Stem Cell Collection & Cryo Preservation

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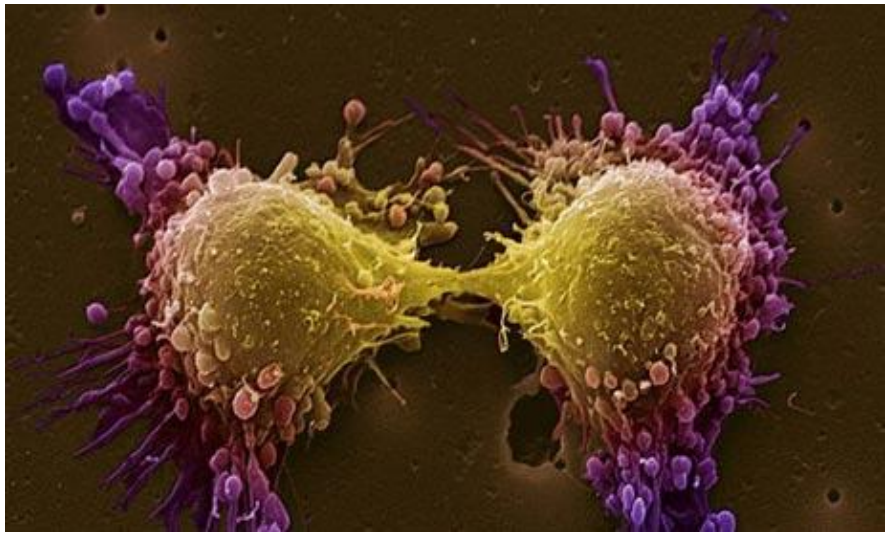
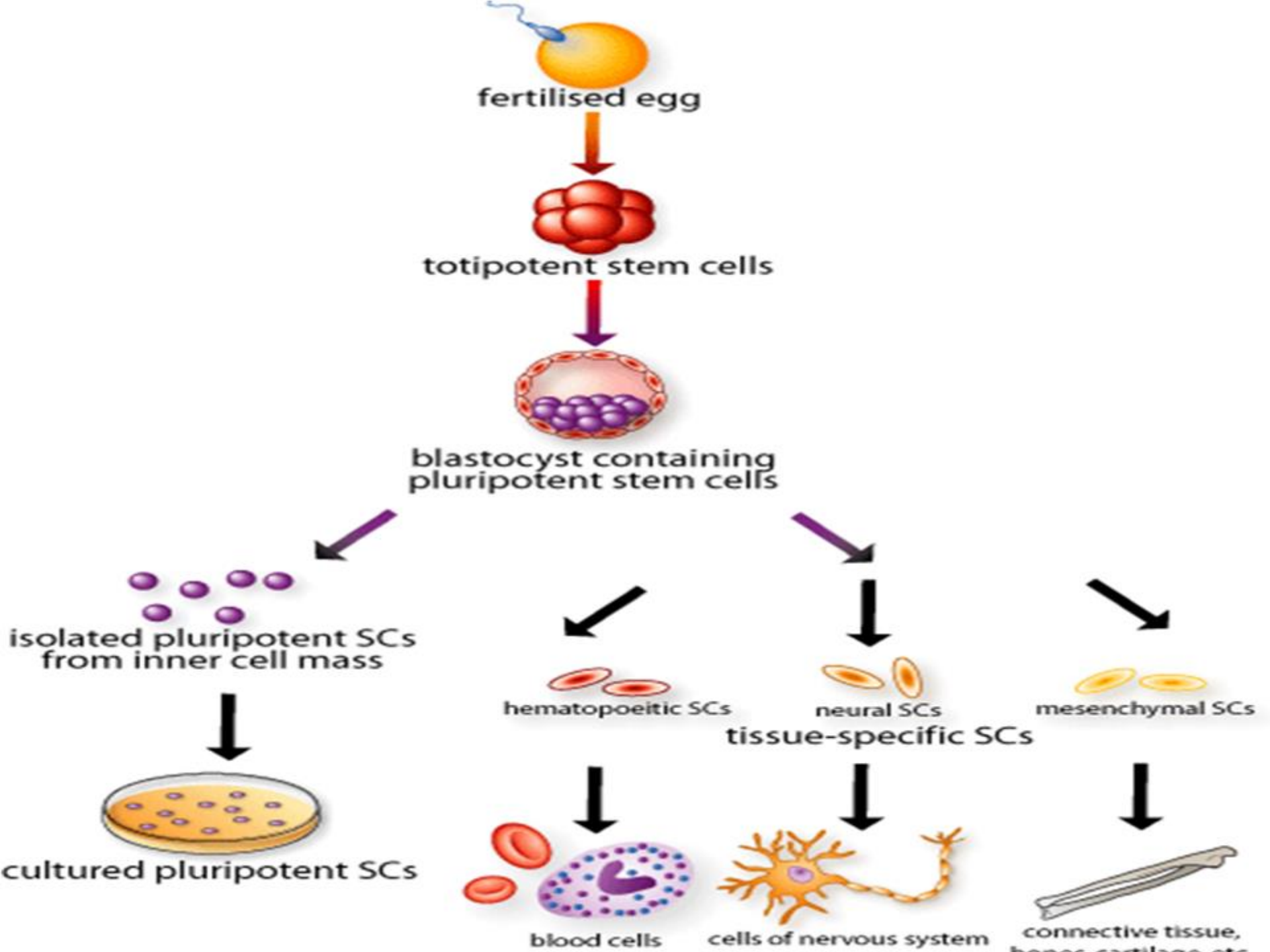
Stem Cell
– *Definition*



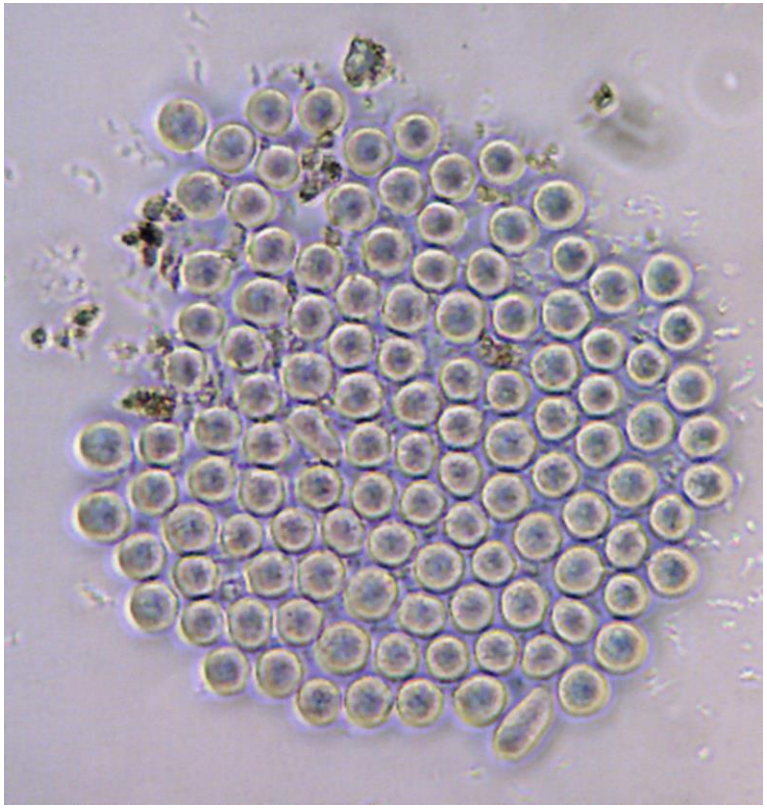
- ‘Blank cells’ (unspecialized)
- Capable of dividing and renewing themselves for long periods of time (proliferation and renewal)
- Have the potential to give rise to specialized cell types (differentiation)

Kinds of Stem Cells

Type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5 to 14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem cells


fertilised egg
totipotent stem cells
blastocyst containing pluripotent stem cells
isolated pluripotent SCs from inner cell mass
cultured pluripotent SCs
hematopoeitic SCs
neural SCs
mesenchymal SCs
tissue-specific SCs
blood cells
cells of nervous system
connective tissue,

Hematopoietic stem cells

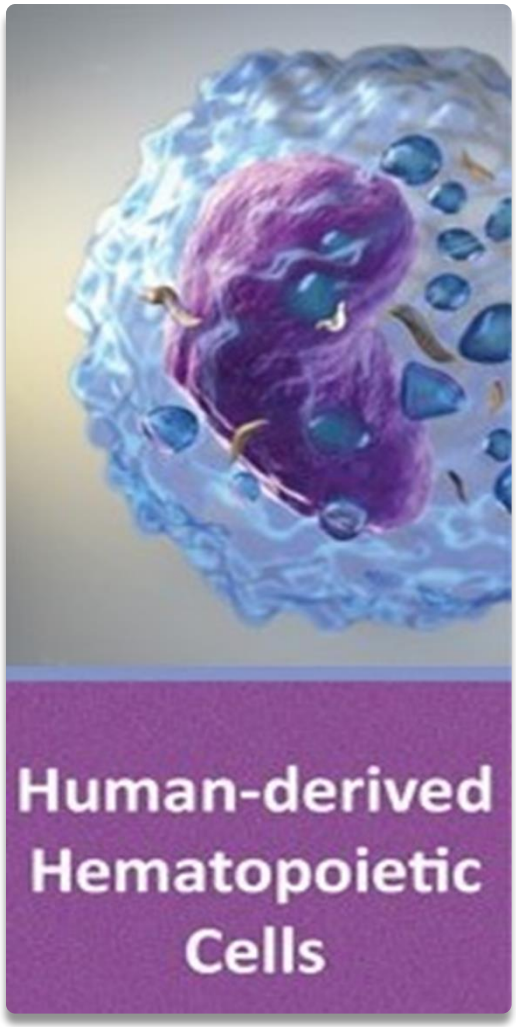


Blood, 15 Jan 2004

Charakteristic:

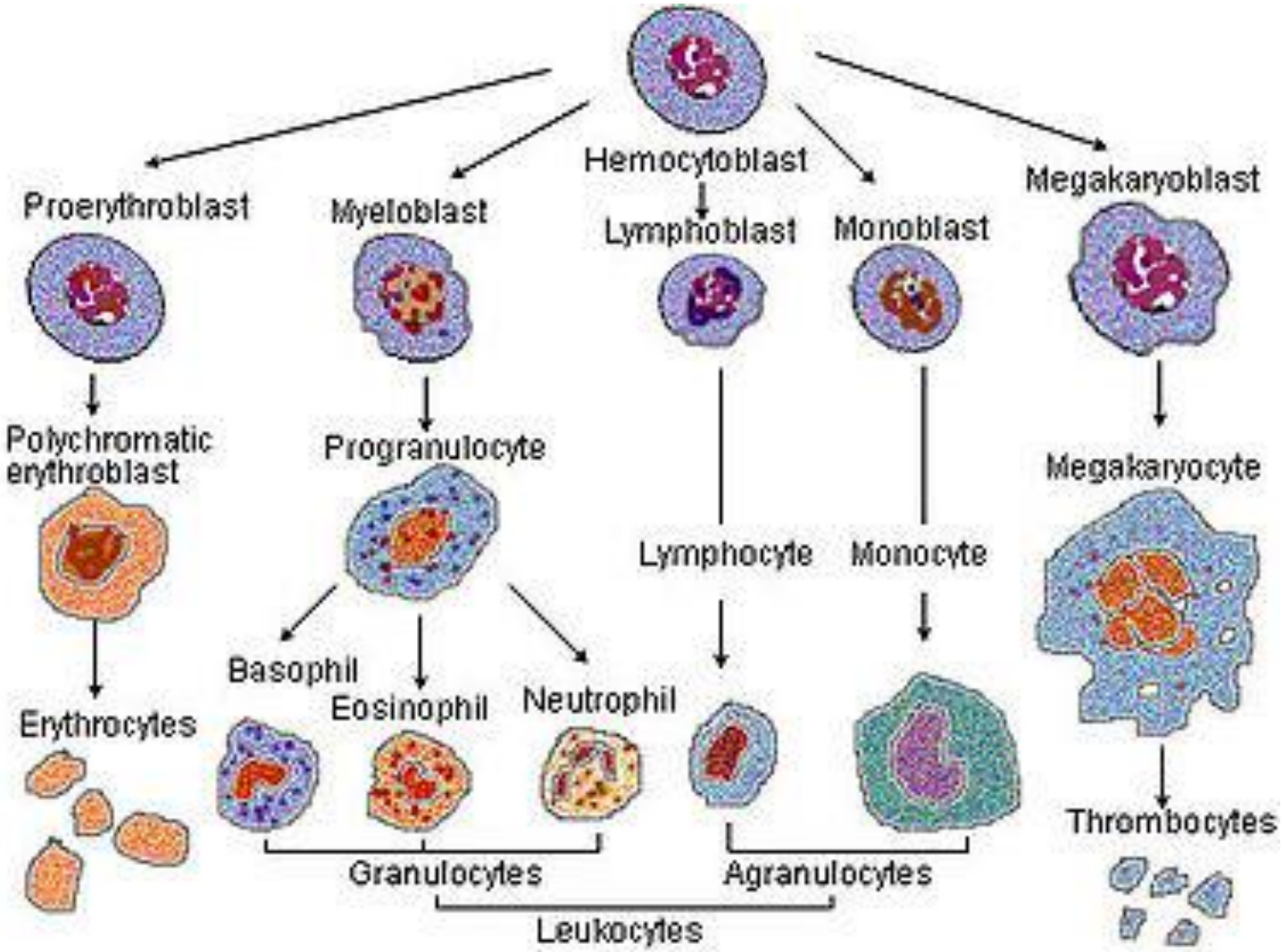
- CD34
- CD133
- Lin^-
- C-kit (CD117)
- BCRP

Stem cell roles in Transplants



- **CD34 –** Blood forming cells
- **NK cells –** Killing targets, produce cytokines (interferan, TNF and others)
- **Dendritic cells –** Immune responder
- **Mesenchymal cells -** GvHD
- **T cells – TCR a/b and g/d -** GvHD
- **B cells –** Immune activator

Blood Cell Formation



Sources of Stem Cells

Three main sources

- Adult stem cells
  - Main reservoir in the bone marrow- BMH,PBSC
- Cord blood stem cells
  - Circulating stem cells in umbilical cord blood- placenta
- Embryonic stem cells
  - Derived from fertilized embryos during early phases of development

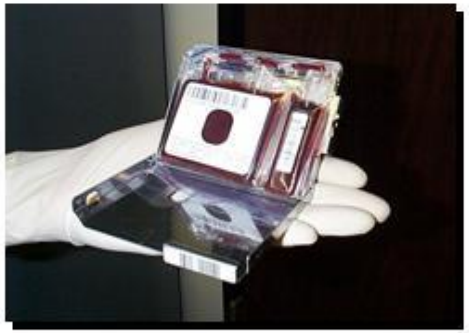
NCBP COLLECTION, PROCESSING AND STORAGE

1 Baby is born!

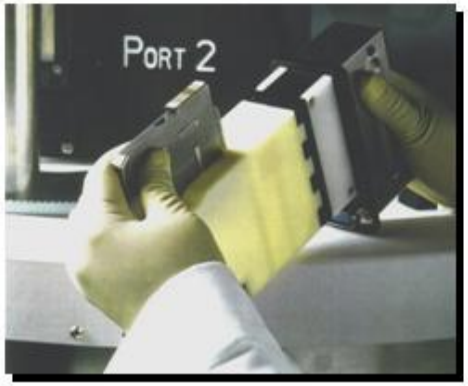
2 Collect umbilical cord blood

3 Remove most red blood cells and plasma and isolate stem cells into 20 ml using the AutoXpress device and add 5 ml cryoprotectant

4 Place 25 ml CB in quarantine overwrap and insert in canister



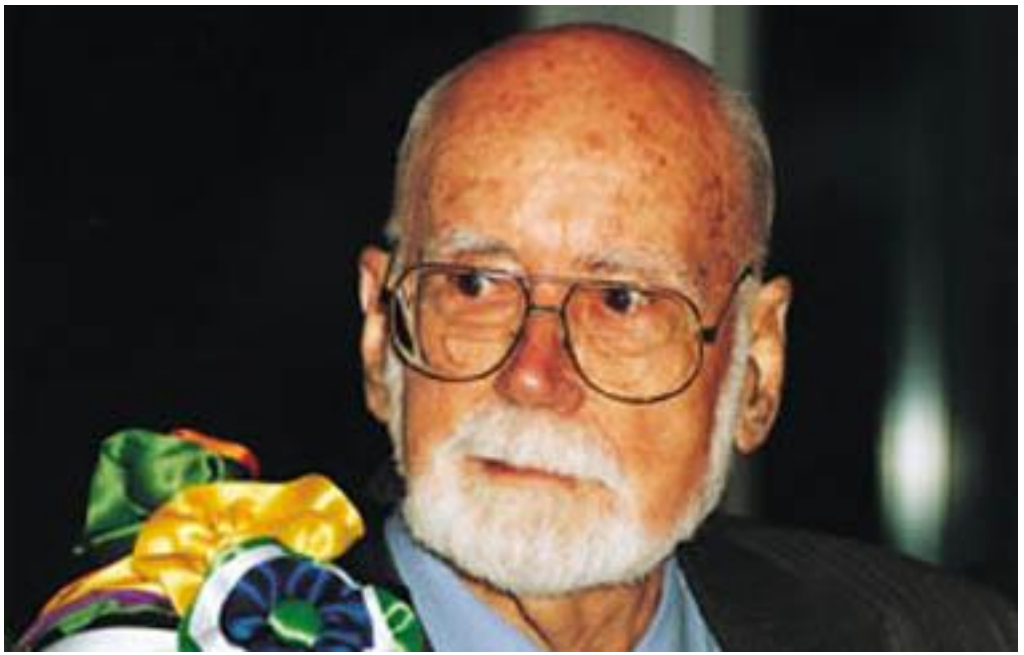
5 Insert NCBP unit into Controlled Rate Freezing module



6 Initiate automatic Controlled Rate Freezing and archival of NCBP unit in BioArchive System

The Nobel Prize, 1990

E. Donnall Thomas



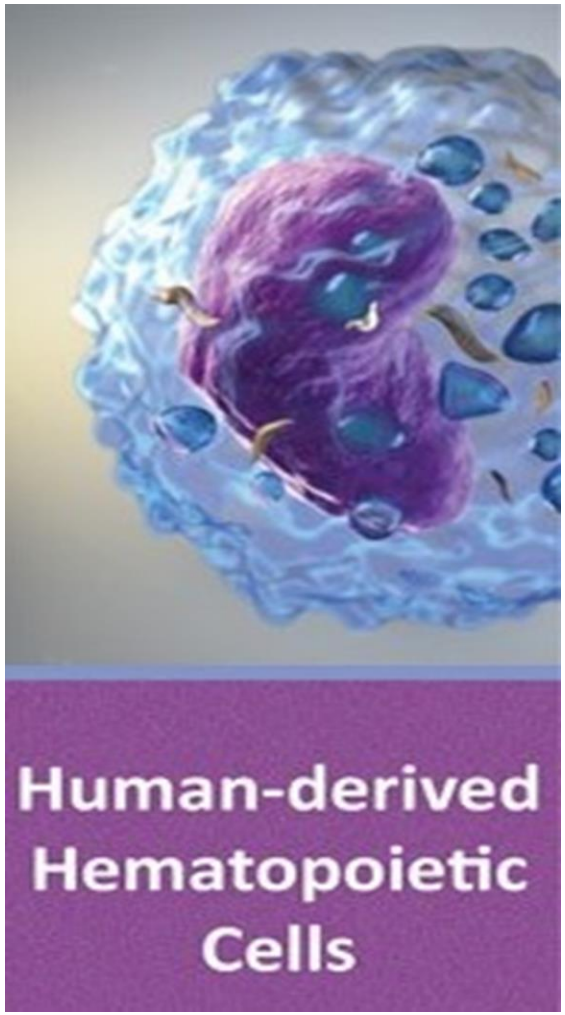
first succsessful HSCT in treatment of acute leukemias

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.

Highlights in Stem Cell Transplant

- 1957: Marrow safely **infused intravenously**
- 1958: Reports of **successful identical twin** transplants
- 1969: **Cytoxan** added to radiation
- 1970: Bone marrow harvests perfected to obtain stem cells
- 1989: Peripheral blood stem cells harvested
- 1990: First successful cord blood transplant
- 1996: First non-ablative transplant

Types of stem cell Transplants



- Autologous —the cells come from patient
- Allogeneic —the cells come from a matched related or unrelated donor
- Syngeneic —the cells come from your identical twin or triplet
- Haplo Identical – the cells from parents or child

Autologous Transplantation

Stem cell infusion as “rescue” from high dose chemo
“marrow lethal dose”

- The success of a transplant depends on the type and stage of the disease and your age and general health
- The original disease may come back after the transplant. If relapse occurs after Autologous transplant, chemotherapy or other treatments may be used
- Transplant related mortality is very low

Autologous

- Relapsed Hodgkins Disease
- Relapsed Non Hodgkins Lymphoma (NHL)
- Stage IV Neuroblastoma
- Relapsed Ewings Sarcoma
- AML/ALL with no donor
- Investigational
  - Metastatic Ewings Sarcoma
  - Medulloblastoma, other brain tumors
  - Autoimmune Diseases (SLE)

Allogenic Transplants

High dose immunotherapy
"rejection" of the cancer and building better immunity

- Need to find HLA matched donor
- Higher in Transplant related morbidity and mortality
- GVHD
- CMV, EBV, and others
- Graft rejection or relapse

Allogeneic Transplant Indications

Malignant Diseases

- AML CR1 – Matched Sibling,Relapsed or Refractory AML or ALL
- Chronic myelogenous leukemia
- Juvenile myelomonocytic leukemia
- Myelodysplastic syndromes

Non-Malignant Diseases

- **Inherited metabolic disorders** - Adrenoleukodystrophy, Hurler syndrome, metachromatic leukodystrophy, osteopetrosis, and others
- **Inherited immune disorders** - Severe combined immunodeficiency, Wiskott-Aldrich syndrome, and others
- **Inherited red cell disorders** - Pure red cell aplasia, sickle cell disease, beta-thalassemia, and others
- **Marrow failure states** - Severe aplastic anemia, Fanconi anemia, and others

Haplo Identical Transplant

The advantages of Haploidentical SCT are that nearly all patients have an immediately available donor and that a stronger Graft- versus-Tumor effect can be realized with partial HLA disparity.

T cells and B cells depleted graft-CliniMACS Technology

Two strategies based on Clinical grade Antibodies for CD34, CD56,CD3,CD19

Passive Depletion – CD34+ Enrichment ,In autologus setting CD34+ cell enrichment is used for the depletion of tumor cells from the graft

Active Depletion – CD3 / TCR a/b / CD19,T cells and B cells depleted product contains CD34+ stem cells, CD34- stem cells and NK cells, Monocytes, myeloid precursor cells and other progenitor cells.

Post transplant cyclophosmide

cy given after 60-72 hrs post stem cell infusion

Developments in Graft-engineering techniques for T-cell depletion of mobilised PBSC 's for haploidentical transplantation

1995 — CD34+ Selection of purified stem cells

2003 — CD3/19 Depletion Stem cells + effectors (NKs)

2011 — TCRαβ/CD19 Depletion stem cells + effectors (NKs + γδT-cells)

Courtesy: P. Lang

Elements of Stem Cell Transplants

Patient Evaluation

Recipient Age

- Autologous: "0" to 70 years
- Allogeneic:
  - Matched Related 55-60 years
  - Mismatched or Unrelated Donor: 50-55 years
    - Risk of GVHD significantly increased age >45
  - Dose-Adjusted Transplantation for older, or ill patients
    - Reduced intensity myeloablative
    - Non-myeloablative
      - Indicated based on extensive pre-transplant evaluation for candidacy

Patient fit & accepted for transplant

Plan for Transplant

Type : Allo, Auto

Source : BM/PBSC/UCB

Conditioning :
Myeloablative / Reduce intensity / NMA

HLA Matching in SCT

Selection of donor

- Based on tissue typing of 6-10 HLA antigens in allogeneic transplantation
- Tissue typing unnecessary in autologous transplantation

HLA and Marrow Transplantation

- Histocompatibility Locus Antigens (HLA) are determinants of immunologic “self” and “not-self”
  - Immunologic “password”
  - Allows for effective immune response against infections, cancer
- T cell reaction to foreign HLA molecules (donor) is a major problem of transplantation (alloreactivity)
  - Need good donor and recipient match for HLA sites
  - Cause of acute rejection in organ transplant, and of GVHD in BMT.

HLA Typing in BMT

- Family members typed with patient for HLA A, B and DR
  - Likelihood of 6/6 or 5/6 match depends on frequency of recipient HLA haplotype
- Likelihood of unrelated donor match related to haplotype frequency in general population
  - Some HLA combinations more frequently found among ethnic groups

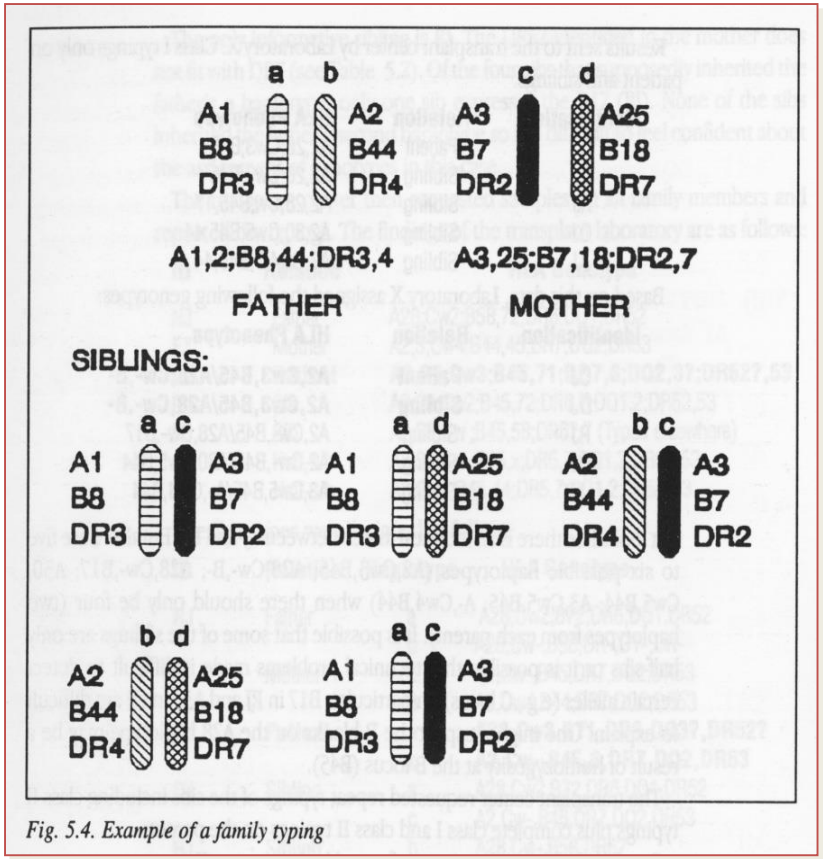


Fig. 5.4. Example of a family typing

What happens if donor and patient are not well matched?

Rejection

The patient's immune system
attacks and destroys the transplant

Graft vs Host Disease (GVHD)

The transplanted tissue
attacks and destroys the patient

DONOR Selected for Transplant

Screening of Donor

I-Infectious Disease Markers-Screening

- HBsAg
- Anti HBc
- HBc- IgM
- Anti HCV
- HIV-1/HCV/HBV NAT
- HIV 1 &2
- HTLV 1&2 Antibody
- CMV IgG and IgM
- VDRL / RPR
- Malarial Parasite

II- Hematological Evaluation:

- Complete Hemogram (WBC, Hb, Platelet, differential count)
- Renal Function Test
- Electrolyte
- Liver Function
- Coagulation Profile (PT, PTT, TT, factor 13 assay),Fibrinogen
- Blood Grouping and Rh Typing
- HB Electrophoresis
- LDH
- Ferritin,Vitamin B12
- Vitamin D,Calcium

Screening of Donor

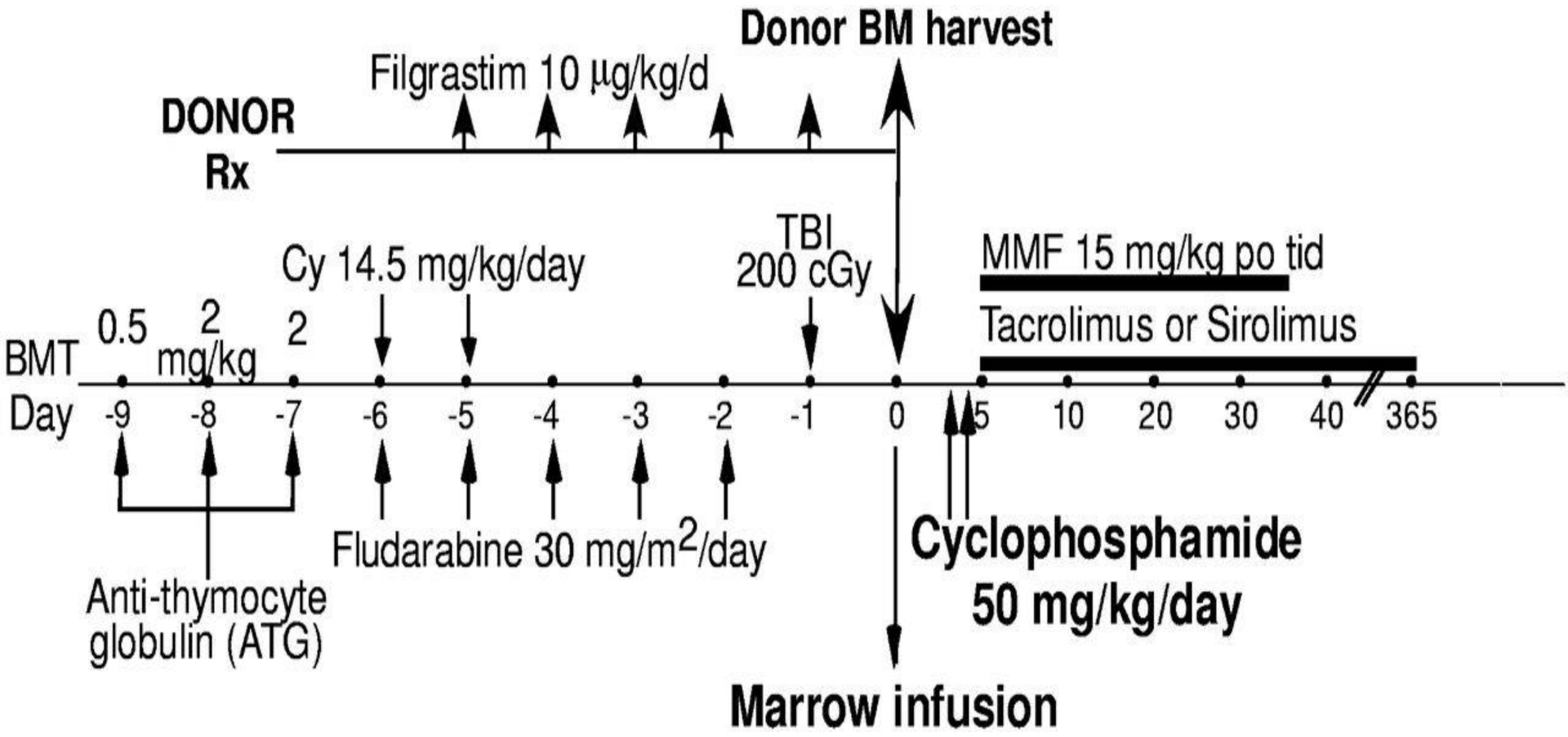
III –Imaging Studies:

- ECG
- Cardiac ECHO
- Chest X-Ray
- Ultrasound of Whole abdomen
- Pregnancy Test (for female)

- Once donor fit for donation of stem cells

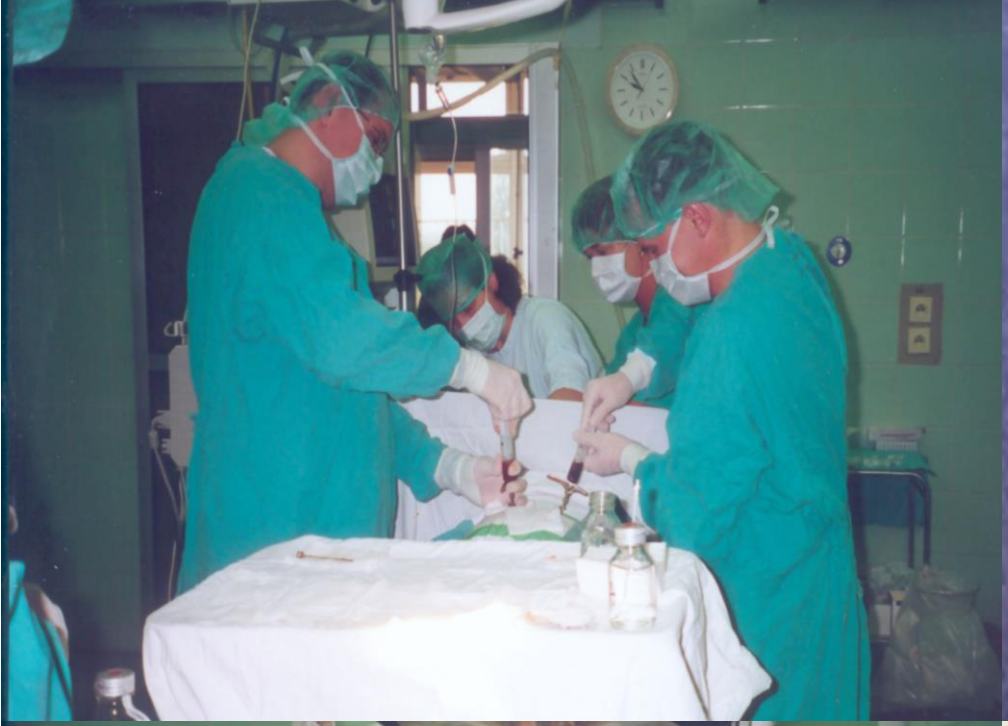
- Plan dates and prepare protocol

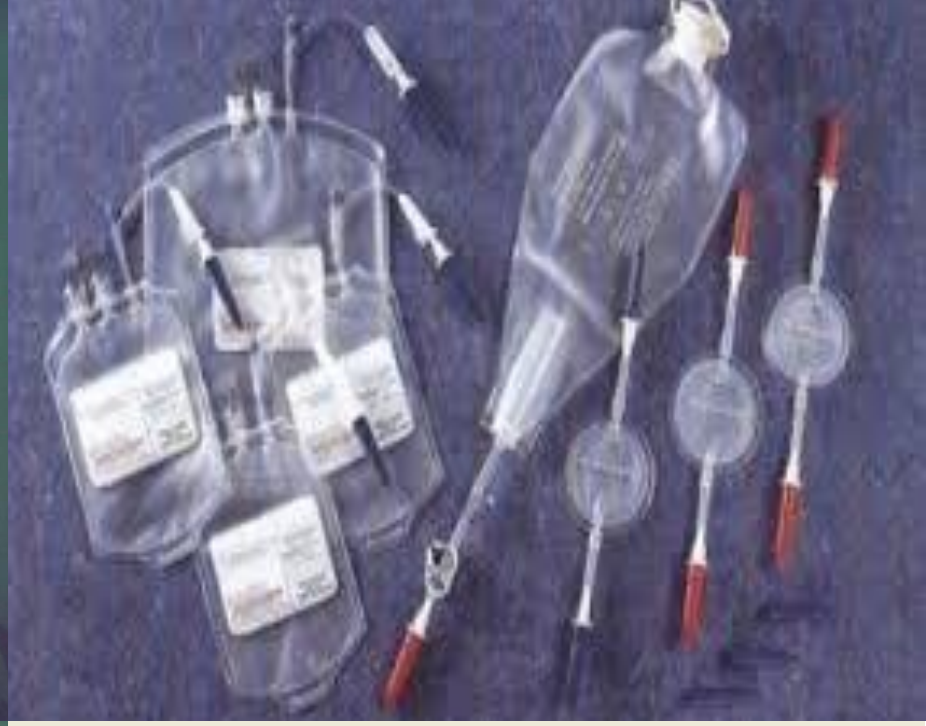
PBSC/BM Mobilization and conditioning

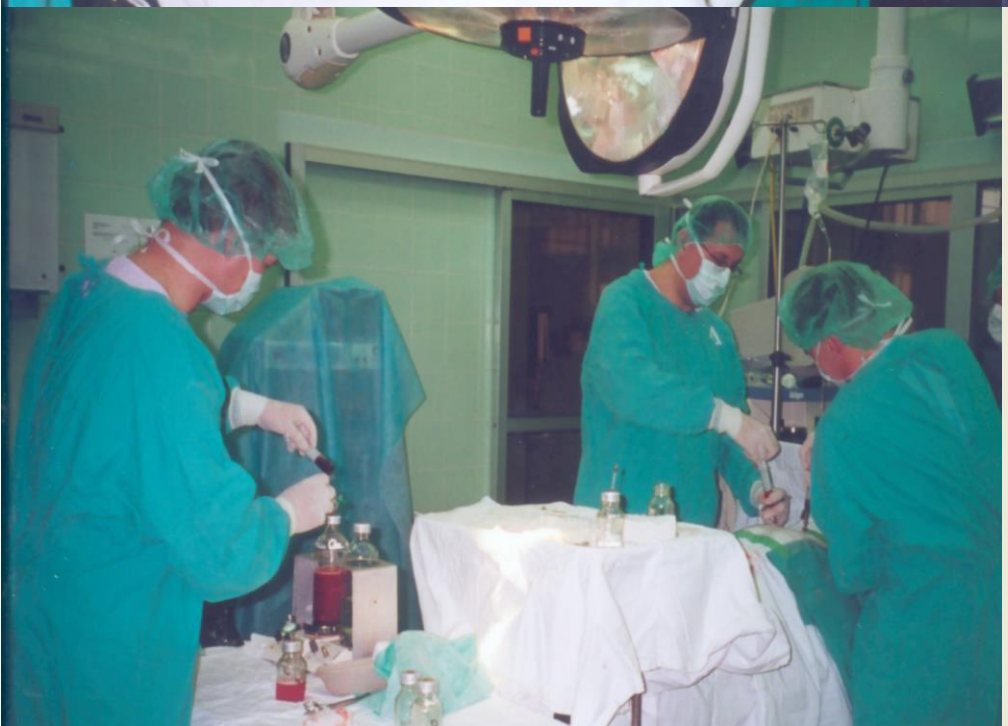


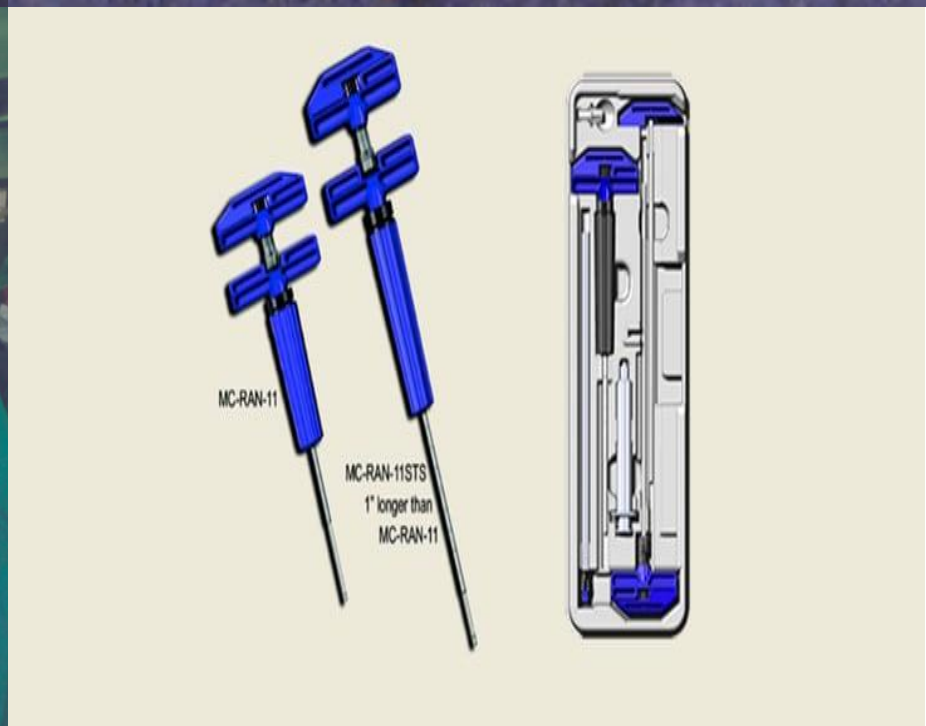
Bone Marrow Harvest

- Used to be standard procedure for harvesting stem cells
- Requires general anesthesia
- No mobilization needed
- Multiple needle punctures into the Posterior iliac crest to extract liquid bone marrow
- Amount harvested depends on size of recipient, usually no more than 10%-15% of donor blood volume .
- Used mostly with pediatric population rarely, for adult donors.
- Heparine used anticoagulant to draw through sryringe
- ACD used as anticoagulant in the collection bag 1:7 ratio
- Adult stem cells obtained by large volume marrow biopsy/aspiration (1-2L)








MC-RAN-11
MC-RAN-11STS
1" longer than
MC-RAN-11

PERIPHERAL BLOOD STEM CELLS COLLECTION BY APHERESIS

Stem cell Mobilization

PBSC Collection

Stem cell mobilizaion option

Growth factors

G-CSF (filgrastim,lemograstim)

↓SDF-1α gene expression,

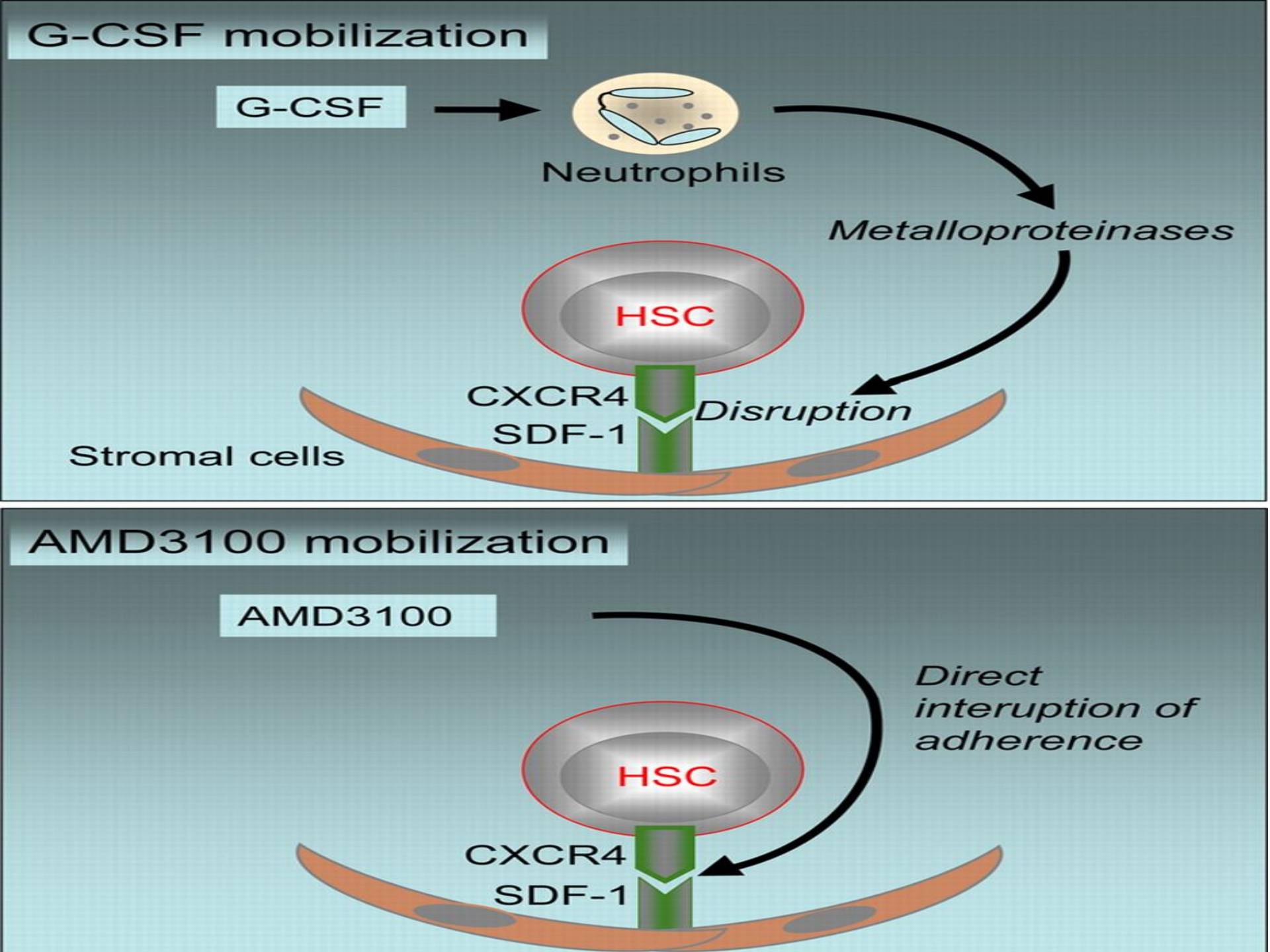
↑ proteinases level (cleave between ST and bone marrow)

CXCR4 antagonist(plerixafor)

inhibts CXCR4 and SDF-1α

DOSE - 10 mcg/kg

5mcg/kg with chemo


G-CSF mobilization
G-CSF
Neutrophils
Metalloproteinases
HSC
CXCR4
SDF-1
Disruption
Stromal cells
AMD3100 mobilization
AMD3100
Direct
interuption of
adherence
HSC
CXCR4
SDF-1

What next with Donor

- Mobilization by GCSF /Plerixafor
- Dose – 5-10 microgram/Kg body weight for 5 days
  - Manage side effects - Bone pain
  - Headache
  - Nausea
  - Local reaction at injection site
- PBSC collection on 5th day
- By peripheral line or central line for 3-4 hrs
- Processing of product

What next with patient/recipient

- Stabilizing and getting fitness for transplant
- Conditioning of patient
- Propylax management for GVHD in allo transplant
- management if infections

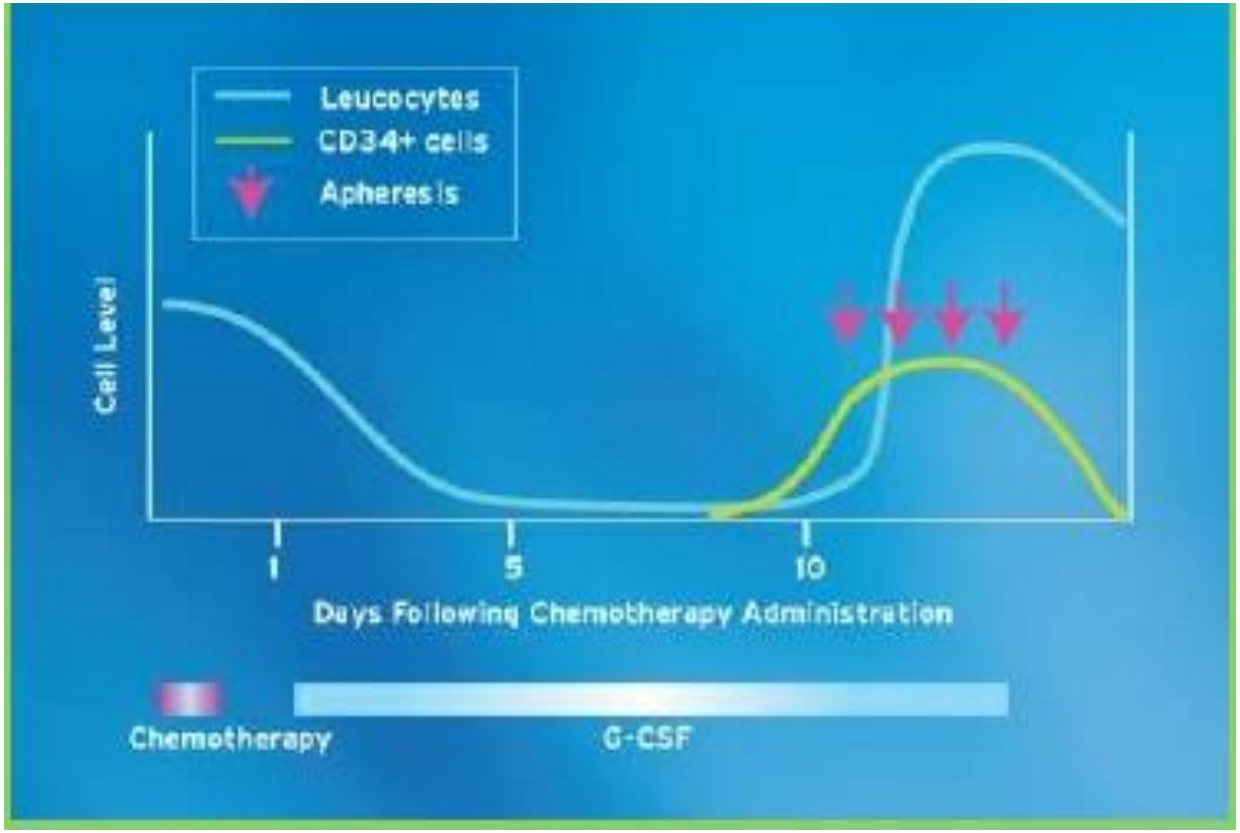
Agent Adverse Events

- Filgrastim - Musculoskeletal pain
- Lenograstim
  - Bone and back pain
  - Leucocytosis and thrombocytopenia
  - Transient increases in liver function test , Elevated LDH, Headache
- Plerixafor
  - Diarrhea and nausea
  - Injection and infusion site reactions

2*Chemotherapy - cyclophospamide, etoposide

Exact mechanism for chemotherapy is still not clear

Possible action may be due to induced damage to stromal cells of bone marrow leads to increase in CD34cells in peripheral blood



COMPLICATIONS OF CHEMO MOBILIZATION

- Short-term Side Effects
  - General malaise
  - Infertility
  - GI symptoms
  - Skin and mucosal effects (rash ,alopecia, mucositis)
  - Myelosuppression (thrombocytopenia, leucopoenia)
  - Infusion-related side effects (such as hypotension, flushing, chest pain, fever, diaphoresis, cyanosis, urticaria, angio-oedema, and bronchospasm)
  - Allergic reaction, Signs of an infection,

- Long-term Side Effects
  - Decreased urination, signs of congestive heart failure
  - Secondary malignancies

Goals for Collection

- Autologous HSCT

  Minimum CD34 cells: 2 x 10^6/kg

  Minimum MNC : 2x10^8/kg

- Allogeneic HSCT

  Minimum CD34 cells: 2-5 x 10^6/kg

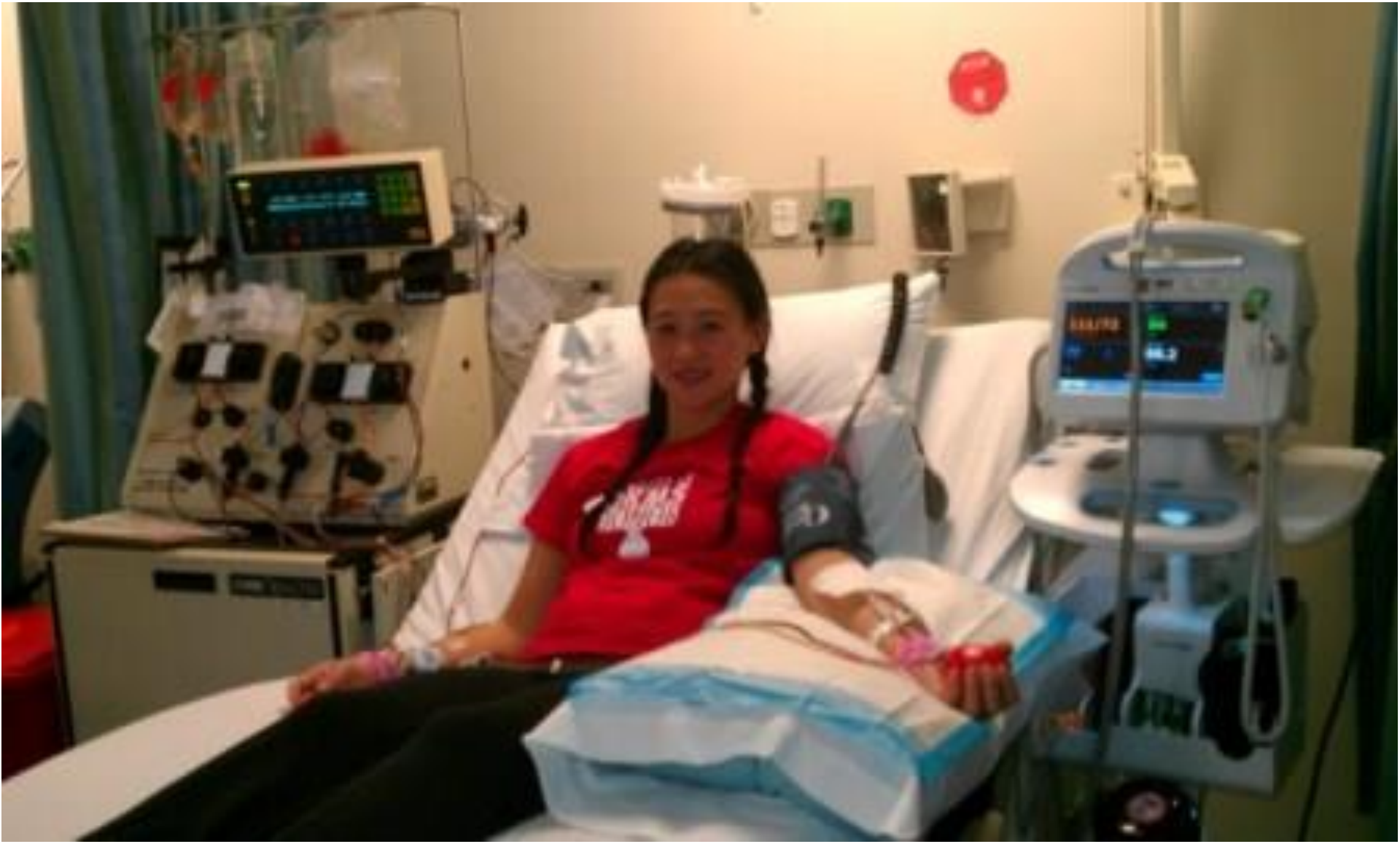
  Minimum MNC : 2-5x10^8/kg

- Cord blood HSCT

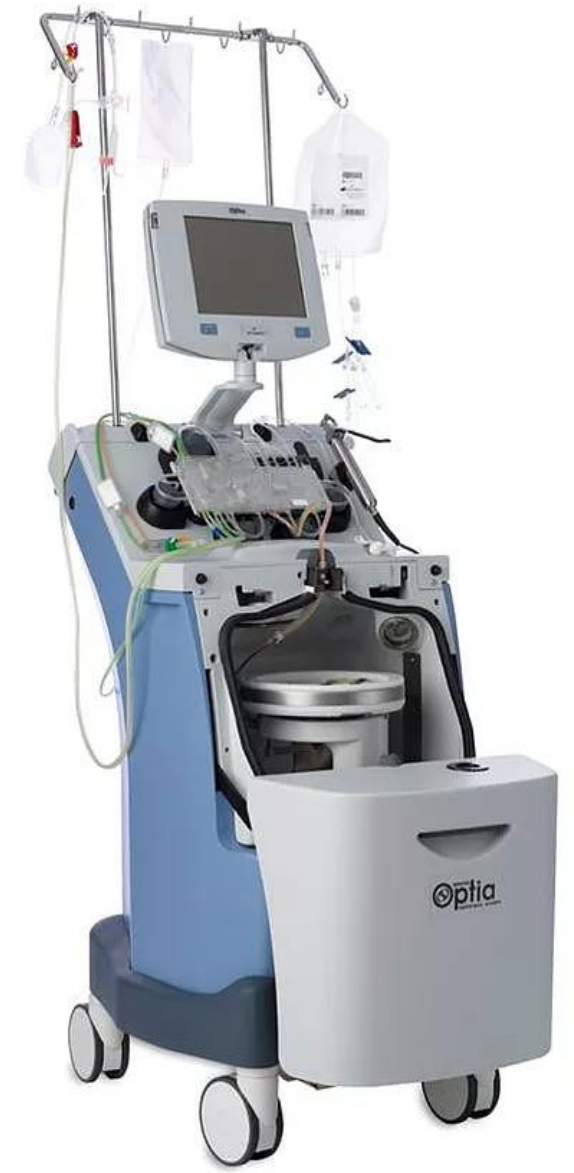
  cell dose of >2.5 × 10^7 Nucleated Cells/kg or

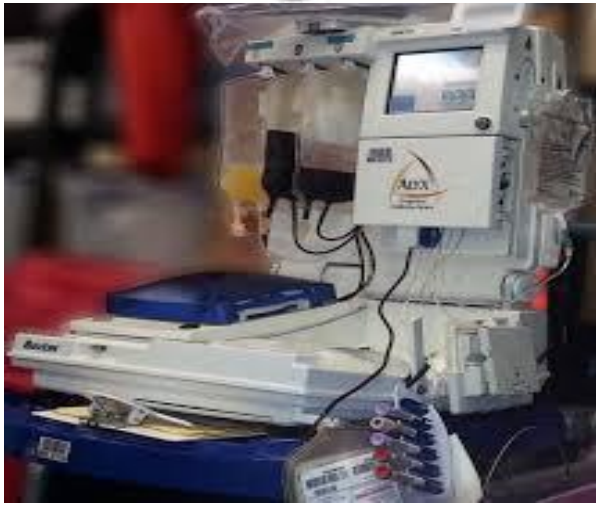
  ≥2×10^5 $CD34^+$ cells/kg;

Collection of peripheral blood stem cells



CELL SEPERATORS - APHERESIS





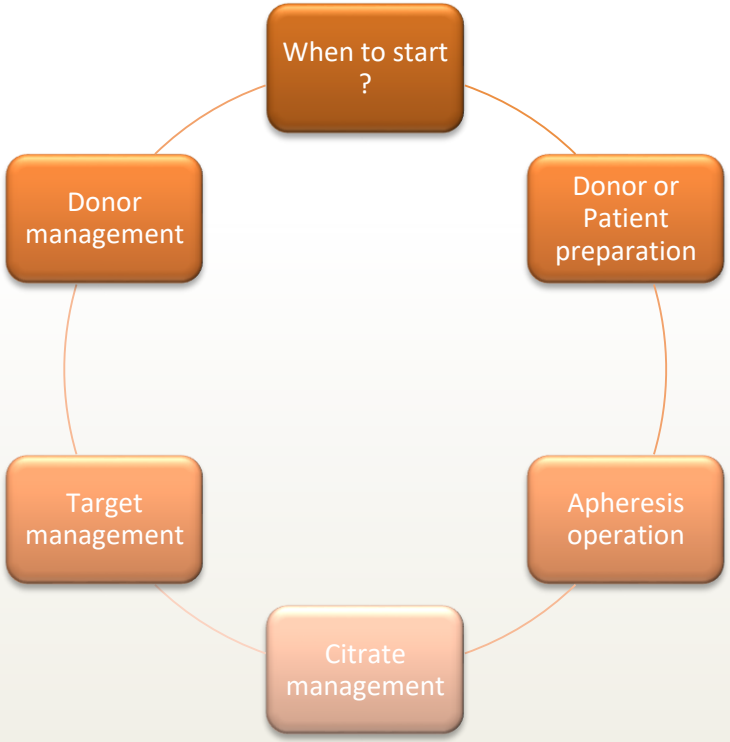
Stem cell Prediction

Pre CD34 count - 10 cell/microlit

Pre CD34 count

CD34 count may not be related to total WBC count

Stem cell collection (Apheresis)



PBSC Collection

- Collection done on 5th day, if needed 6th day also after 3-4hr of GCSF dose
- Peripheral or central line (femoral line/neck/chest)
- Calcium replacement - oral stat dose/iv in need
- Injection heparin 100iu/kg stat and reduce ACD flow ratio
- Heparin in ACD bag if needed

Advantages & Disadvantages of Haematopoitic Stem Cell collection methods

Collection Method	Advantages	Disadvantages
Bone Marrow	Single collection No need for central line No need for GCSF	Need general Anaesthesia slower Engraftment ↑ morbidity and mortality Tumor cell contamination of product
PBSC	No Anaesthesia can be done as out patient Fast Engraftment Lower rise of morbidity & mortality Less tumor cell contamination	collection may take extra days in some cases sometimes need central line IV comp possible Haemorrhage embolysis Infection

PBSC Method Complications

Adverse Effect	Cause	Signs&Symptoms	Corrective action
Citrate Toxicity	Anticoagulant Citrate	Hypocalcemia Tingling around mouth,hands muscle,witching cramps,Tetany	slow the procedure calcium replacement therapy
Hypovolaemia	Due to extracorporeal blood & plasma volume	Hypotension Fatigue Dizziness	Slow procedure stop fluid replacement
Thrombocytopenia	Platelet adherence to kits	low count bruising	Transfusion
Cathetral fluid	Clot on position	↓ flow inability to flush sometimes	reposition catheter gently flush
Infection	Catheter /site	fever,chills fatigue,Hypotension	Treatment Antibiotics remove catheter

Stem Cell Processing

- **Volume reduction**

Centrifugation-removal of excess plasma **for minor mismatch** blood group

- **Removal of red blood cells**

Enables transplant of **ABO/Rh mismatched** stem cells

Sedimentation-

Red Cell Reduction Techniques- blood cell separators Cobe Spectra, Fresenius AS 104, Fenwall CS 3000

Achieve 60-85% MNC recovery , less than 20 ml of RBC remain

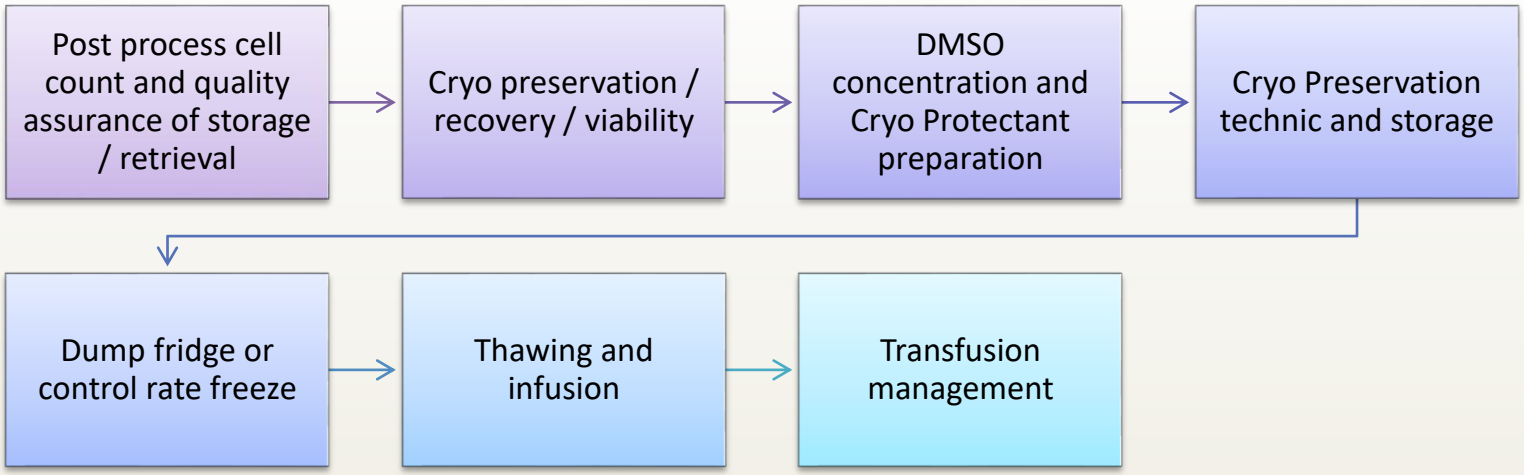
Reduction - Gravity sedimentation

BM diluted 1:8 with 6% HES , PBSC with 1:4

RBC removal 99% in 1-3 hrs

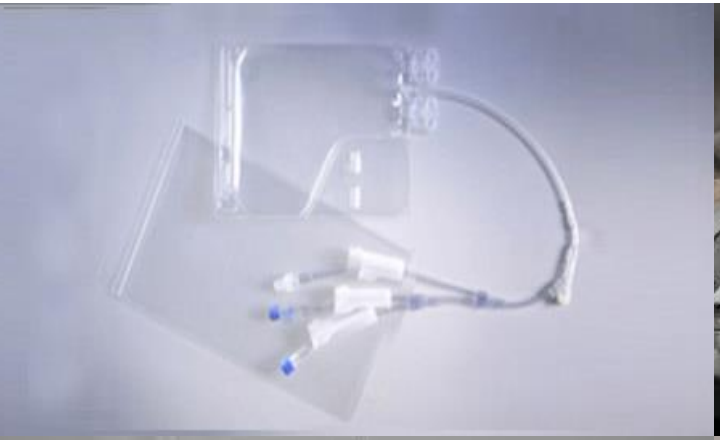
MNC recovery about 75%

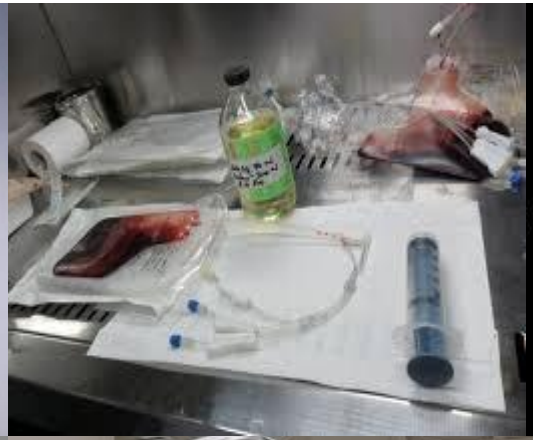
Stem cell process

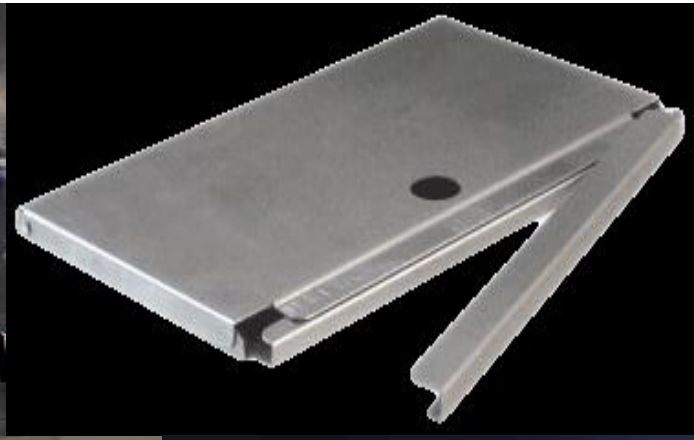


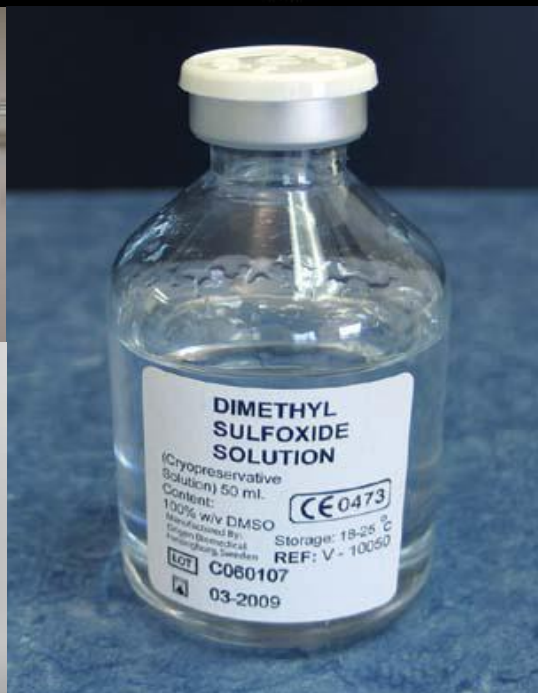
Stem Cell Cryopreservation

- Done when stem cell is not going to be infused within 72 hrs
- HSCs should be cryopreserved, in a cryopreservation solution (usually 5%- 10% dimethyl sulfoxide (DMSO) in autologous plasma, hydroxyethyl starch (HES) or albumin).
- DMSO can readily permeate across cell membranes to both inhibit intracellular ice formation and to prevent cell injury triggered by severe dehydration, as extracellular ice causes withdrawal of water from the intracellular milieu
- After controlled freezing, cells can be stored in liquid nitrogen for up to at least 10 years
- critical process – controlled rate freezing with cooling rate around 1-2 ^{o}C/min.
- Storage in liquid nitrogen at a temperature of – -156^{o}C (vapor phase) – -196^{o}C (liquid phase)








DIMETHYL
SULFOXIDE
SOLUTION
(Cryopreservative
Solution) 50 ml.
Content:
100% w/v DMSO
CE 0473
Storage: 15-25 °C
REF: V - 10050
LOT C060107
03-2009

DMSO

The biological mechanisms that cause ARs after cryopreserved HSC infusion are complex and not yet completely understood. The likely factors include:

- Post-thaw cell aggregation and dead cell debris.
- Lysis of RBCs, with release of Hb, electrolytes and membrane fragments.
- Low temperature of infused products.
- Electrolyte imbalance.
- Premedication given before transfusion, for example, antiemetics, corticosteroids, diuretics and antihistamines, which are used to neutralize DMSO-induced histamine release but may cause bradycardia at the same time.

Maximal dose of DMSO to be infused should be adjusted to bodyweight (1 g DMSO per kg/day)

Complications of DMSO

- Allergy - Flushing, rash, pruritus, erythema, edema, bronchospasm
- GI-vomiting, discomfort, diarrhea, cramp or pain and taste
- Unpleasant smell, respiratory distress
- Acute renal failure
- Cardiovascular-hypo/Hypertension, arrhythmias
- Hepatic - progressive jaundice
- Neurological - Mydriasis, miosis Numbness,
  muscle weakness,
  severe encephalopathy, infarction

Temperature and DMSO Toxicity Management

PBSC should be processed immediately for volume / Platelet reduction

PBSC should be kept at 2 – 4 ‘C till cryopreservation done and maximum of 72 hours

Cryopreserved product should not be brought in to RT level

Cryopreserved product should be transfused at 4’C environment

Cryopreserved product should not be thawed completely for a transfusion

Transplant Process (5 steps)

1. Conditioning
2. Stem cell infusion
3. Neutropenic phase
4. Engraftment phase
5. Post-engraftment period

Conditioning Regimens

- **Principles**
  - Provides marrow “space”
  - Eradicates malignant cells
  - immunosuppression for Bone marrow failure states

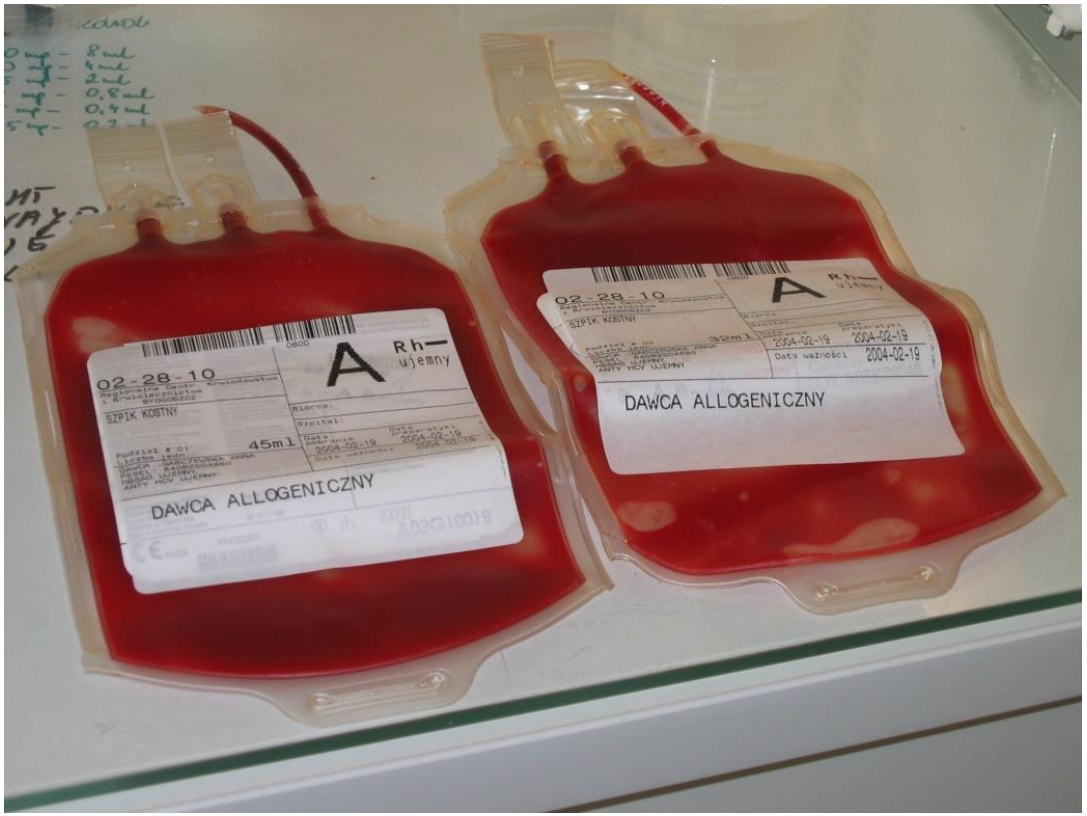
Immune deficiency without empty marrow leads to rejection.

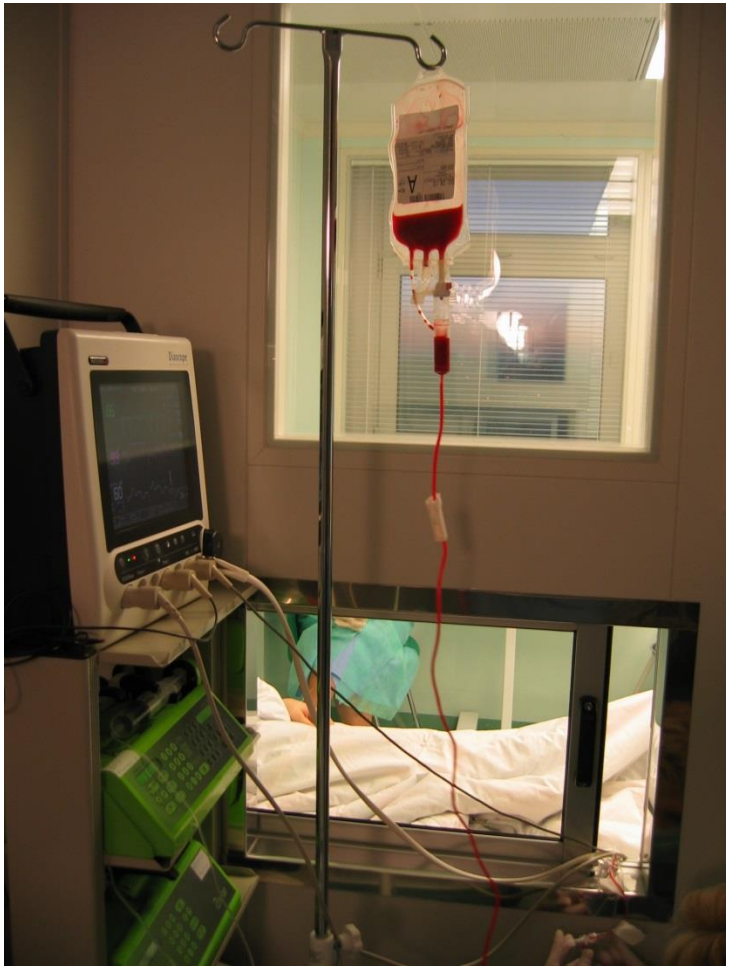
- **Strategy**
  - Ablative therapy-radio/chemo
    kills” hematopoietic stem cells .
  - Reduced intensity therapy-radio/chemo
    likely myeloablative but better tolerated than traditional ablative
  - Non-myloablative therapy-radio/chemo
    does not “kill” hematopoietic stem cell

Stem cell Infusion

- Infused through a CVL, much like a blood transfusion by blood set . No leuco depletion or irradiation
- Infusion - 20 minutes to an hour, varies depending on the volume infused
- Premedication with acetaminophen and diphenhydramine to prevent reaction
- Anaphylaxis, volume overload, and a (rare) transient GVHD are the major potential complications involved
- Stem cell products that have been cryopreserved contain dimethyl sulfoxide (DMSO) as a preservative thawed and washed if needed

Hematopoietic stem cell infusion

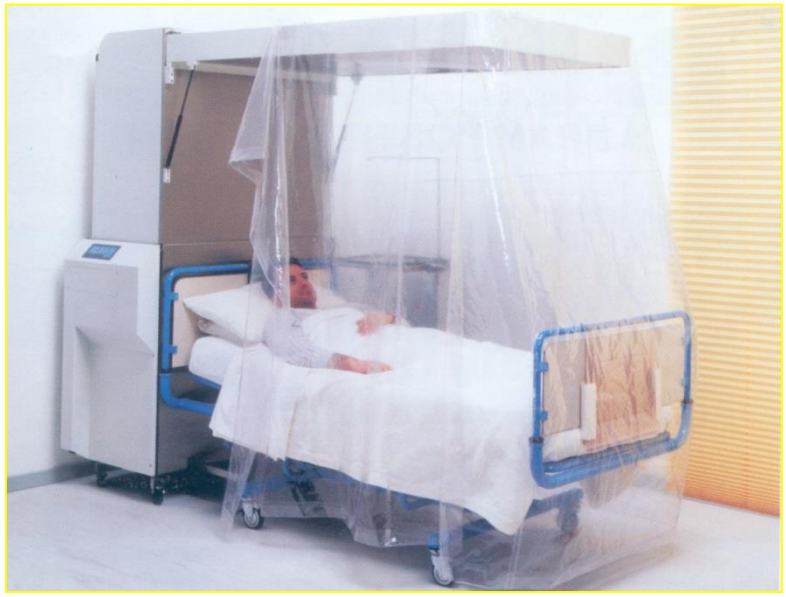


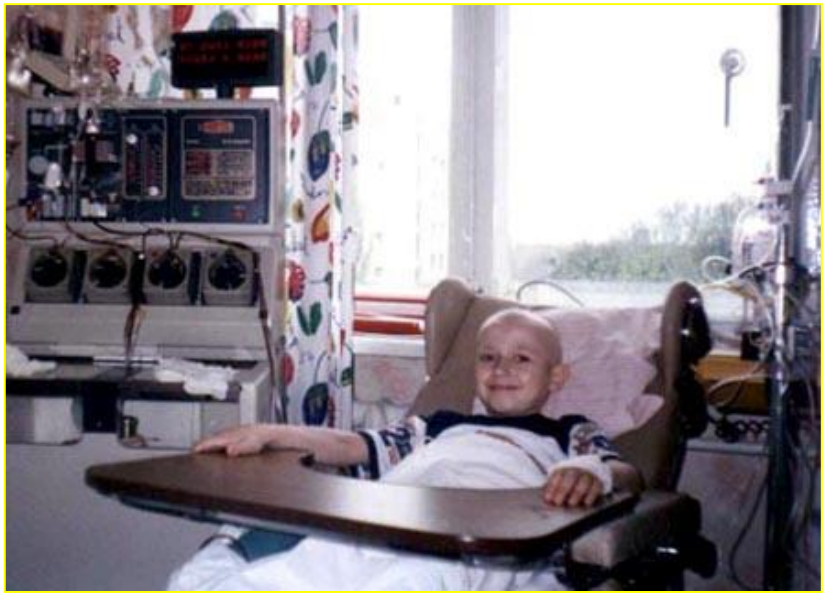


Neutropenic Phase

- During this **period (2-4 wk),** the patient essentially has no effective immune system.
- **Healing is poor**, and the patient is very susceptible to infection.
- **Supportive care and empiric antibiotic therapy** are the mainstays of successful passage through this phase.

Bone marrow transplantation unit





Engraftment Phase

- Engraftment is defined as the attainment of
  - sustained neutrophil count of **>0.5 × 10^9/L;**
  - transfusion independent platelet count of **>20,000 × 10^9/L.**
- The time to achieve engraftment will vary from patient to patient, depending on:
  - type of transplant (allogeneic usually longer than autologous)
  - cell dose of graft (quicker with higher cell dose);
  - product infused (BM usually longer than PB);
  - type of post-transplant GVHD prophylaxis (methotrexate delays)
  - post-transplant use of growth factors (e.g. GCSF) (speeds engraftment);
  - presence or absence of other features such as infection in the recipient.
- most patients will achieve engraftment 2–4 weeks post-transplant.
- Failure to engraft is now uncommon.

Post-engraftment Phase

This period lasts for **months to years**. Hallmarks of this phase include the gradual development of **tolerance, weaning off of immunosuppression, management of chronic GVHD,** and documentation of immune reconstitution.

Chimerism Analysis

- Percent of donor and recipient cells in the body•
- Determining the mix of cells, or chimerism , provides information for therapeutic interventions like withdrawal of immunosuppressive drugs, DLI infusion

- Methods for analaysis

  cytogenetic

  isoenzyme analysis

  blood grouping phenotype

  sex chromosome differentiation using fluorescence(FISH)

Factors influencing the outcome of HSCT

- **Disease factors**
  - stage
- **Patient - related factors**
  - Age
- **Donor - related factors**
  - Histopompatibility (HLA)
  - Sex
  - Viral status (CMV positivity)
- **Peri-transplant factors**
  - Conditioning
  - GVHD prevention
  - Stem cell source and content
- **Post-transplant factors**
  - GVHD

Complication

- **Allogeneic**
  - **Early**
    - infection
    - aGVHD
    - bleeding
    - toxicity
    - graft failure
  - **Late**
    - chGVHD
    - infection
    - relapse
    - gonadal failure
    - secondary malignancy
    - toxicity
- **Autologous**
  - **Early**
    - infection
    - bleeding
    - toxicity
  - **Late**
    - relapse
    - infection
    - gonadal failure
    - secondary malignacy
    - toxicity

Blood Transfusion Support in Stem Cell Transplant

Role of transfusion services

- Basic transplant issues that impact blood bank policies
- Recognize common serologic problems encountered in transplant recipients
- Appropriate blood products when transfusion is needed

Basic Transplant Issues

Recipient-Donor ABO compatibility

ABO and Rh compatibility are not required for the successful outcome of BMT

Special Blood Requirement

- **Irradiated-prevents graft versus host disease in an immunocompromised recipient.**
- **Leukocyte-Reduced-**

  Alloimmunization

Prevention of Febrile Non Haemolytic Transfusion Reaction.

Replacement of CMV negative blood components.

- **CMV Negative**
- **Saline-washed or volume reduced**

Pre-transplant Considerations

- Is this a major or minor ABO incompatibility?
- How high the donor/patient's antibody titers against the patient/donor's ABO group?
- Will the patient require special conditioning? Will the HPC collection require processing?

Recipient- Donor ABO Compatibility

- ABO Major Mismatch: Recipient is O-Donor is A
  - Acute hemolysis at infusion
  - Delayed hemolysis from persistent patient antibodies
  - Delayed onset of hematopoiesis

  Red cell depletion
- ABO Minor Mismatch: Recipient is A- Donor is O
  - Acute hemolysis at infusion
  - Delayed hemolysis from donor antibodies

  Plasma depletion
- ABO Major-Minor Mismatch: Recipient is A-Donor is B

  Both red cell and plasma depletion

ABO Compatibility

DONOR

RECIPIENT

Blood Group	O	A	B	AB
O	Compatible	Major	Major	Major
A	Minor	Compatible	Major and minor	Major
B	Minor	Major and minor	Compatible	Major
AB	Minor	Minor	Minor	Compatible

Transfusions following bone marrow transplantation

- Compatible transplant-no special requirements
- Minor incompatibility-recipient type plasma and platelet until recipient cells have disappeared
- Major incompatibility-recipient type red cells until recipient isoagglutinins have disappeared
- Major and minor incompatibilities- group AB plasma, group AB or washed platelets until recipient cells gone; group O red cells until recipient isoagglutinins have disapeared.

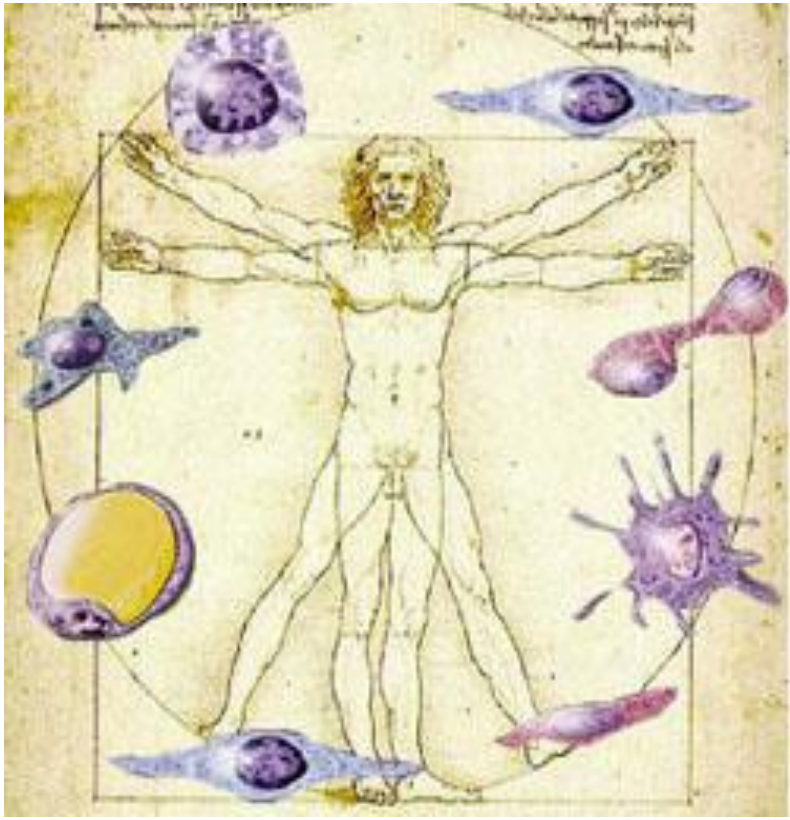
Blood Selection when recipient/donor are not ABO identical

Patient ABO	Donor ABO	RBC	FFP 1st Choice plt	2nd Choice plt
O	A B AB	O O O	A,AB B,AB AB	B,O A,O A,B,O
A	O B AB	O O A,O	A,AB AB AB	B,O B,A,O A,B,O
B	O A AB	O O B,O	B,AB AB AB	A,O A,B,O B,A,O
AB	O A B	O A,O B,O	AB AB AB	A,B,O A,B,O A,B,O

Conclusion

- Stem cells are seed cells with no characteristics, can multiply and circulate from one tissue to other.
- They are totipotent, pluripotent,multipotent
- Three main sources of stem cell adult SC (BM,PBSC),CBSC,ESC (not legalized)
- Two type of transplant based on source of SC are auto/allo SCT

To get through the hardest journey
we need take only one step at a time,
but we must keep on stepping”



Thank you