Bone marrow and PBSC Harvest

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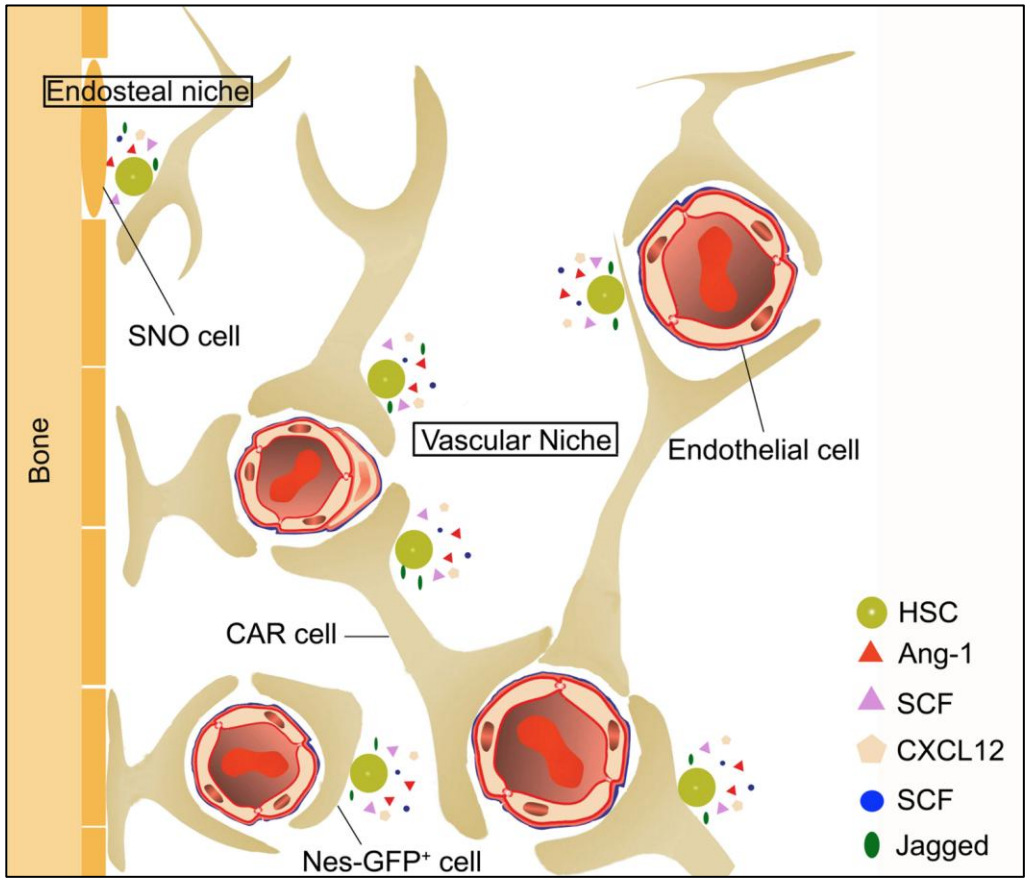
Bone Marrow Microenvironment



Bone marrow niche is a housing site as well as regulator for HSCs

Endosteal niche: Quiescent HSCs in G0 state

Vascular niche: Active dividing and differentiating HSCs

HSCs may ‘mobilise’ to the vascular niche and circulation or ‘home’ back to the endosteal niche

Endothelial, mesenchymal cells and CXCL12 reticular cells release factors to support HSCs

Hematopoietic Stem Cell Transplantation

Intravenous administration of healthy hematopoietic stem cells

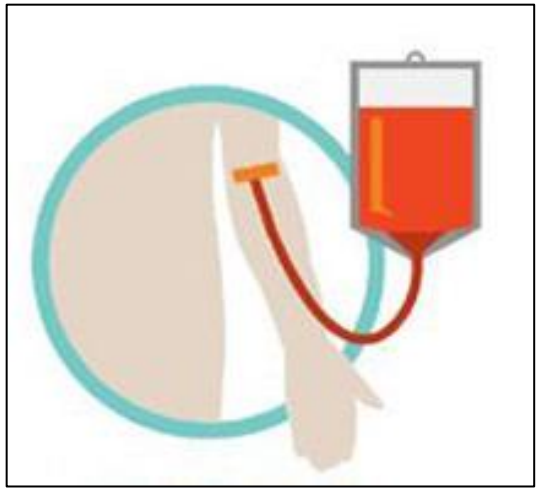
In patients with dysfunctional or depleted bone marrow

To re-establish marrow function

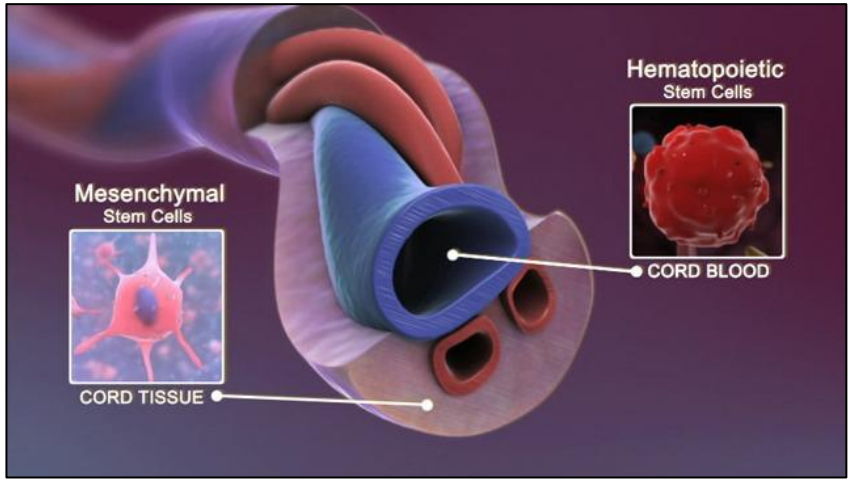
No longer called “bone marrow transplant” as other stem cell sources are available

Sources of Hematopoietic Stem Cells

BONE MARROW



PERIPHERAL BLOOD
(following mobilization)



CORD BLOOD
(umbilical vein cord blood at the time of delivery)

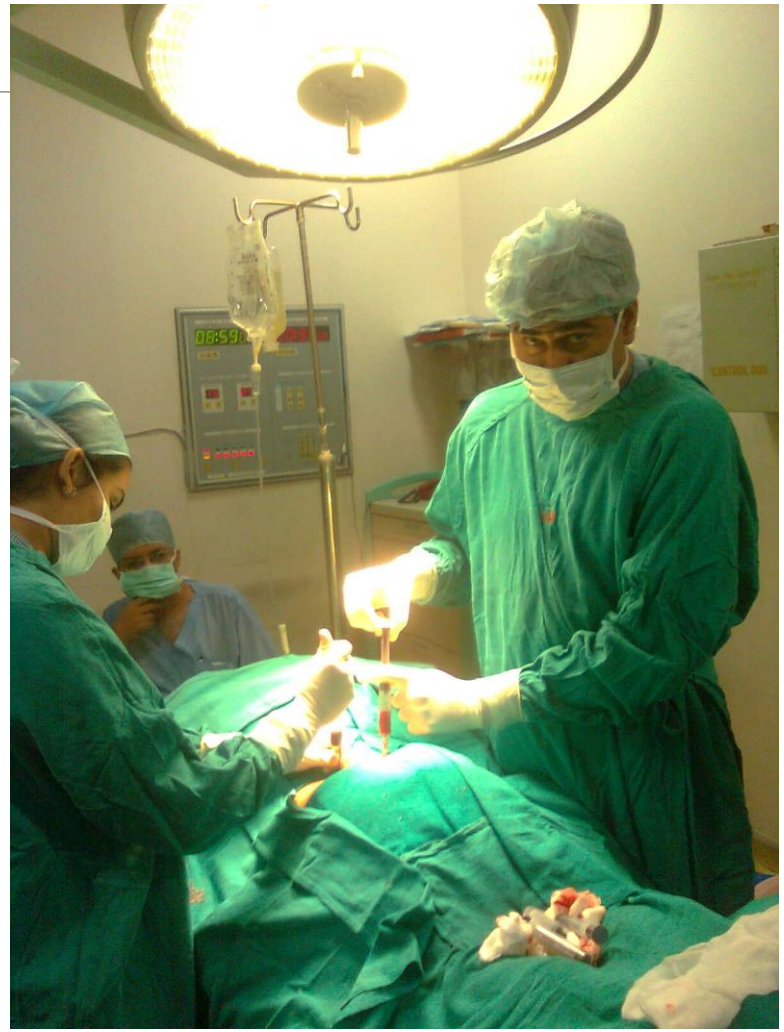
Harvesting HSC from bone marrow

Bone marrow aspirated under regional/general anesthesia

Goal: To collect 10-15 mL of marrow/kg recipient body weight

Can collect max 20 ml/kg from donor

Choice in pediatric transplants for non-malignant conditions.



Procedure

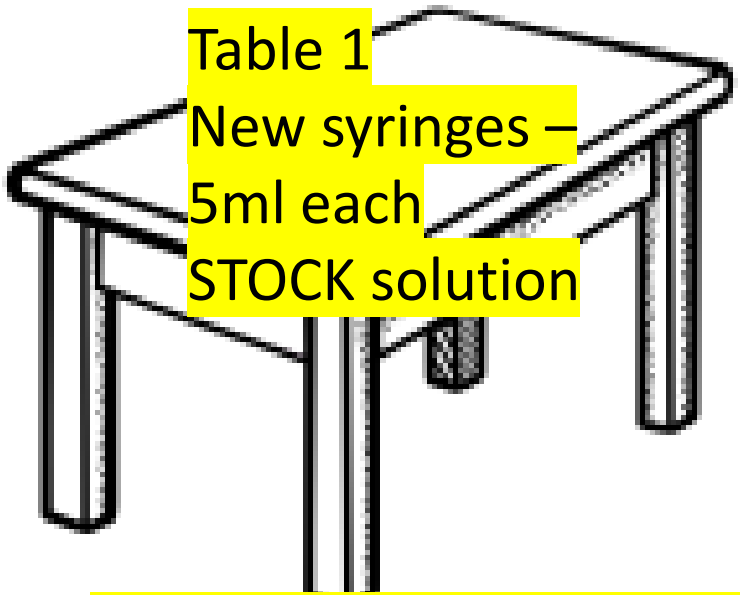
Preparation: In a sterile operating room, the donor should be positioned in a prone position – with a pillow under the abdomen, ankles and groin. Monitor and avoid compression of the breasts and genitalia.

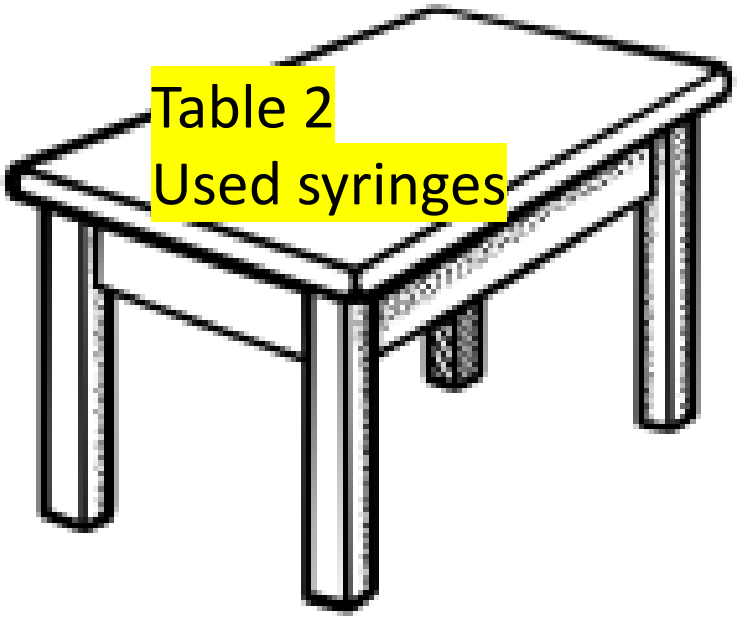
Anesthesia: usually, general.

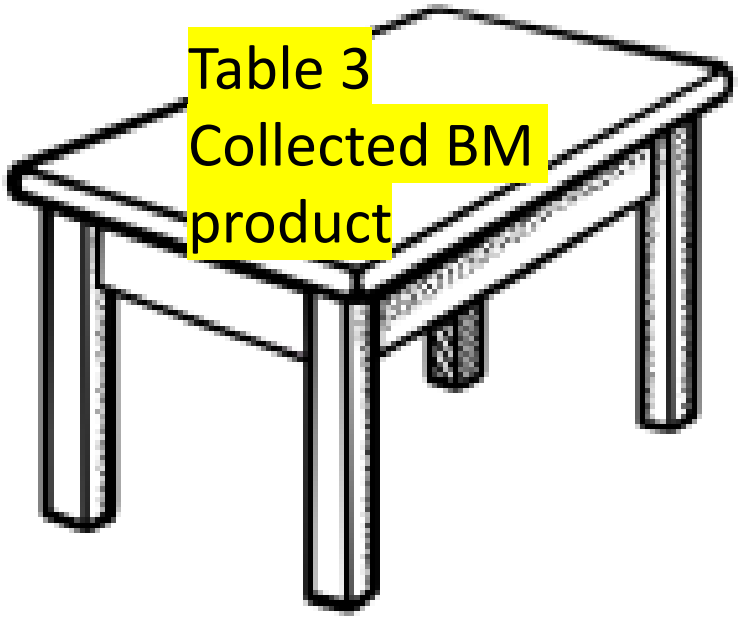
Preparation of the area: Disinfect the skin of the posterior pelvic region with povidone iodine, and, then, dry with a sterile gauze. Cover the donor with sterile drapes, leaving the upper part of the posterior pelvis free.

- Donor Hb 10 g/dl
- Not more than 4-5 aspirates from one prick site
- Use 5 ml syringes – not more than 5 ml – haemodiluted sample
- Max volume of collection – 20ml/kg of donor
- Post procedure – Bupivacaine (5mg/ml) → 5 ml to be infiltrated each side. Check CBC, SE post procedure.

Preparation







Stock solution – 500 ml NS + 5000 U Heparin – 1:10 solution

One person dedicated to calculate BM volume collected

Bags for BM collection –

CPDA anticoagulant – ratio of 1:6-7

Commonly, Frasenius blood bags used for collection

BT set filter (170-200 microns) is used to remove any bony spicules/clots

If weight disparity between donor and recipient – use G primed BM.

Target CD34 – 3-5 million cells/kg

TNC target – 3-5 x 10^8 cells/kg

Pros and cons of BM harvest

BM	For the patients	For the donors
Disadvantages	Lower hematological and immunological recovery and consequently: – A higher risk of infectious complications – More days of hospitalization	They need: – To be anesthetized – Hospital admission – Autotransfusion
Advantages	Lower incidence of chronic GVHD and consequently: – A lower risk of late infections – Less late complications due to prolonged IST (e.g., osteoporosis, aseptic necrosis, etc.) – A better quality of life – A higher risk of relapse in neoplastic diseases	– No need to administer G-CSF, avoiding possible short- and long-term secondary effects [1]

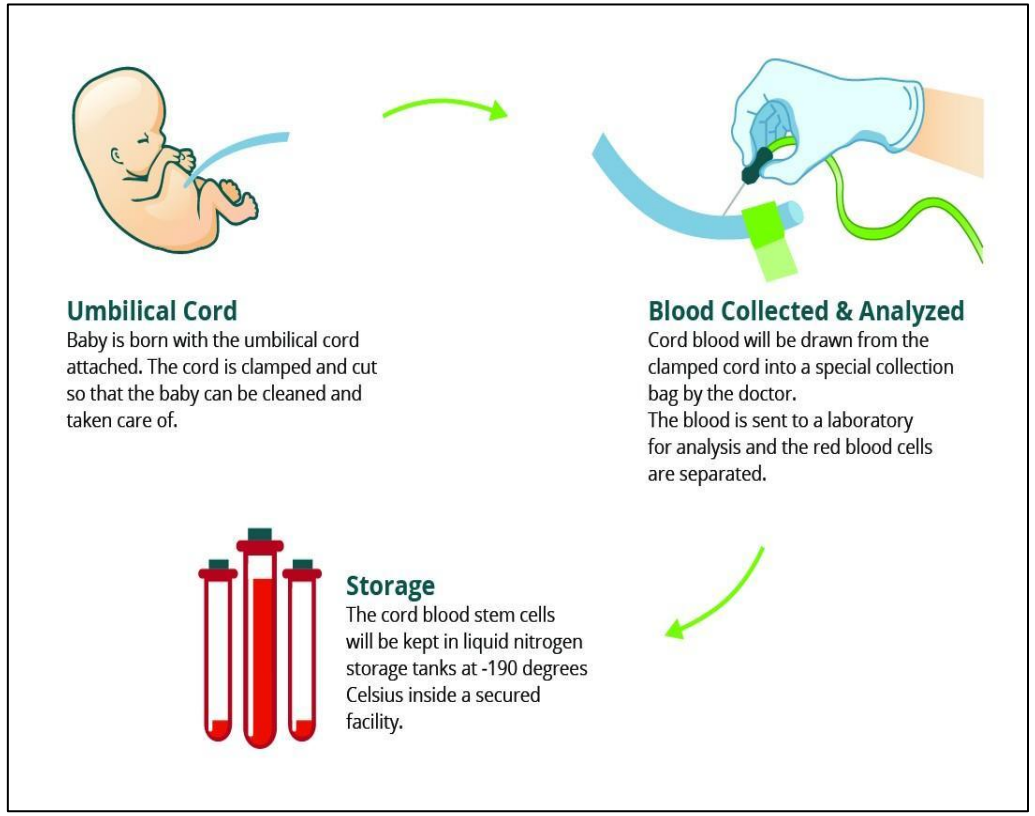
Cord Blood Stem Cells

Cord blood banks process and cryopreserve cord blood for HSCT

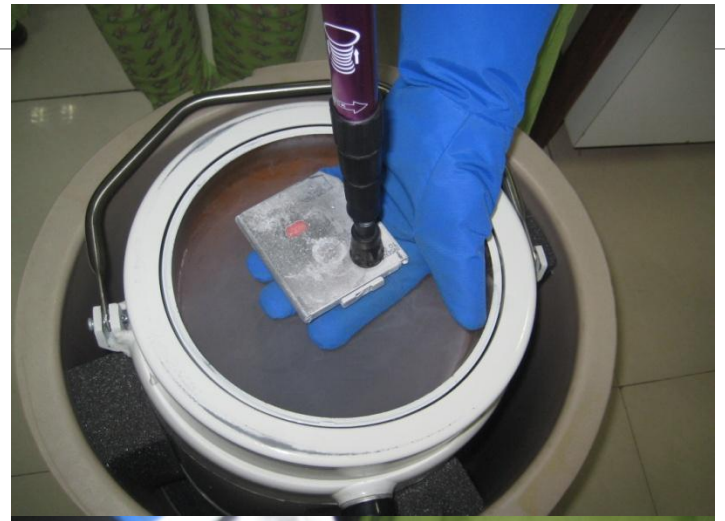
Total nucleated cell dose (TNC) to be calculated before preserving.

T cells are lower in number and immune reactivity so less GVHD.

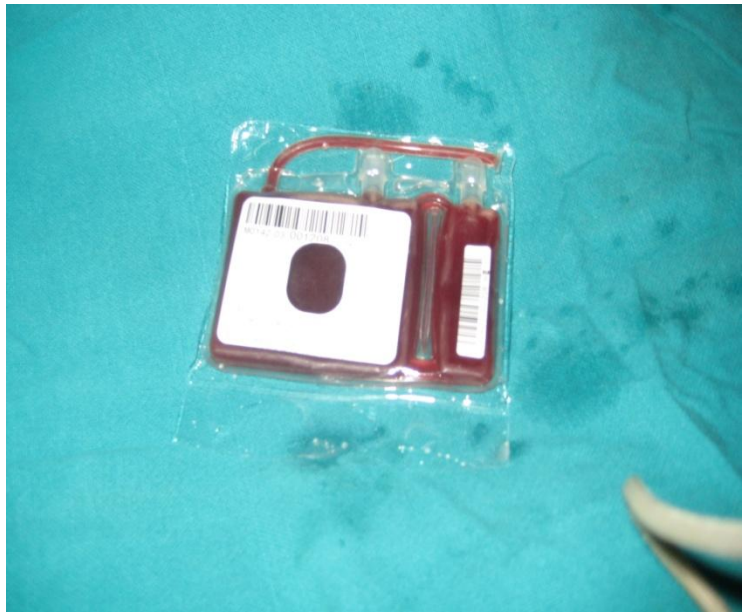
Limited number of progenitor cells; so high risk of graft failure and delayed engraftment and immune reconstitution.



Umbilical Cord Stem Cell



Umbilical Cord Stem Cell transport
in liquid nitrogen



Umbilical Cord Stem Cell bag

Peripheral Blood Stem Cell Collection

PBSC harvest

Why is it preferred ?

Early engraftment

Easier to perform

No need of GA

But is it all good?

Requirement of HD catheter

Chronic GVHD

Precautions – GCSF in SCD → VOC

- can cause splenic enlargement if already splenomegaly +

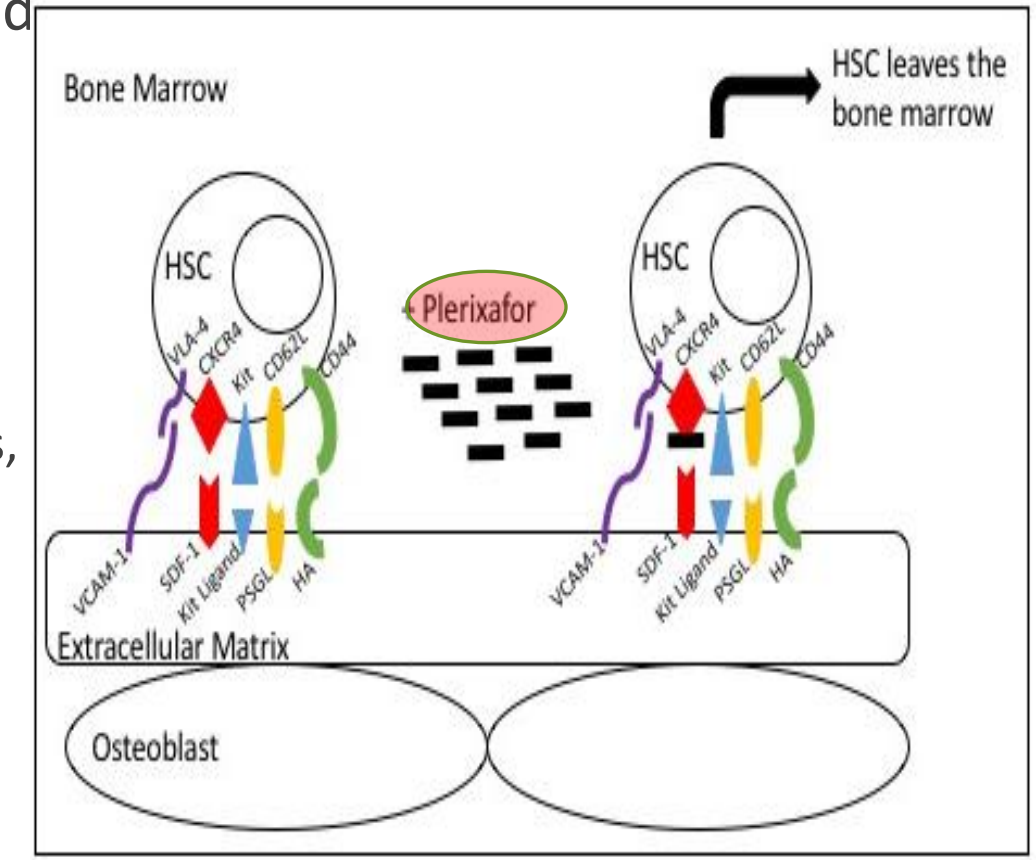
What is stem cell mobilization?

- Stimulating HPSCs to move from BM into peripheral blood , making them accessible by apheresis.

- Agents:

1. GSCF – started 4-5 days before apheresis (10mcg/kg/day)
2. Plerixafor – 11 hrs before apheresis, 4-6 hrs in poor mobilisers, 0.24 mg/kg- It releases marrow HSCs by inhibiting CXCR-4 SDF-1 interaction.
3. Chemomobilization- CY 2 g/m2, ICE, CHOP

New agent – Motixafortide (CXCR4 antagonist with long receptor occupancy- used in MM, not for kids)



Targets, when to start apheresis?

MNC count:

- ALC+AMC
- Correlates better with CD34
- Not fully accurate
- MNC >10-15k/µL- good mobilisation

WBC count:

- D4- to decide for plerixafor
- 1% rule- CD34 count- 1% of total WBC count
- Poor correlation with CD34 count (especially for poor mobilisers)

Others- Immature granulocyte count, HPC count

CD 34+ cell count by flowcytometry- GOLD STANDARD

≥ 20/µL- good mobiliser, Avoid apheresis if <10

Mobilization failure

What is mobilization failure?

- Not having achieved CD34 > 20 microL within 6 days of GCSF at 10mcg/kg/day
- CD34 < 2 million cells/kg bw of recipient in ≥ 3 apheresis sessions

Risk factors for poor mobilization: older donor (>60 yrs) and low CD 34+ circulating cells

Scenarios in which you expect poor HPC mobilization?

- multiple lines of CT ,
- pelvic or spinal radiotherapy,
- low Platelet count,
- poor nutritional status (PEM),
- Poorly controlled T2DM,
- Advanced disease,
- BM cellularity <30%,
- extensive BM involvement
- (Adults-> old age, Lymphoma diagnosis, prolonged exposure to lenalidomide, prior exposure to melphalan)

When to use plerixafor?

- Needed for patients at perceived high risk of mobilization failure
- Low WBC or MNC count after GCSF alone
- CD34+ <10-20/µL before apheresis

Vascular access specifications

PERIPHERAL

- Wide bore (16G)
- In both arms (inlet & outlet)
- May be difficult to attain, especially in pediatric donors
- Long procedure
- Not preferred

CENTRAL

- Avoid PICC, HICKMANN
- Double lumen
- Non-collapsible
- Can support high flow rate

HD catheter- preferred

Body size/ weight dictate French size of double lumen central venous catheters

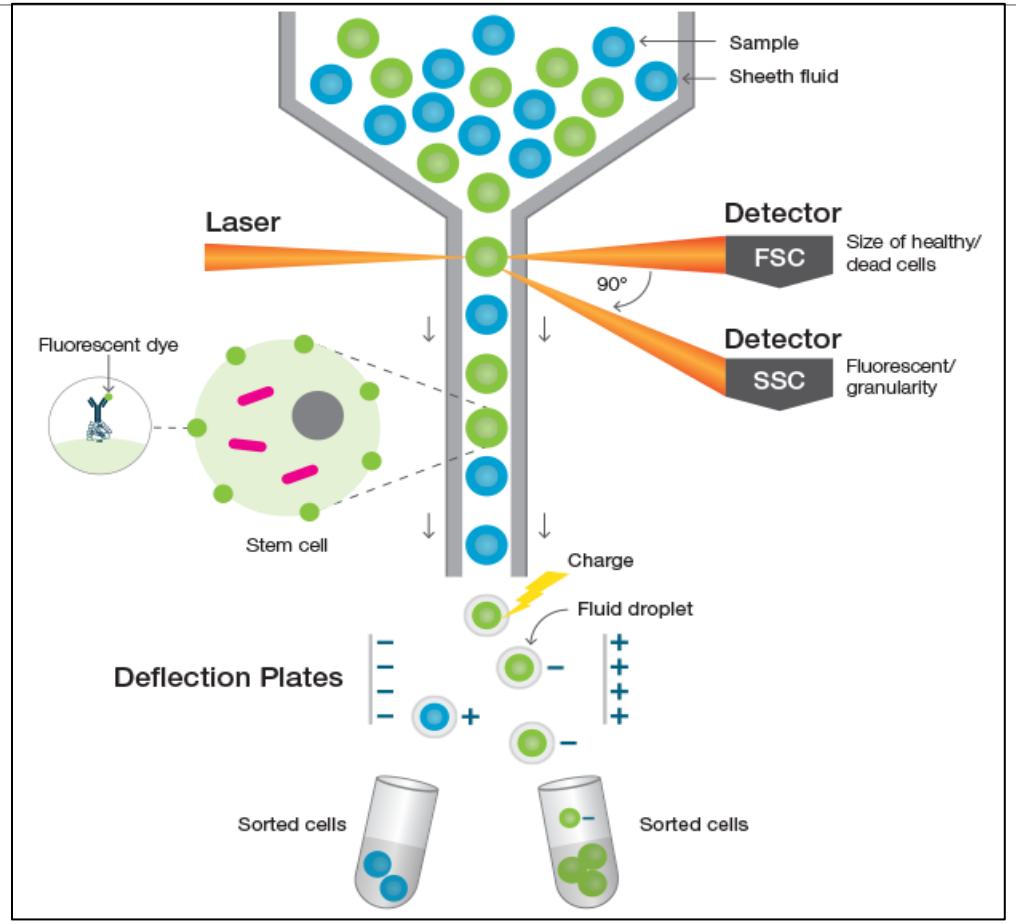
Weight (kg)	**Size of catheter**
<3	Two single lumen catheters (5 French)
3-10	7 French double lumen
10-20	8/ 9 French double lumen
20-50	9/ 10 French double lumen
>50	11.5/ 12/ 13.5 French double lumen

Identification and isolation of HSC

HSCs are CD34+ and CD38-.

Technique used for separation of stem cell-Flow cytometry

Other methods of identification include- total nucleated cell count(TNC) and colony forming units(CFU).



Cell separators

- Centrifugation based
- Separates blood components based on specific gravity
- Available cell separators- OPTIA, AMICUS, COM.TEC

AMICUS

COM.TEC

Priming

- To prevent hemodynamic instability
- Indications:

-BW<25 kg

-ECV>15% of estimated TBV

-TBV<1000 ml

-Hb<8g/dl

- Plasma, PRBC or albumin can be used

- Leucodepleted Irradiated RH, Kell, Blood group compatible

Adverse events

- Citrate related-

-Citrate used as anticoagulant can cause hypocalcemia

-Mild- headache, shivering, paraesthesias

-Moderate- Nausea & vomiting, abdominal cramps, tetany, hypotension

-Severe- Arrhythmia, seizure

- Vasovagal
- Catheter related
- Thrombocytopenia

Pre-harvest calculations

TOTAL BLOOD VOLUME				
	Obese (ml/kg)	Thin (ml/kg)	Normal (ml/kg)	Muscular (ml/kg)
Male	60	65	70	75
Female	55	60	65	70
Infant/ Child			80/70	

Blood volume processed:

- Standard- 1-3 x TBV
- Large volume- 3-6 x TBV
- Usual- 3-4 x TBV

Pre-harvest CD 34+ dose prediction:

$$\frac{\text{Pre-harvest CD34+ count x BV processed X CE x 1000}}{\text{Recipient weight (kg)}}$$

Example

Pre-harvest CD 34 + count- 34/µL

Donor weight 70 kg (male)

Volume to be processed- 3 x 70 x 70 = 14700 ml

Collection efficiency- 75%

Recipient weight- 30 kg

Predicted dose= $\frac{34 \times 14700 \times 0.75 \times 1000}{30}$ = 12.4 million/kg

Post-harvest calculation

$$\text{Total CD34+ cell dose} = \frac{\text{Post-harvest CD 34 + count (apheresis product) X Volume infused x } 10^3}{\text{Recipient weight (kg)}}$$

Example:

CD34+ cell count- 1235/µL

Volume infused- 100 ml

Body weight 20 kg

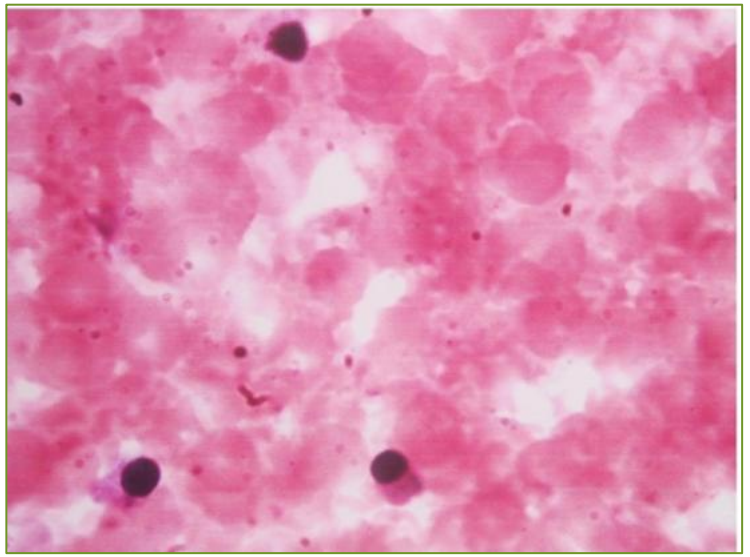
$$\text{CD34+ dose given-} \frac{1235 \times 100 \times 1000}{20} = 6.1 \text{ million/kg}$$

Optimum cell dose

Nucleated cell dose of 2 x 10^8/kg adequate for peripheral blood/marrow.

Rates of hematopoietic recovery, transplant-related mortality, and five-year survival better when CD34+ cell dose was ≥3.0 x 10^6/kg

Single unit cord blood transplant require $CD34^+$ cells ≥2 × 10^5/kg



E Bahçeci et al, British Journal of Hematology, 2000.
NMDP, CIBMTR guidelines 2019.

CD 34+ dose

Type of HSCT	Optimum dose (million/kg)
Autologous	3-5
Allogeneic HSCT	4-6
Haploidentical HSCT (TCR αβ)	15-18

Storage of Stem Cells

Kept in liquid state at 4°C if stored <96 h since cryopreservation may cause loss of CFU-GM progenitor

If stored for > 4-5 days, cryopreservation is required with thawing before use

Cryopreservation in either liquid or vapor phase of nitrogen at -196°C

10% DMSO used. Dump freeze (short term) or controlled freeze (long term)

Comparison of HSCTs from different sources

	Peripheral blood	Bone Marrow	Cord Blood
Ease of collection	Donor may have some discomfort during mobilisation	Donor undergoes marrow aspiration for several hours under anesthesia	No danger to mother or child
Time to engraftment	Median time=13 to 15 days	Median time=16 to 18 days	Median time=3 weeks
HSC dose	High	High	Low
T cell dose	Highest	High	Low with high proportion of naïve T cells
GVHD	Higher risk of chronic GVHD than bone marrow	Common in mismatched grafts	Uncommon and less severe
HLA mismatch	Requires perfect HLA match	Requires perfect HLA match	Well tolerated
Repeat transplant	Possible	Possible	Not possible

MCQ 1

For which of the following conditions, a bone marrow harvest is preferred

1. Relapsed ALL
2. CML in Blast crisis
3. Severe aplastic anemia
4. Hemolytic PNH

Answer- 3

MCQ 2

Which of the following is not a correct statement?

1. Hypotension, Paresthesia and tingling, Low platelets can be seen with PBSC collection
2. If you have 200 ml of marrow collection, 80 ml ACD-A should be mixed to anti-coagulate
3. DMSO concentration used is 10%
4. Cord blood leads to delayed engraftment and immune reconstitution

Answer- 2