1st International Conference/Workshop on
Stem cell research and applications

Erciyes University

October 7-9th, 2011, Kayseri, Turkey
Pre-conference Workshop: October 6th, 2011

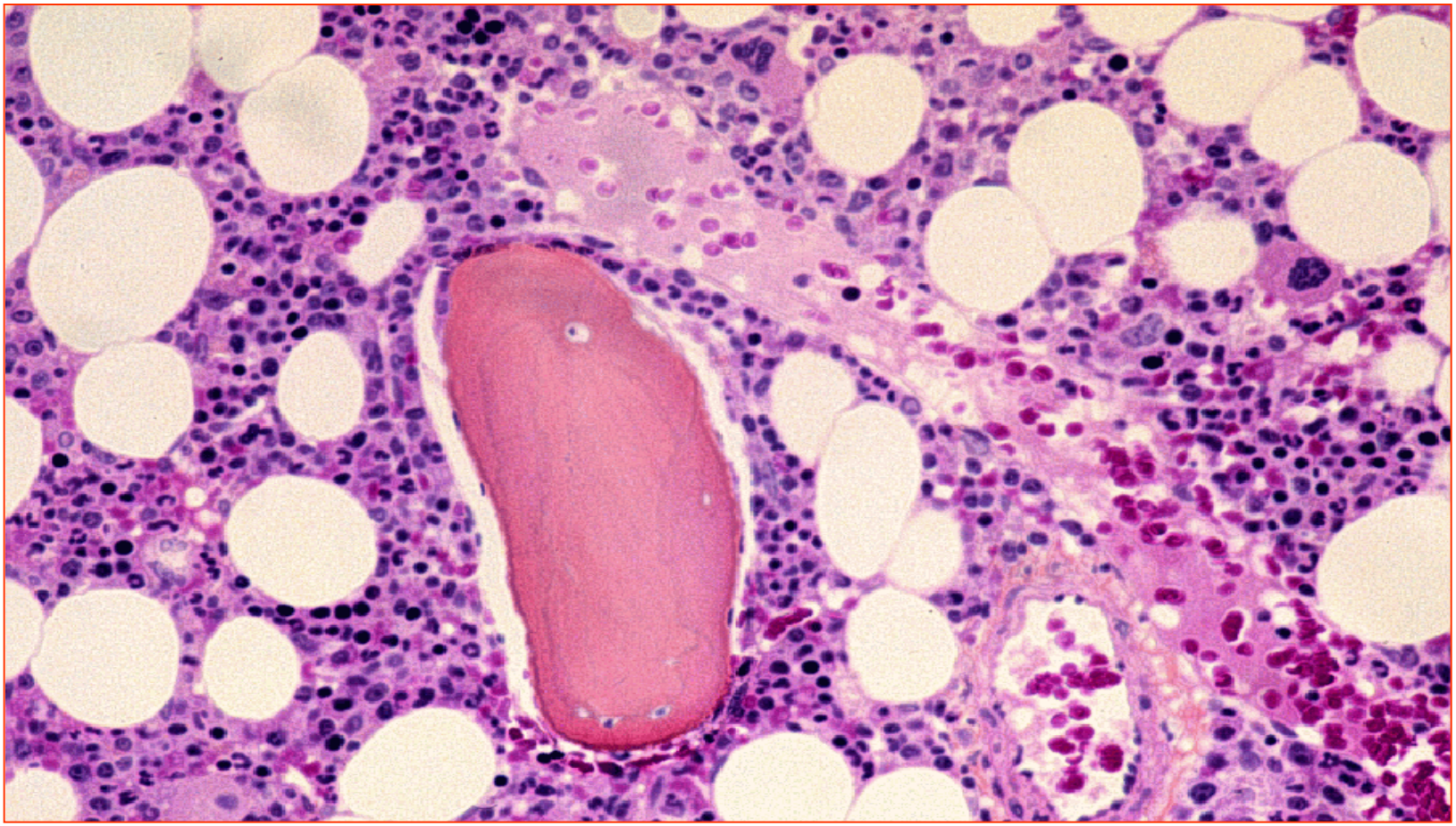
Peripheral Hemapoetic Stem Cell Mobilization

Dr. Mustafa ÇETİN, M.D.
Erciyes University Medical Faculty

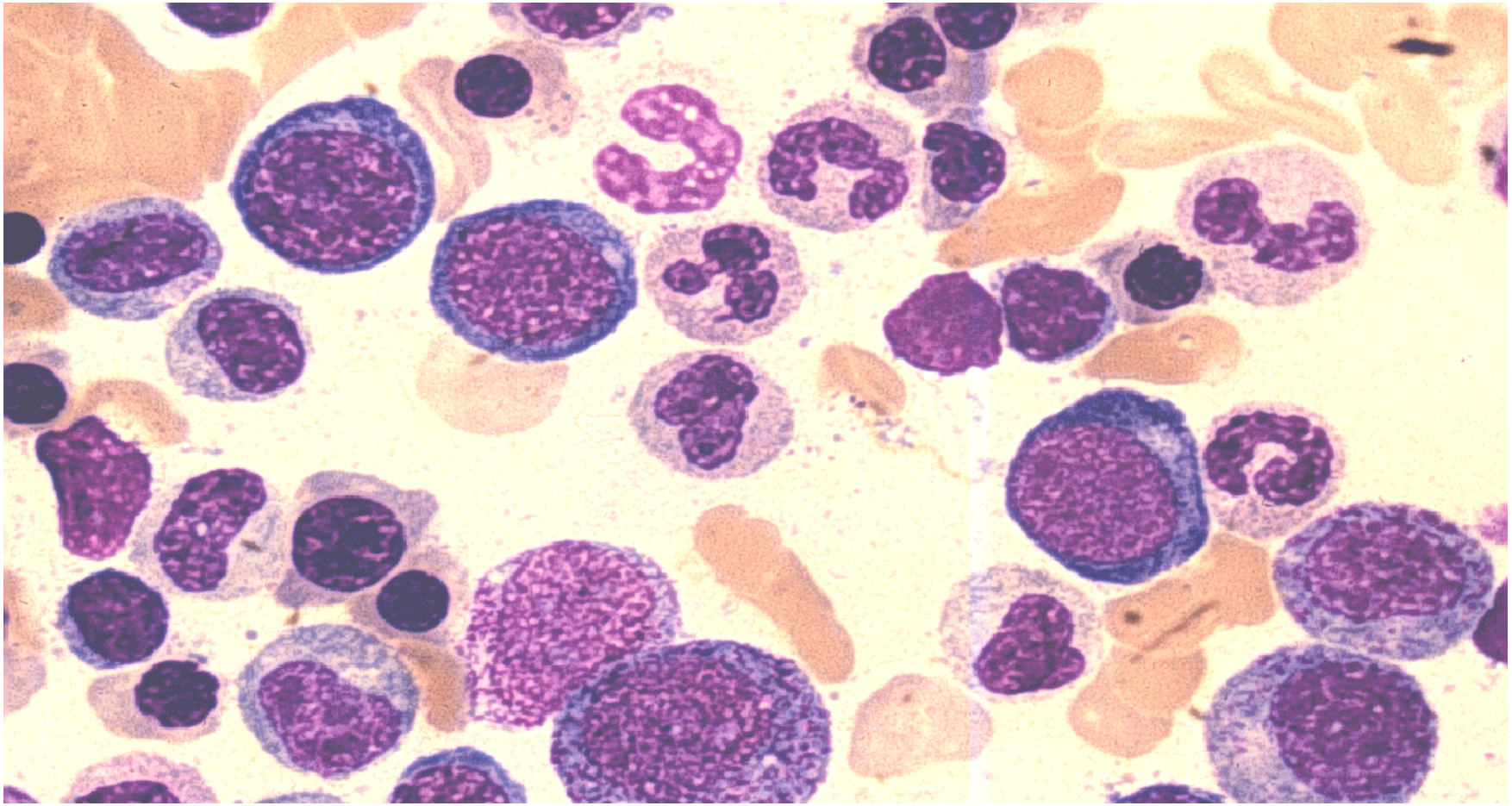
Background

- Bone Marrow Function
- Bone Marrow Transplantion
- Mobilization techniques [molecular basis]
- Mobilization success & failure
- Mobilized product [Clinical aspect]

Bone Marrow: Function



Bone Marrow: Function

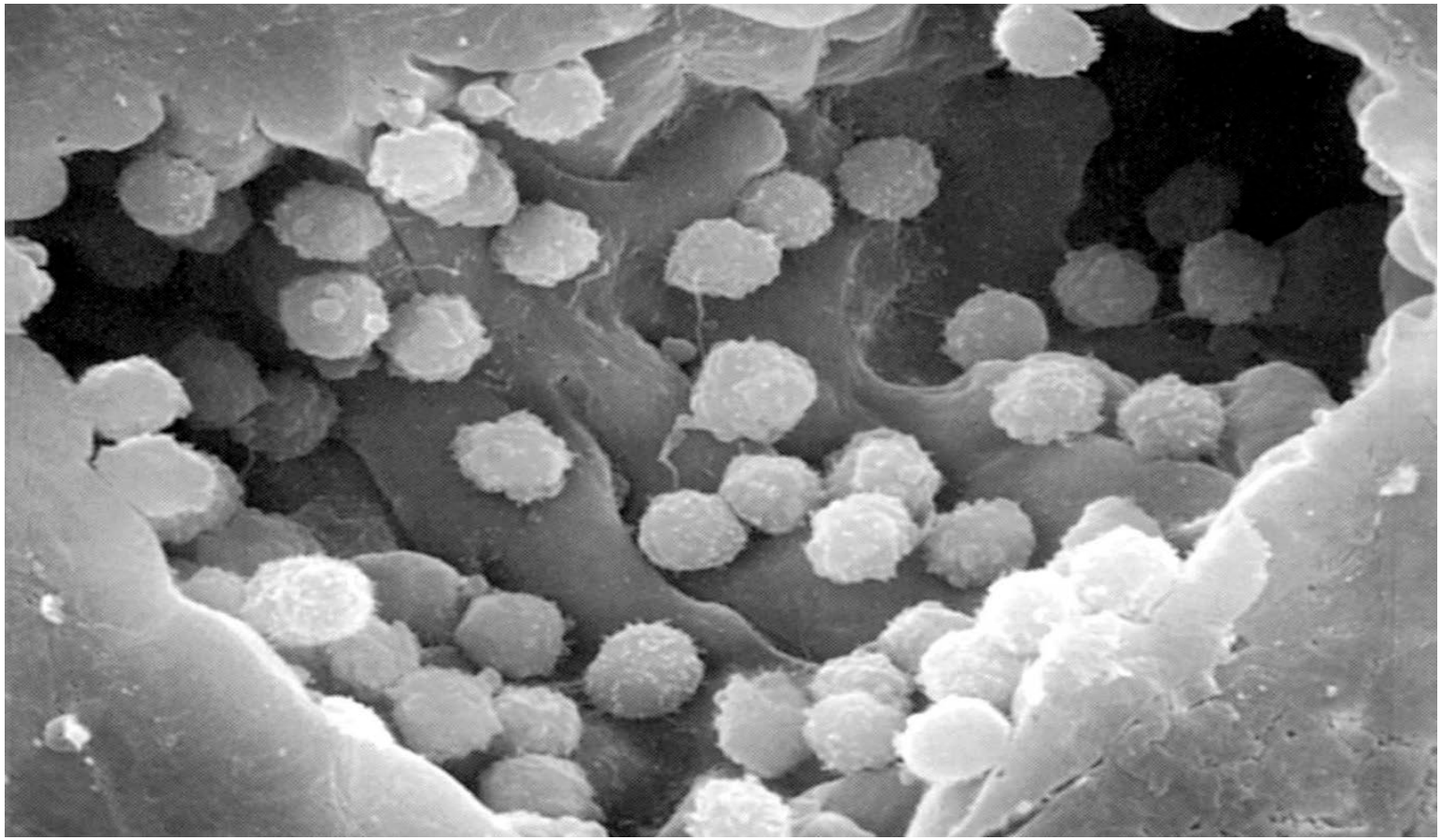


Bone Marrow: Function

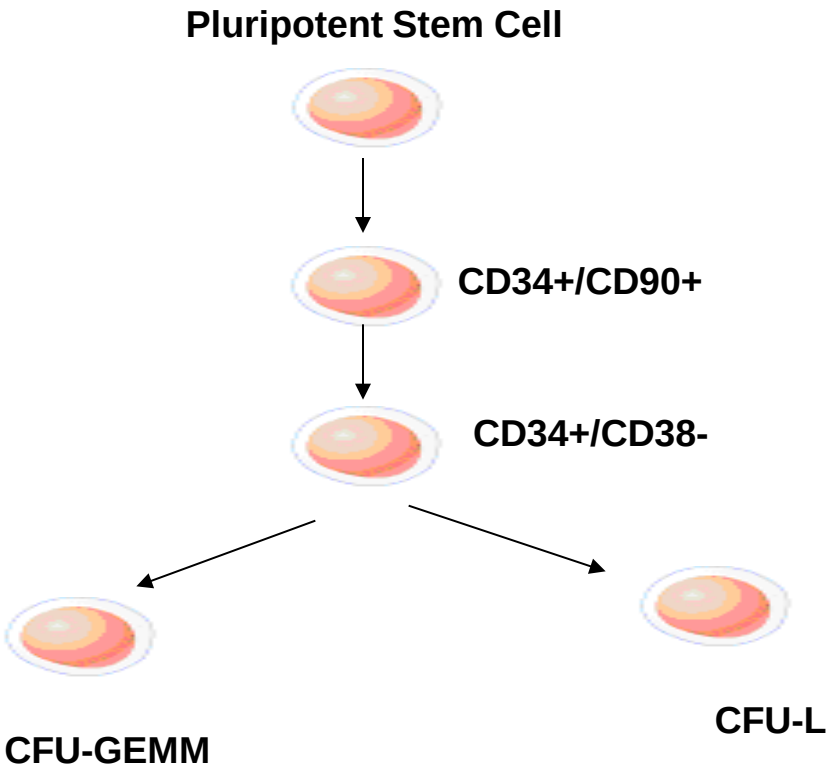
- Production of red blood cells, white cells, and platelets
  - 175 billion red cells/day
  - 70 billion granulocytes/day (neutrophils, eosinophils, basophils)
  - 175 billion platelets/day
  - Capable of 5-10 fold increase in production

*****1- 0.1% human bone marrow cells are early hematopoietic cell precursors**

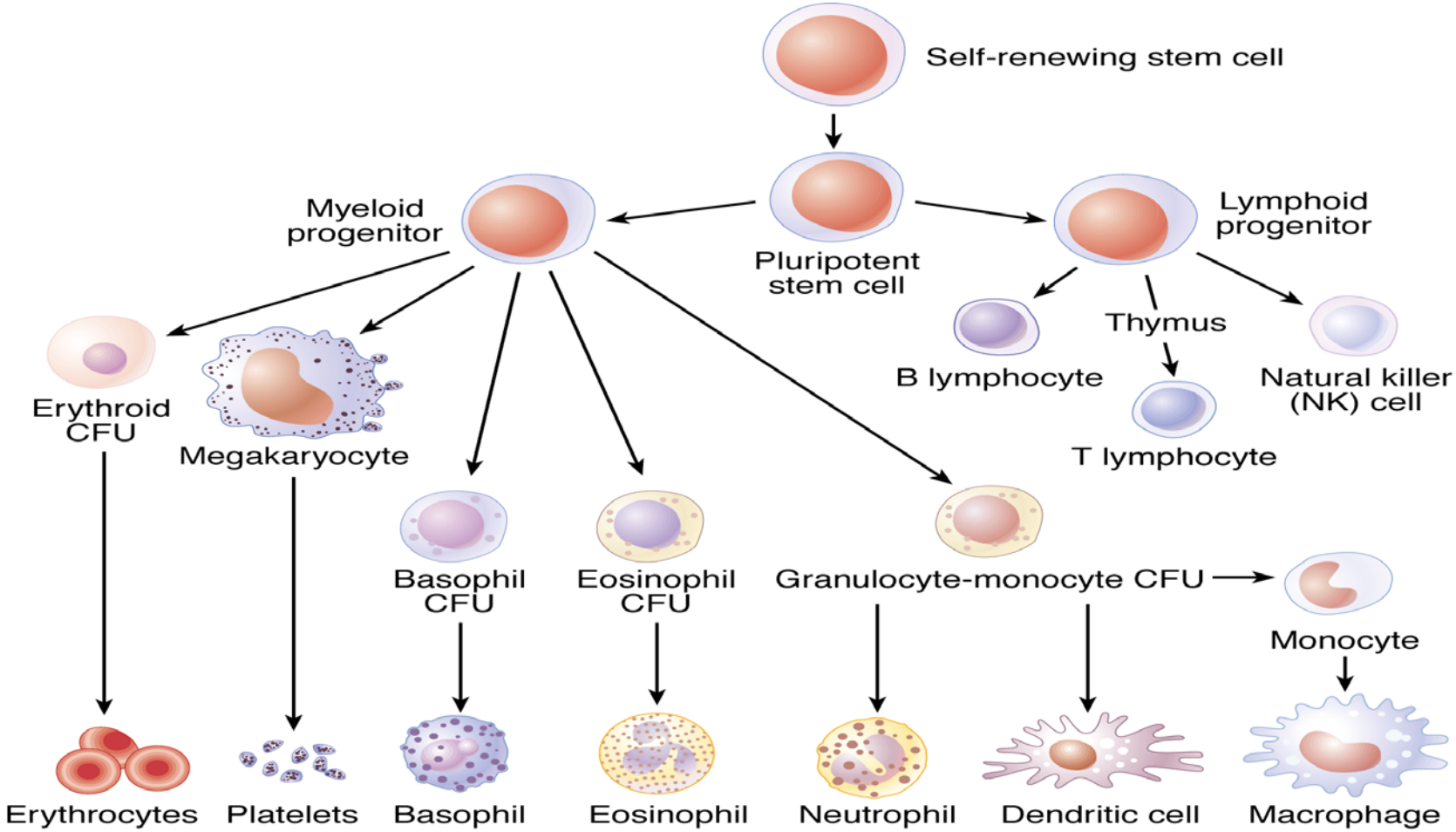
Hematopoetic cell trafficking



Experimental Model of Early Hematopoiesis



Bone Marrow hematopoesis



Types of stem cell for transplantation

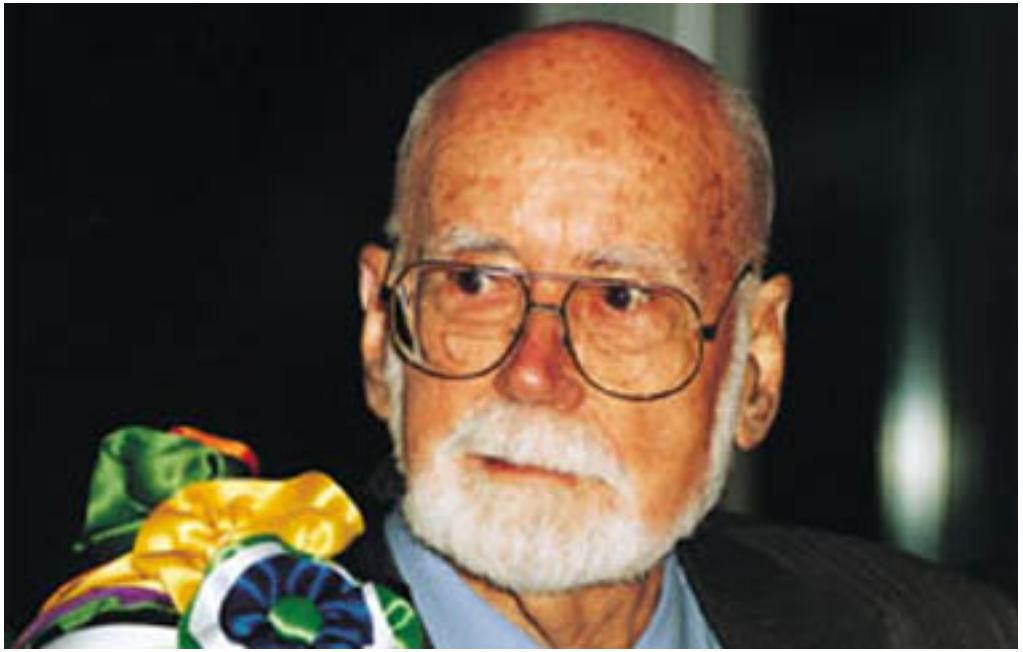
- Autologous adult
- Allogeneic adult
- Foetal cord blood
- Mesenchymal

History of BM transplantation

- 1959 – 1st marrow infusion
- 1968 – 1st successful BMT
- 1981 – 1st thalassaemia Tx
- 1988 – 1st cord blood transplant

The Nobel Prize, 1990

E. Donnall Thomas



first succsessful clinical HSCT in treatment of acute leukemias

Thomas' work showed that bone marrow cells infused intravenously could repopulate the bone marrow and produce new blood cells.

Thomas ED, Lochte HL, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N. Engl. J. Med. 1957; 257: 491.

Hematopoetic stem cell harvesting

How can we get hematopoetic stem cell ?

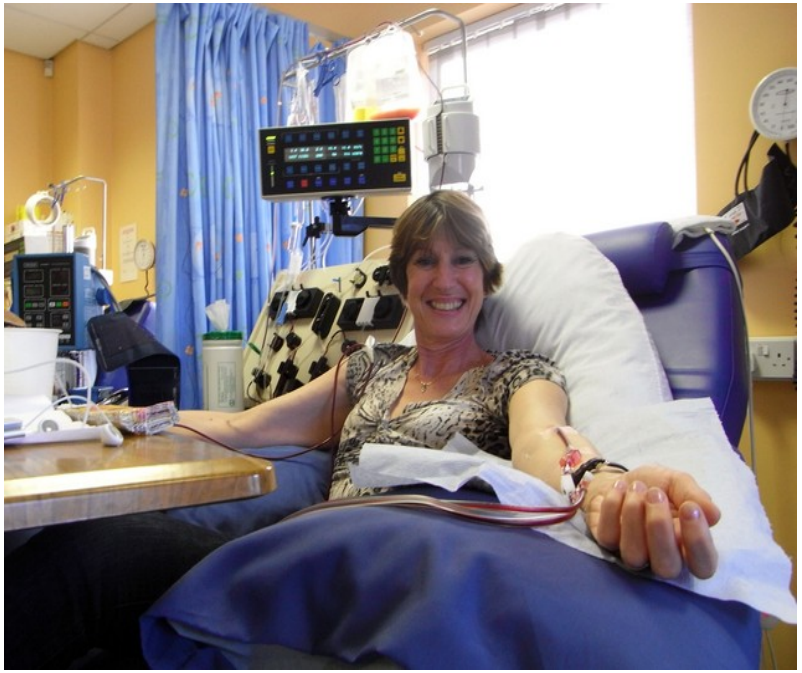
- Bone marrow harvesting
- Peripheral blood harvesting

Bone marrow harvesting

- **General anaesthetic**
- **Marrow aspirated from pelvis (+sternum)**
- **Marrow filtered to remove debris**
- **Marrow may be administered “fresh” or cryo-preserved**

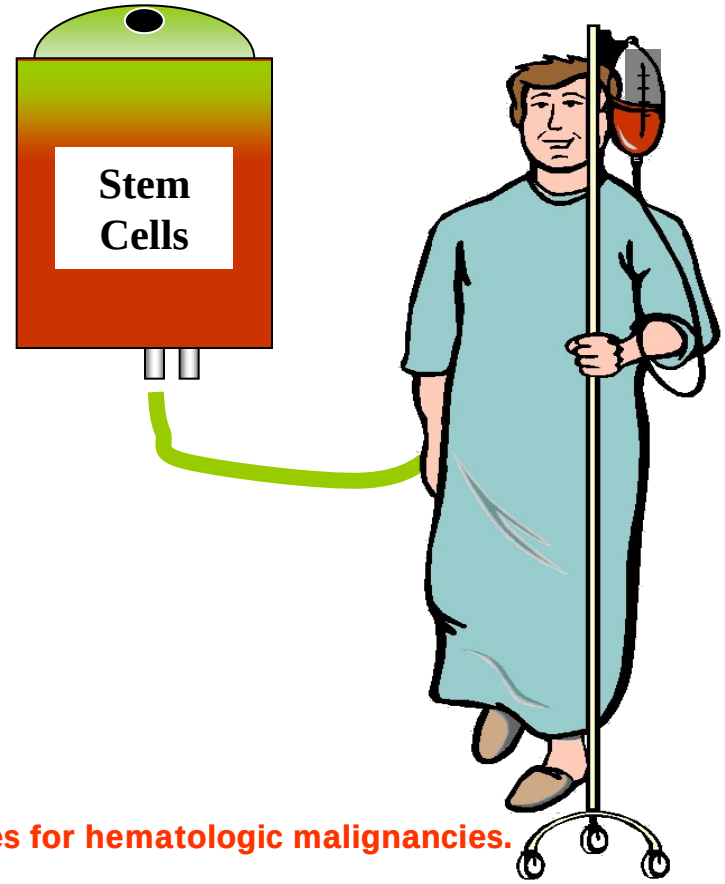
Peripheral blood harvesting

- **Stem cells mobilised –**
  - G-CSF in healthy donors
  - Cyclophosphamide + G-CSF in patients
- **On day 5 (donors), day 10 (patients)**
  - 3 hours session on stem cell collection machine
- **Stem cells are given fresh or cryopreserved**



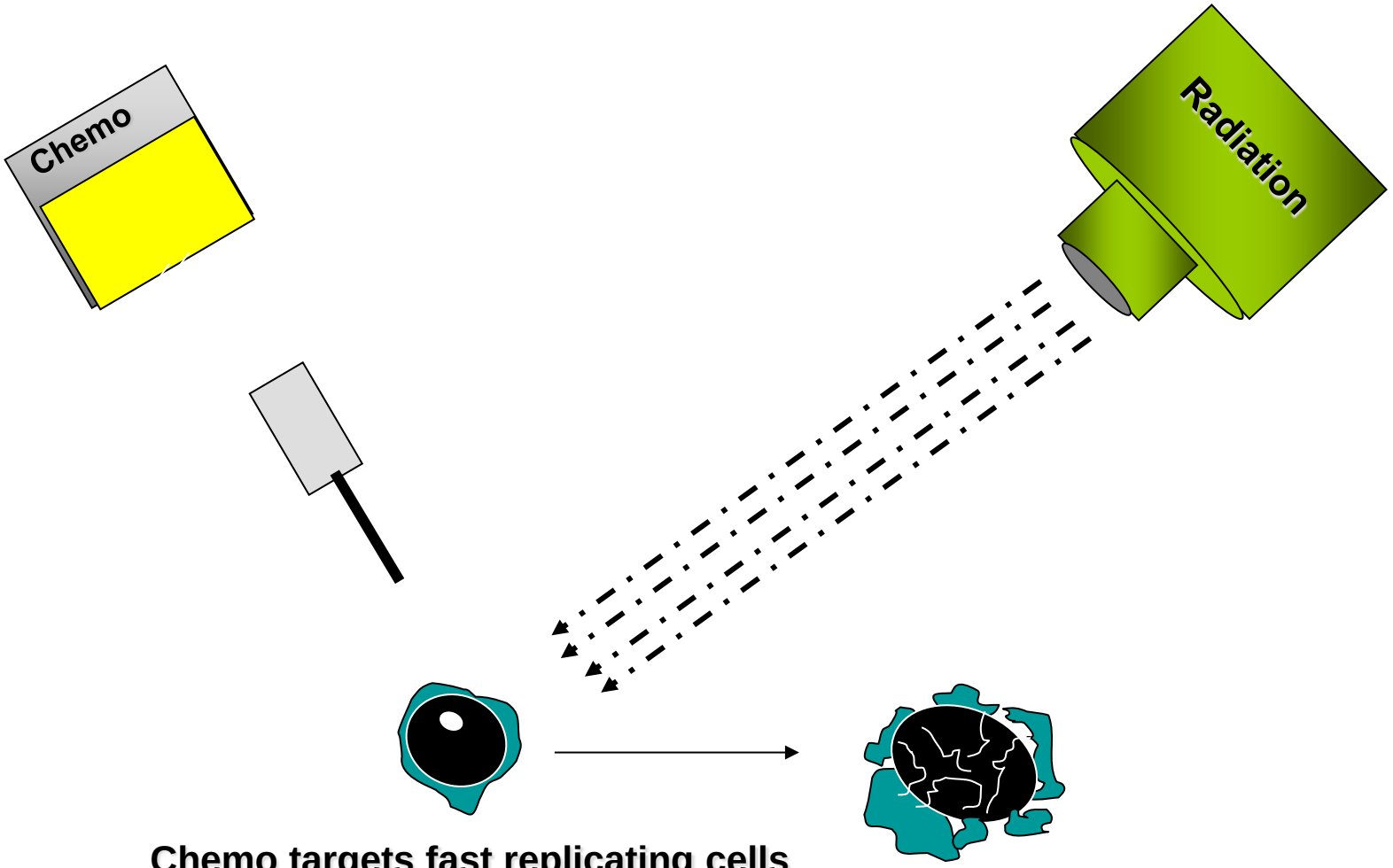
Hematopoietic stem cell transplantation

- Intravenous infusion of autologous or allogeneic stem cells
  - Collected from bone marrow aspiration, peripheral blood or umbilical cord blood
- Re-establish hematopoietic function in patients with damaged/defective bone marrow or immune systems
- Potentially curative for a wide variety of disorders



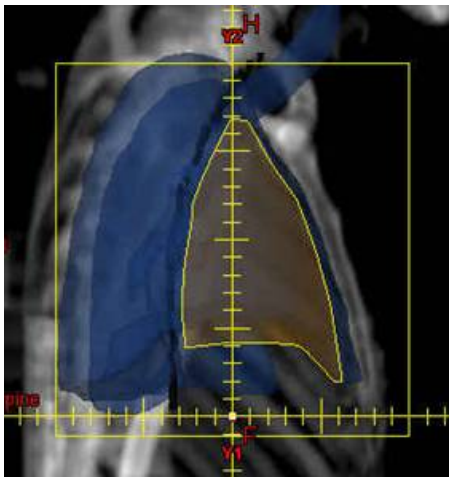
Lazarus HM. Autologous and allogeneic transplantation procedures for hematologic malignancies. Manual of Clinical Hematology, 3rd edition 2002:399-409

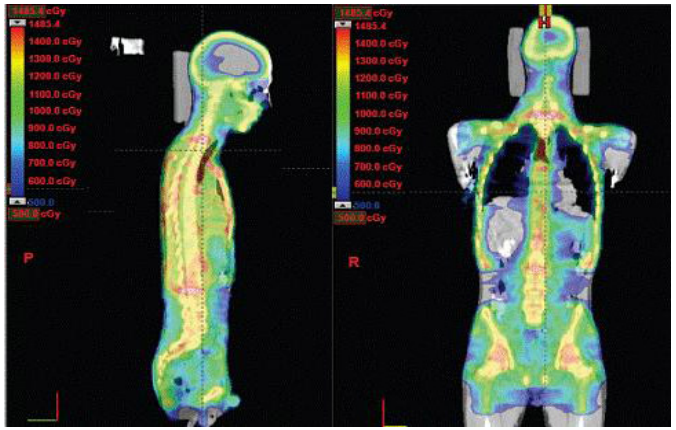
Stem Cell Transplant: Effects of Treatment

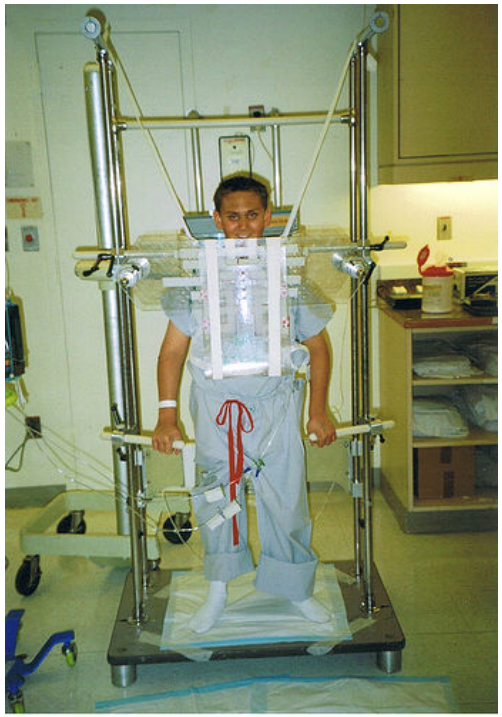


Myelo-ablation

- Total body irradiation 10-12 Gy (fractionated) + cyclophosphamide
- Cyclophosphamide + busulfan

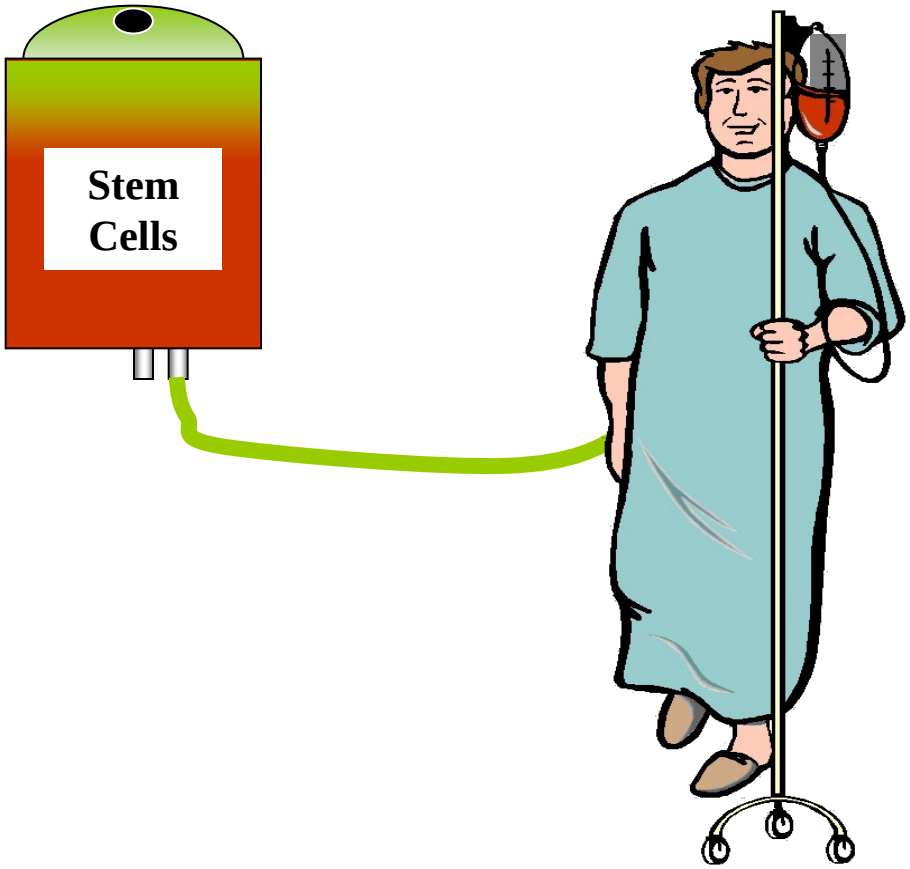


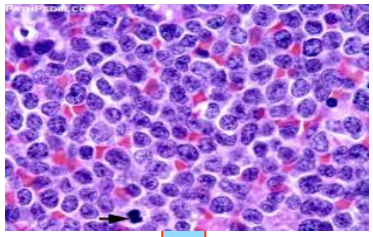




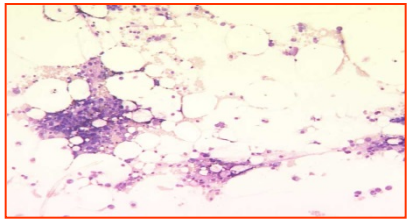
Nb 4.5 Gy fatal in 50% exposed individuals

Engraftment & cure

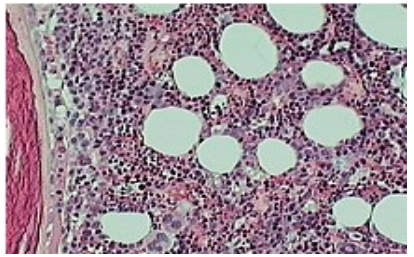




Tumor



Myelo-
aplasia



Graft

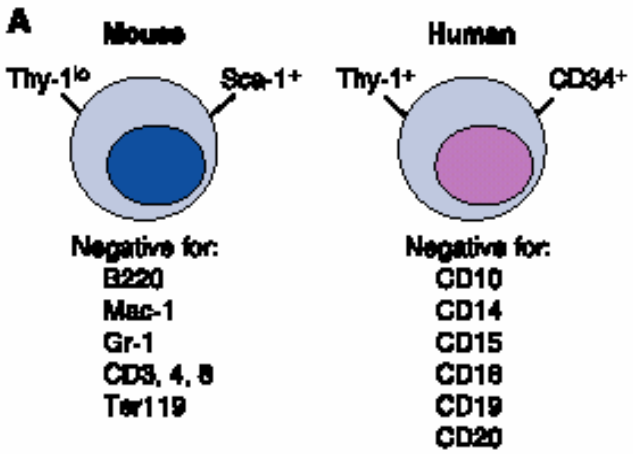
Normal
Marrow

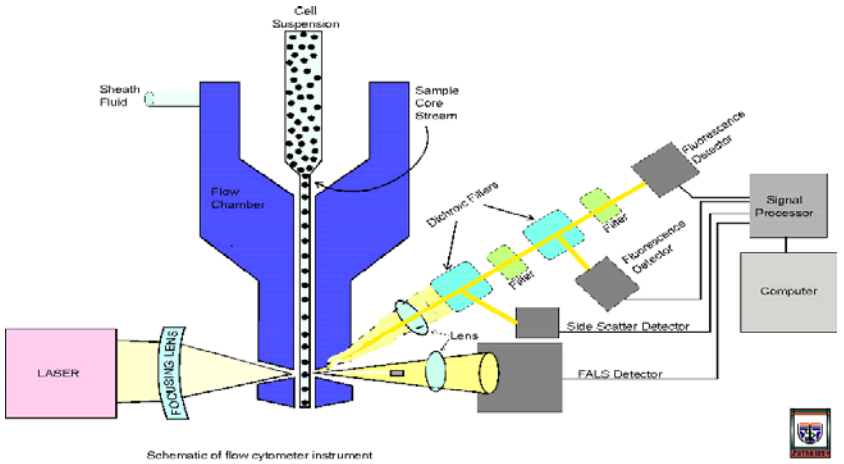
BMT/ PBSC Transplants: Indications

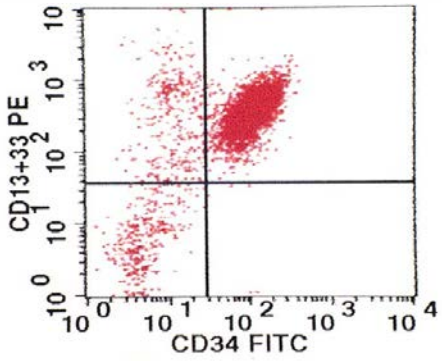
- Leukemias (acute and chronic)
- Lymphomas (Hodgkin's and NHL)
- Multiple myeloma
- myeloproliferative disease
  - (myelofibrosis, polycythemia vera, essential thrombocytosis)
- Aplastic anemia
- Metastatic breast cancer *
- Some metabolic diseases
- Stem cell rescues for many childhood cancers (neuroblastoma, sarcomas, high grade gliomas, Wilm's tumor)
- Congenital immunodeficiencies (e.g., CGD, SCID, Wiskott-Aldrich)
- Rheumatologic/autoimmune diseases (RA, jRA, Lupus, AIHA)
- Hemoglobinopathies
- Metabolic diseases (osteopetrosis, Hurler's, adrenaleukodystrophy)

Hematopoetic stem cell (immunophenotyping)

CD34+, Thy1+, CD38-, HLA-DR-, c-kit+, Lin-, AC133+, CD33-, CD4-, CD8-, CD14-, CD19-,

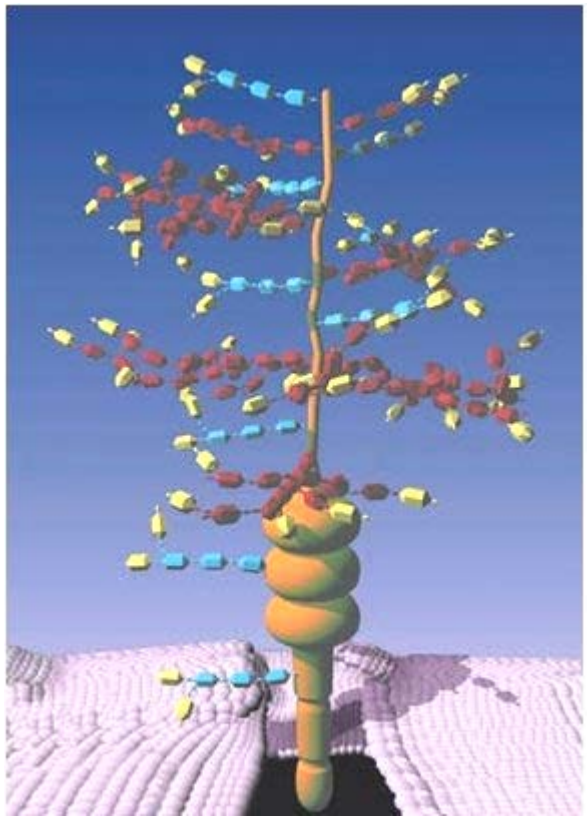






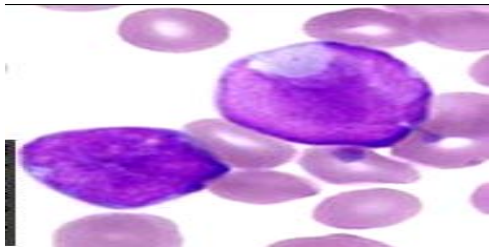
Magic seed:
-HSC
-CD34+ ??

What is CD 34 anyway ?



- 105-120 kDa transmembrane Glycoprotein
- Present in early hematopoietic cell precursors
- Present in 0.1% of peripheral mononuclear cells
  1-4% human bone marrow cells

Probably an adhesion molecule.



CD34 Cells

Number of cells correlates with engraftment

Number or cells correlates with speed of engraftment

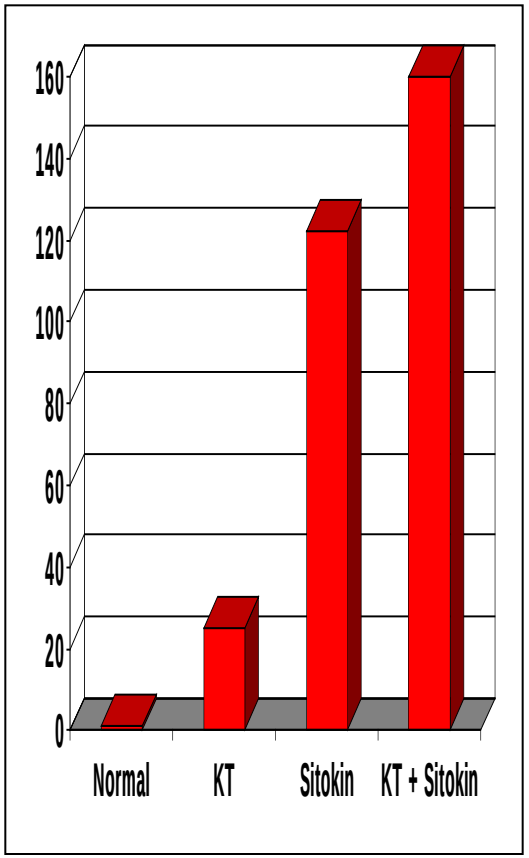
- **1 – 2 x 10^6 / Kg (ideal body weight) is considered "sufficient"**
- **2 – 2.5 x 10^6 / Kg (more acceptable dose for engraftment)**
- **5 x 10^6 / Kg (gives more rapid engraftment and lower incidence of graft failure**

Further increases, decrease the time to platelet engraftment

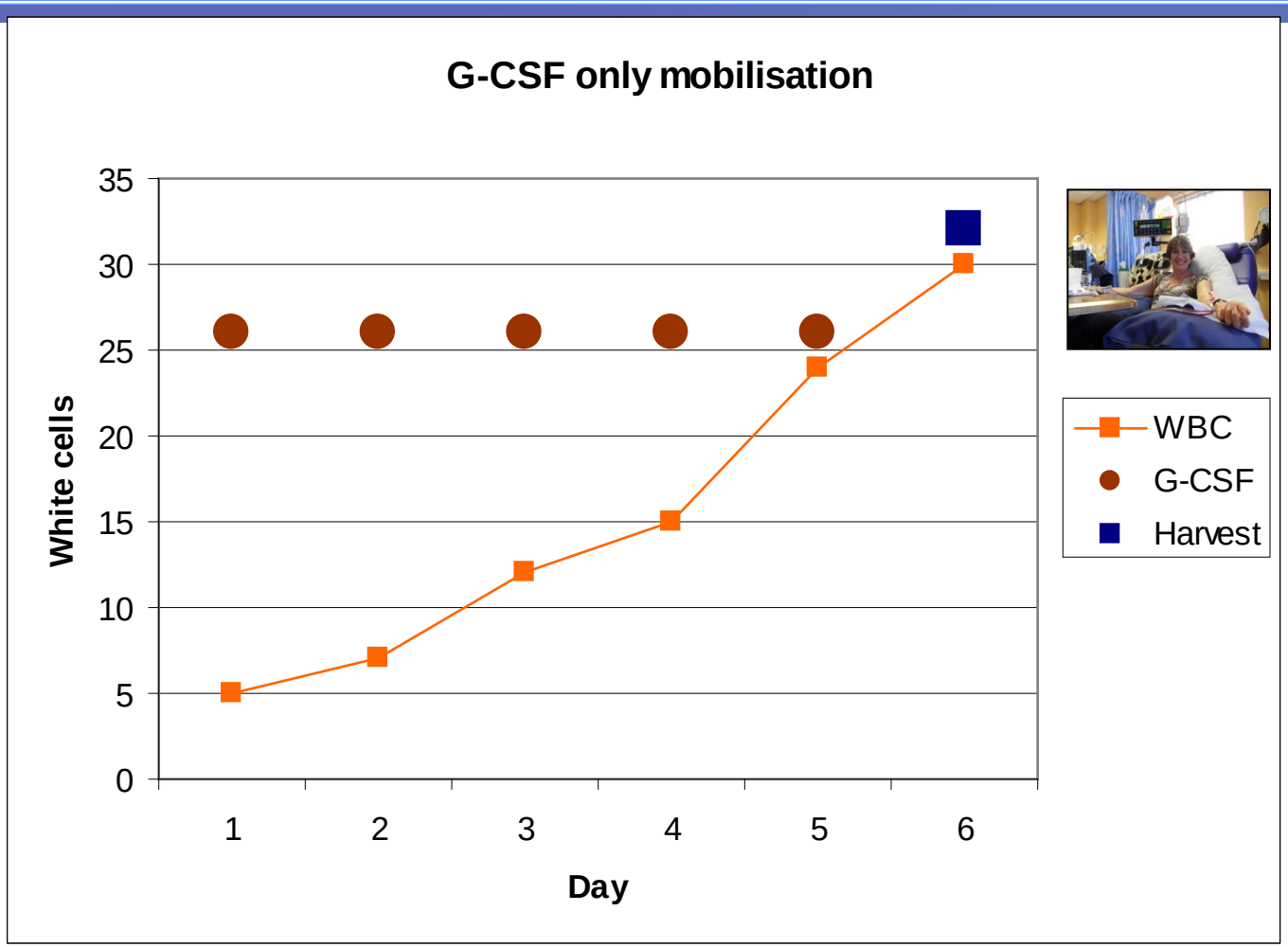
The quantity of viable stem cells given have been shown to correlate with time it takes for the cells to engraft. Basically, the more cells, the faster, up to a certain point.

PBSC Mobilization Regimens

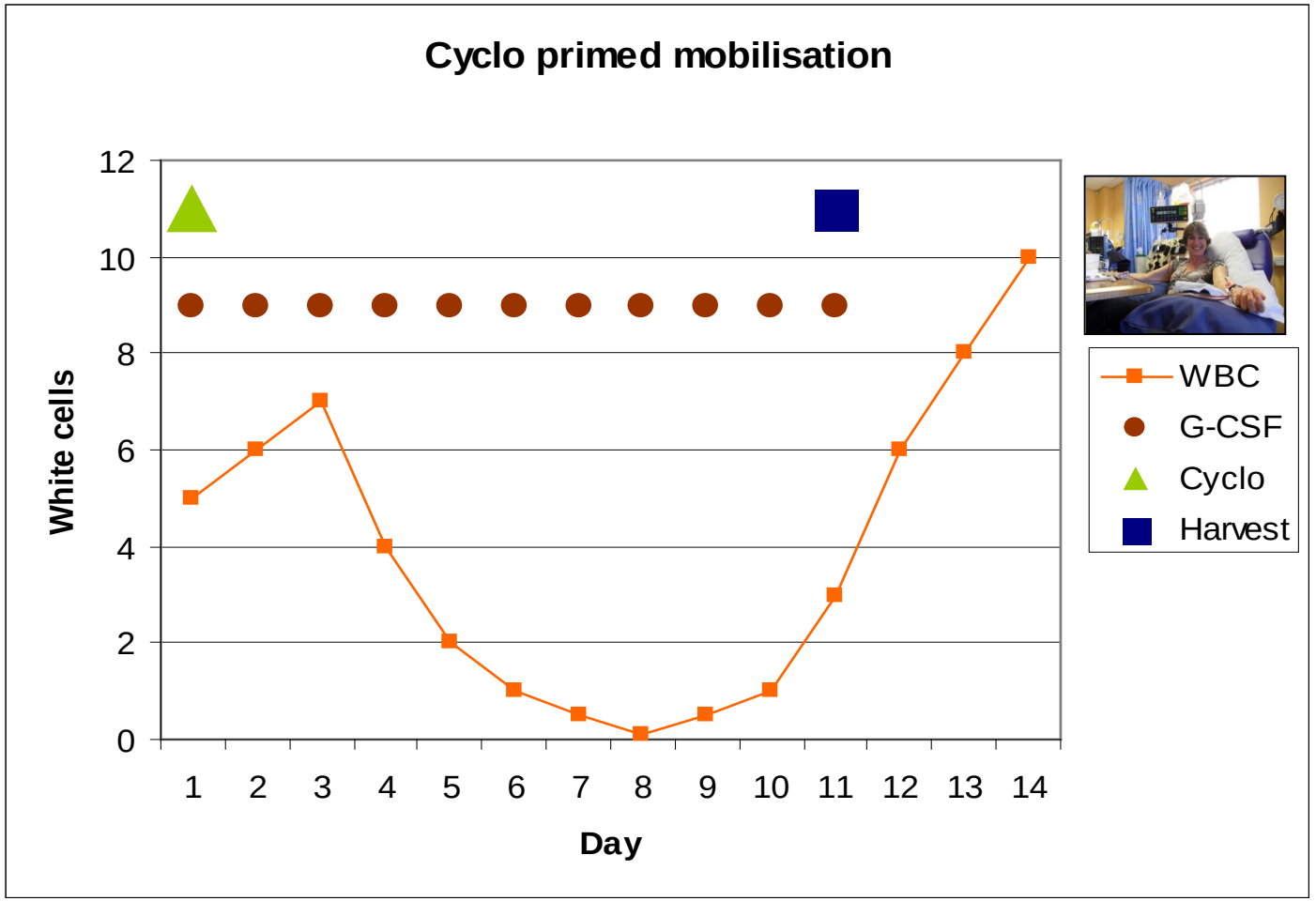
- Haematopoietic growth factors (G-CSF)
- Chemotherapeutic agents + G-CSF
- Plerixafor in combination with G-CSF



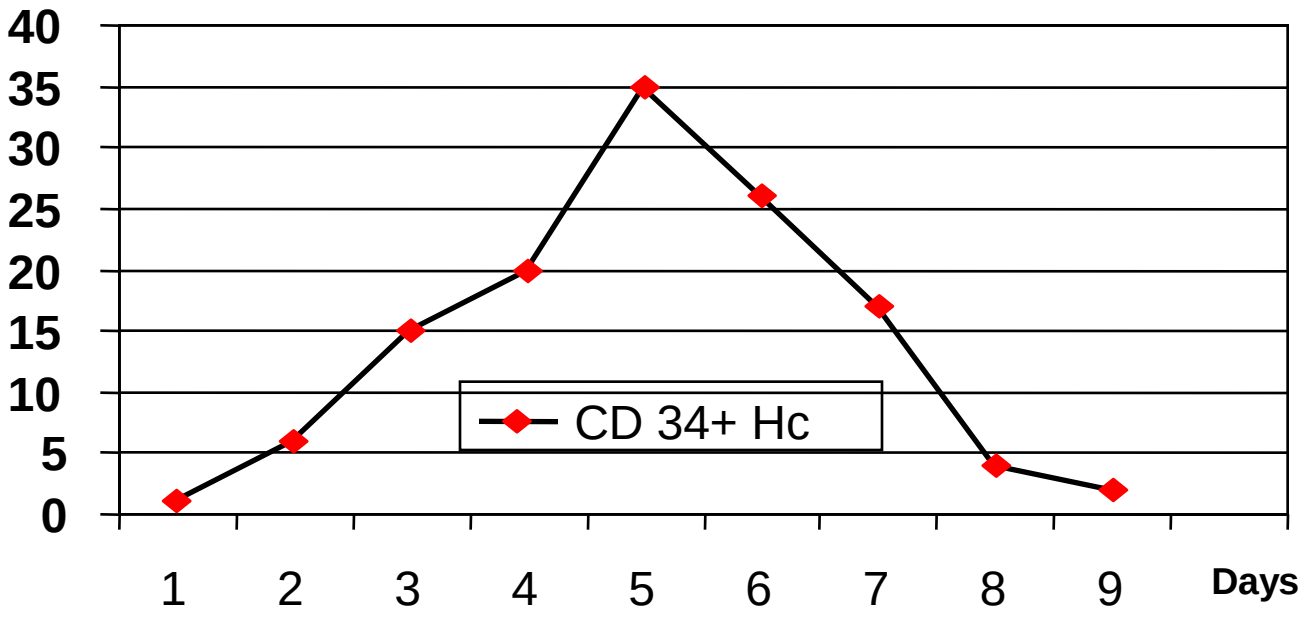
G-CSF mobilisation protocol



Cyclophosphamide mobilisation



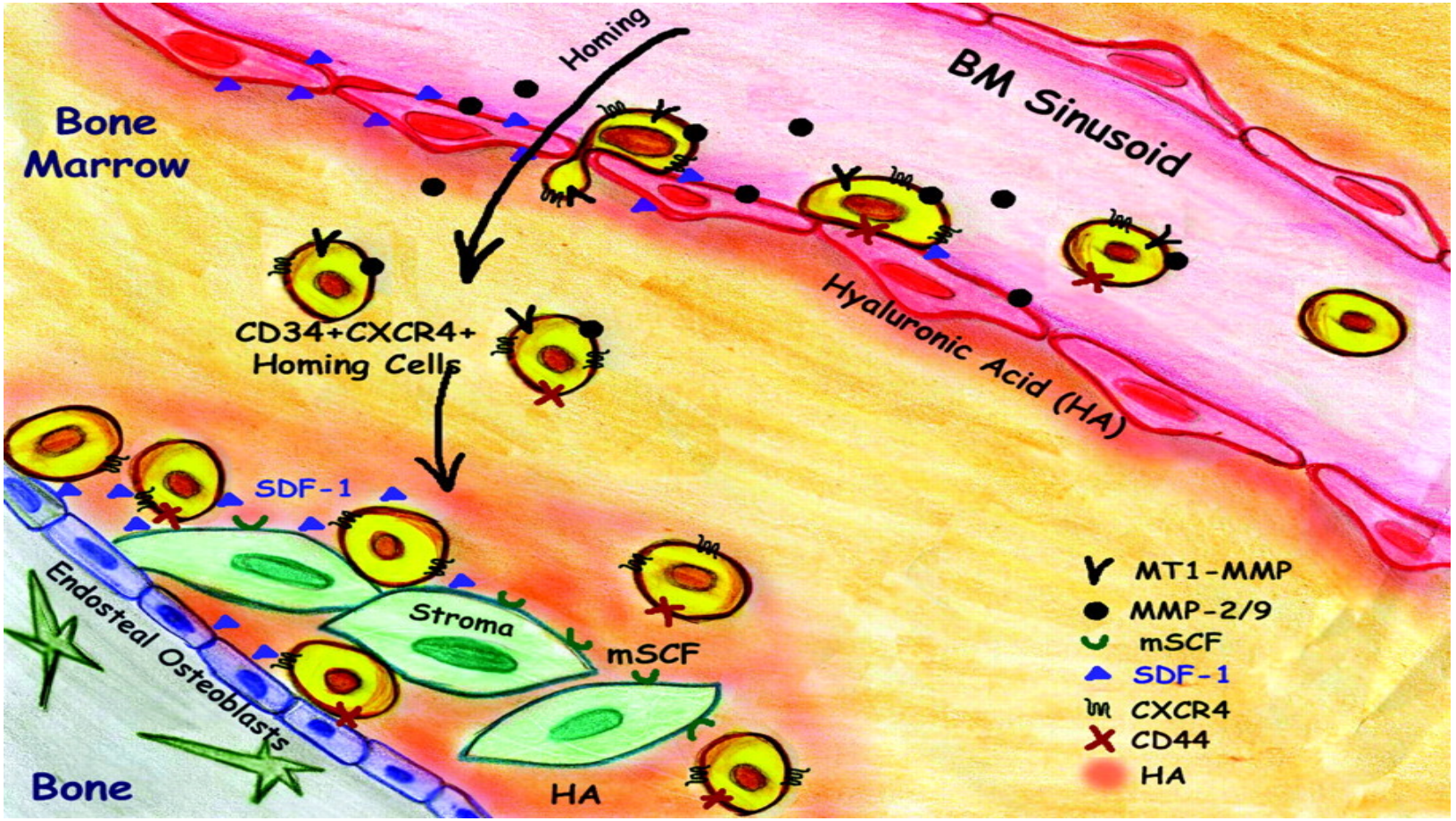
G-CSF mobilization & harvest day



Bishop MR, et al. Allogeneic-blood stem-cell collection following mobilization with low-dose granulocyte colony-stimulating factor. Clin Oncol 1997; 15: 1601-1607

Stem cell homing and stromal adhesion

The role of chemokins (SDF-1, CD44, HA, etc.)



[T. Lapidot et al., 2003]

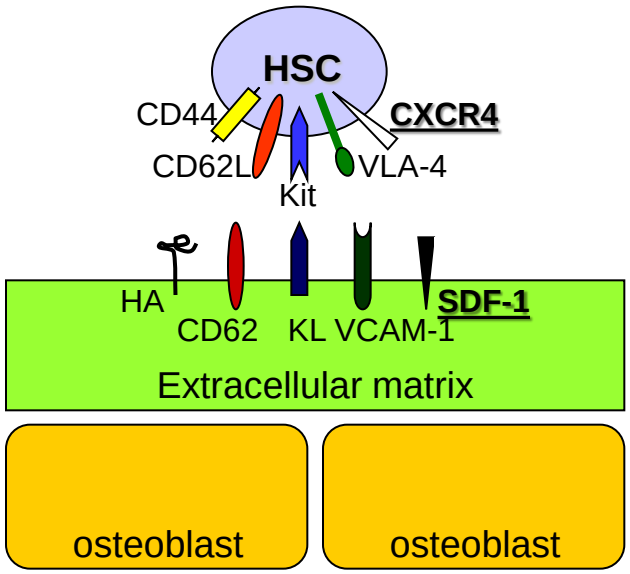
G-CSF mobilization

The role of proteases (NE, CG, and MMP9)

a

adhesive interactions between HSC and BM:

cell adhesions molecules



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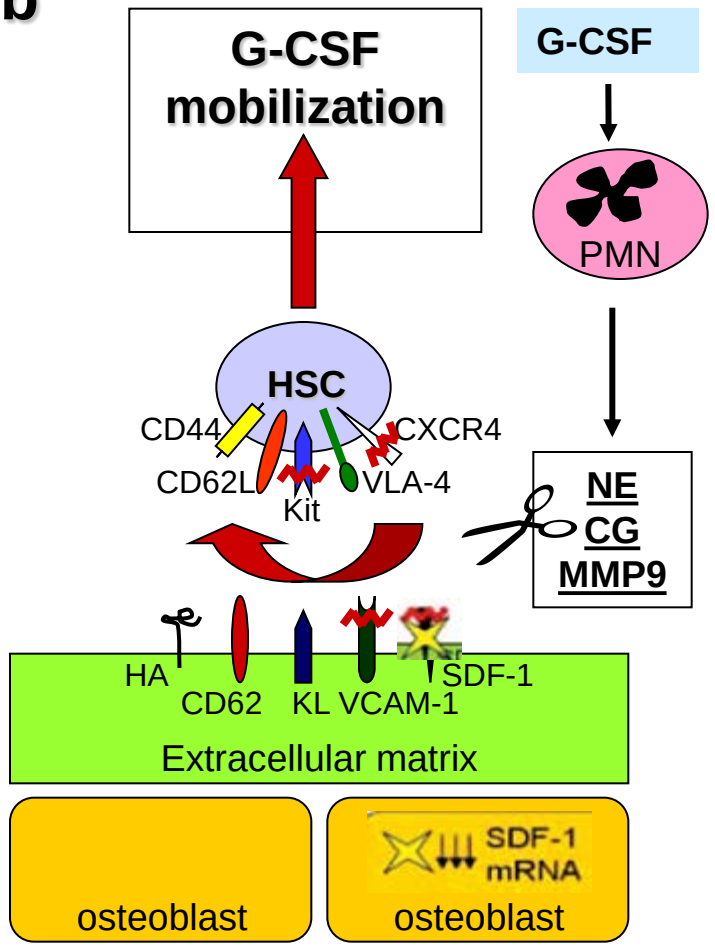
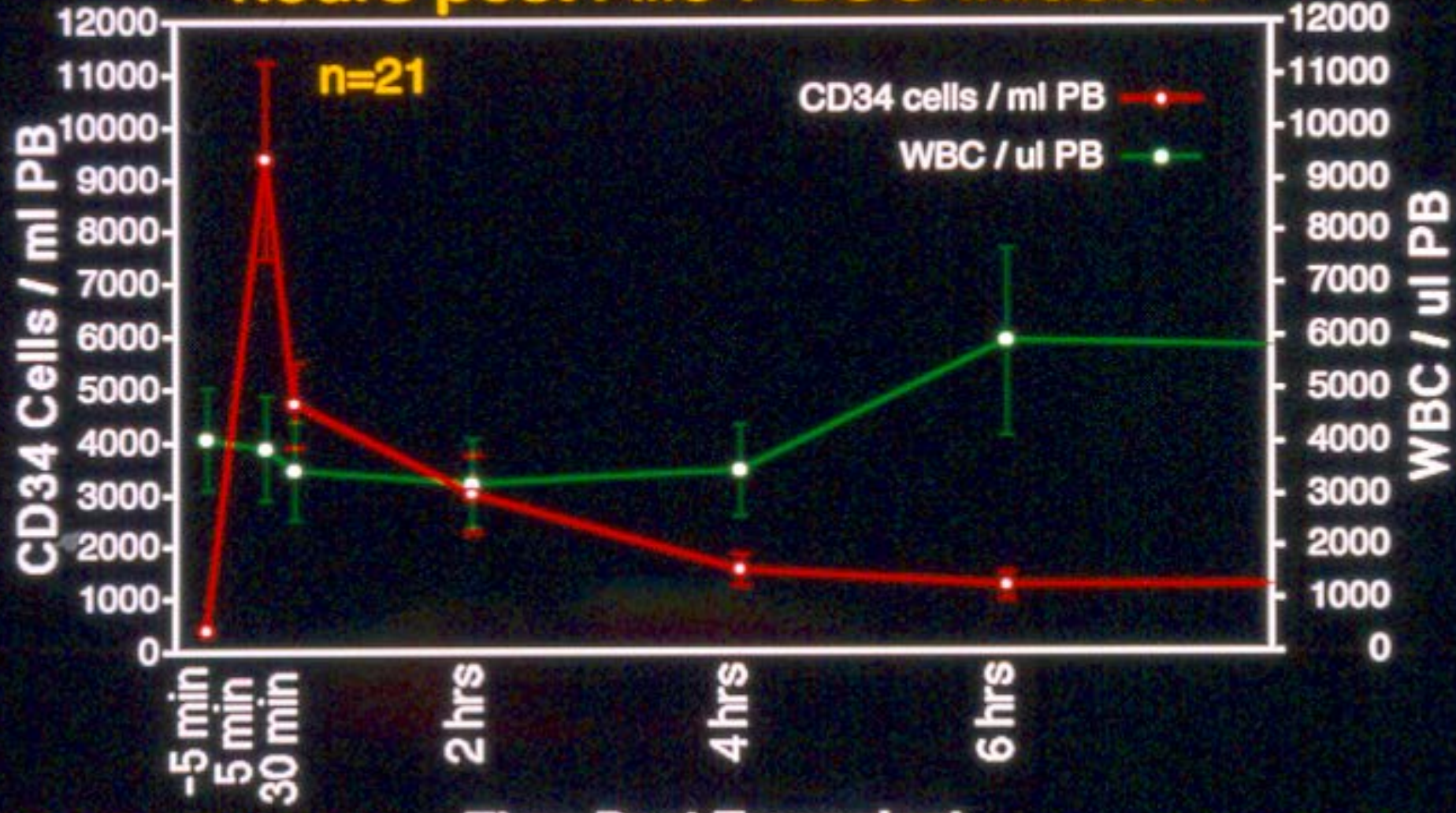


Mobilization Of Stem Cell From Marrow

- Cytokines clearly play a role
- Cytokine administration is used to induce stem cell mobilization
  - Increase the metalloprotease expression
  - Release stem cell factor from stromal cell surface
  - Induce stem cell migration through endothelial barrier
- Homing to the bone marrow
- Mobilizing from the bone marrow
  - Dynamic and continuous

Circulating CD34 and WBC: Zero to 6 hours post Allo PBSC infusion



* Peripheral IV r=.9
* CFU-GM r=.9
* no differential expression of VLA-4 or C-kit

Outcomes of standard mobilisation regimens

- Mobilisation failure rates for MM and NHL are approximately 5% and 20%–40%, respectively

- In a recent retrospective analysis of 1040 patients who underwent aHSCT, failure (defined as collection of $< 2 \times 10^6$ CD34+ cells/kg after 5 days of apheresis) was observed in 5.9–6.3% in MM and 22.9%–26.8% in NHL[1]

- When multiple apheresis sessions are required to collect a sufficient number of CD34+ cells for transplantation, the overall costs of treatment increase

1. Pusic et al. *Biol Blood Marrow Transplant* 2008;14:1045–56

Consequences of suboptimal mobilisation

- Failure to mobilise a sufficient number of CD34+ cells might result in
  - Ineligibility for transplantation
  - Increased number of apheresis days
  - Need for bone marrow harvest
  - Repeated attempts at mobilisation
  - Increased resource utilisation
- Use of suboptimal apheresis product yield may lead to
  - Delayed, partial, or failed stem cell engraftment
  - Potential for increased risk of infections and/or bleeding
  - Increased need for transfusions

Known risk factors for suboptimal mobilisation

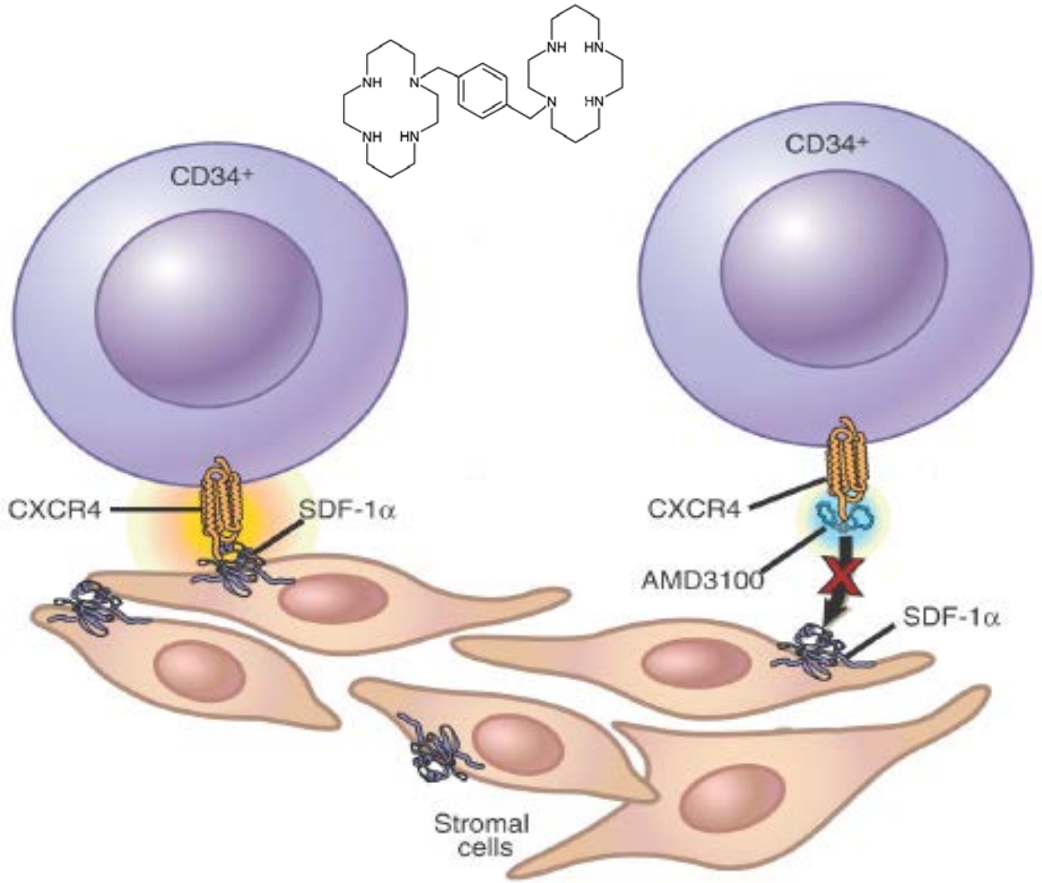
- Patient characteristics
  - Age > 60 years
  - Underlying disease
  - Previous RT and chemotherapy
  - Multiple chemotherapy cycles
  - Previous treatment with melphalan, carmustine, fludarabine
  - Novel induction strategies (e.g. lenalidomide in MM)

Poor Mobilizer

- < 10 $CD34^+$ cells/µL
- < 1 $x10^6$/kg $CD34^+$ cells in 1-2 large volume aphereses
- Alternatives
  - G-CSF (16-20µg/kg)
  - GM-CSF**, SCF**, IL-3**
  - Pegfilgrastim (Neulasta®)
  - Plerixafor (Mozobil®)

** high rates of side effects

Plerixafor: Mechanism of Action



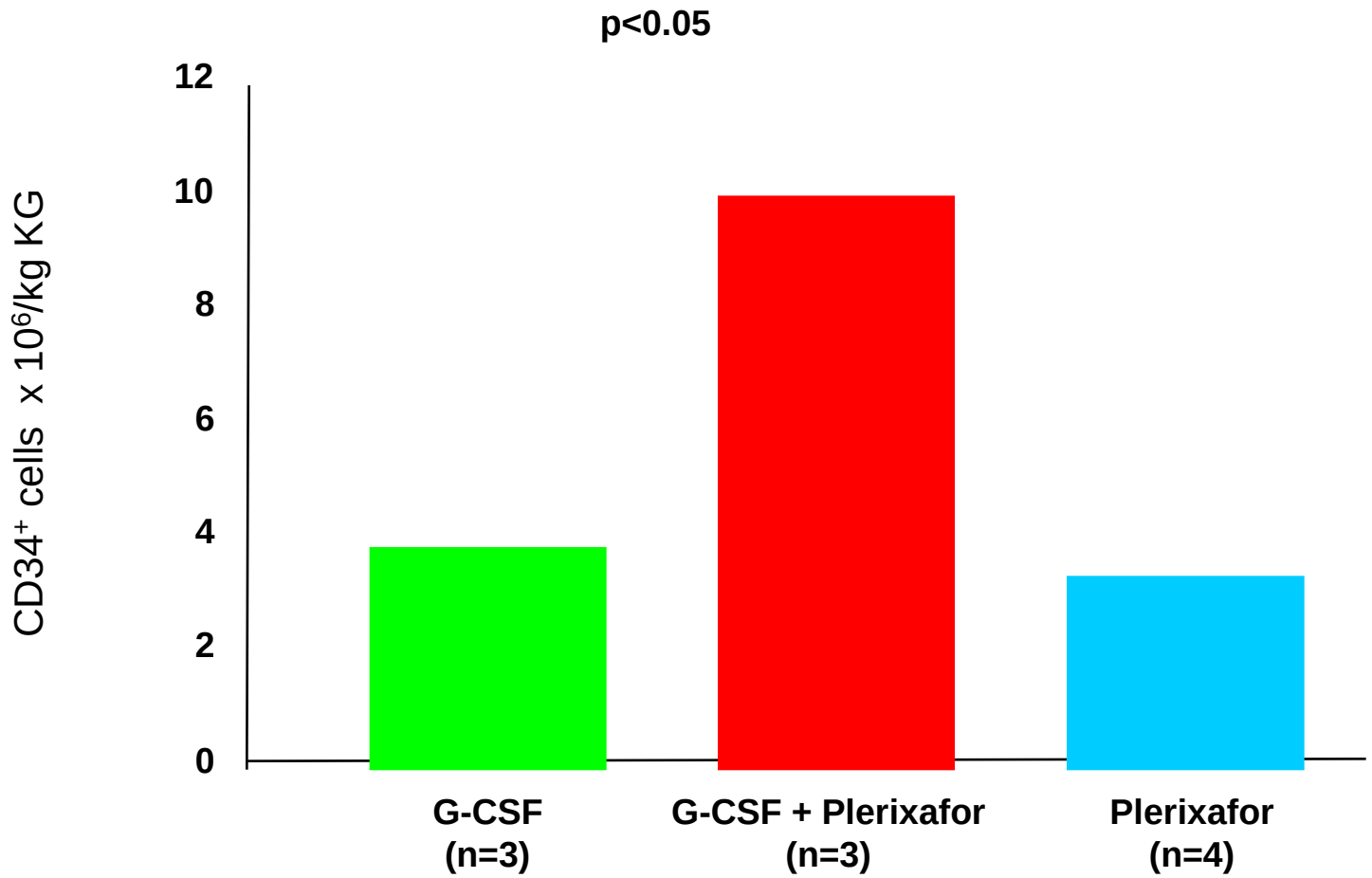
Lapidot T and Petit I, *Exp Hematol*. 2002

SDF-1α and CXCR4 play key regulatory roles in stem cell trafficking to, and retention by the bone marrow.

Plerixafor blocks the CXCR4/SDF-1α interaction, releasing stem cells from the bone marrow into the circulating blood.

Martin et al, *BJH* 2006

Plerixafor increases with G-CSF the CD34+ Collection

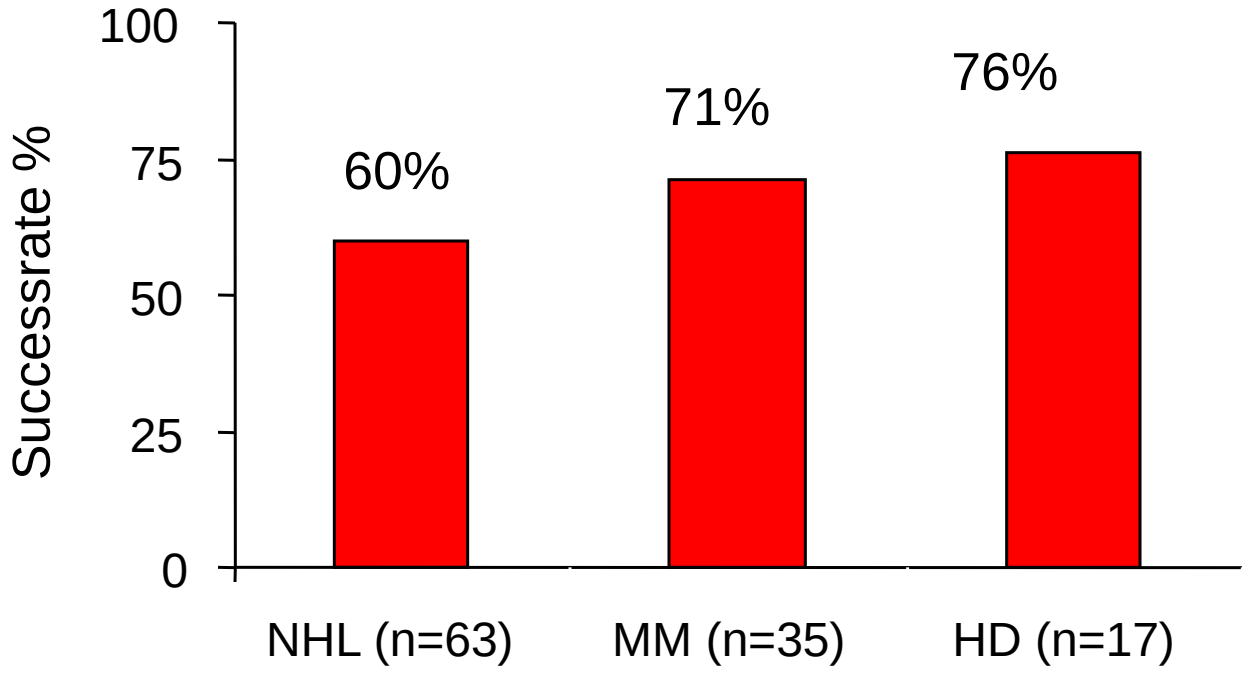


Liles WC, et al. *Transfusion*. 2005;45:295.

Compassionate Use Data (CUP)

>2 x10^{6} CD34^{+} /kg (Plerixafor + G-CSF) in 115 pts.

Overall success 66% after Plerixafor re-Mobilisation



Calandra G, et al. BMT 2008

Conclusions

- HSCT has become a routine procedure in the treatment of a variety of diseases.
- The source and procurement of hematopoietic stem cells has changed over time.
- PBSC donation is a safe procedure as BM.
- The addition of plerixafor to G-CSF ± CHT offers a new approach to reduce mobilisation failures in patients demonstrating poor mobilisation.

1st International Conference/Workshop on Stem cell research and applications

Erciyes University

October 7-9th, 2011, Kayseri, Turkey
Pre-conference Workshop: October 6th, 2011

Thank you

for your

attention