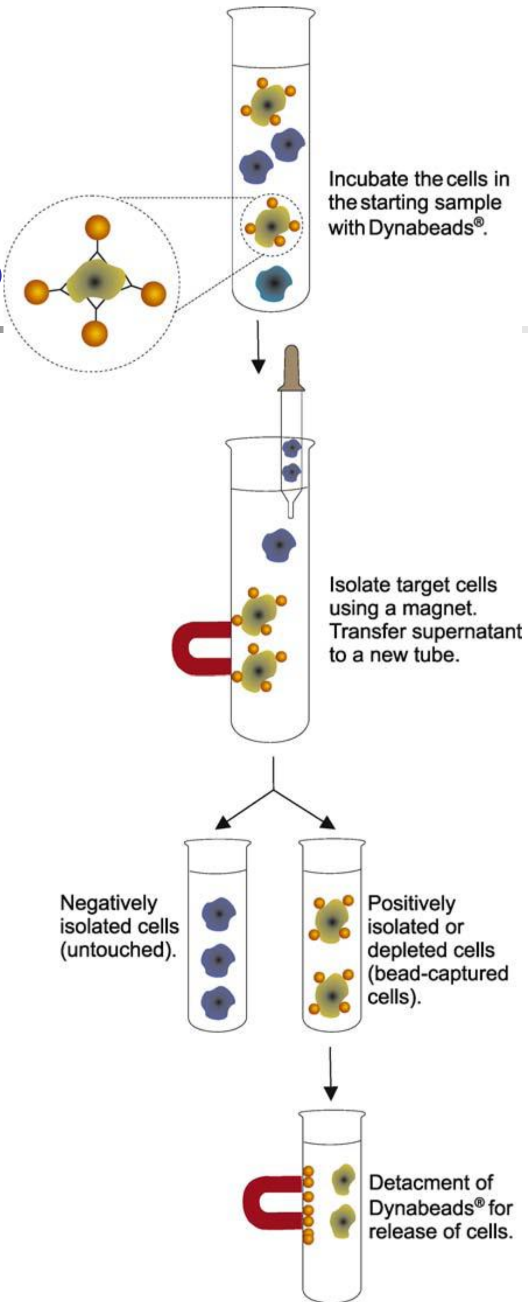
EPO+G: MDS

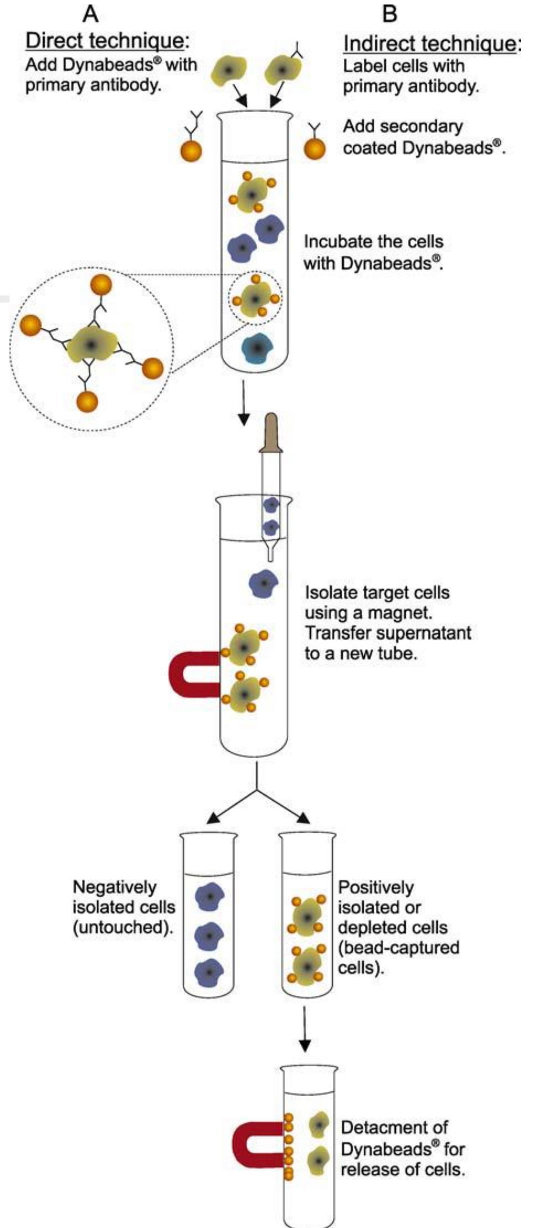
Dual colour combined FISH analysis performed on immunomagnetically sorted CD34+ cells before (A) and after (B) treatment in a MDS patient with del(5q) responding to rHuEpo and G-CSF administration. In B, rHuEpo and G-CSF administration has produced a significant reduction of the proportion of CD34+ cells with the 5q deletion. Pathologic CD34+ cells are identified by the presence of only one red signal (DNA probe to the band 5q31). As control, the two green signals (DNA probe to the band 5p15.2 locus D5S23) indicate the presence of two chromosomes 5.

Rigolin et al, BJH 2004

Dynabeads







Methods of isolation of HSCs: *FACS*

- Fhe flow cytometer may physically sort cells of desired fluorescence or fluorescence pattern, size and granularity characteristics.
- Using a magnetic field, these cells may be iverted to a collection tube during analysis and later analyzed using techniques of molecular and cellular biology.
- FACS may be used to isolate HSCs using both positive and negative selection strategies with fluorescence - labeled antibodies directed against primitive hematopoietic cell antigens.