Hematopoietic Progenitor Cell Collection

Shan Yuan, MD
May 11, 2011
Pluripotent: Can differentiate into blood cells of all three lineages (erythroid, myeloid, and megakaryoblastic)

- Express CD 34+ antigen
  - CD34 most likely an adhesion molecule
- Found in the marrow and in small amounts in the peripheral blood of adults
  - 0.1% of peripheral mononuclear cells
  - 1-4% of bone marrow cells
- Can be harvested from autologous/allogeneic donor and then transfused into myeloablated/myelosuppressed recipient = HPC Transplantation (HPCT)
- HPCT: A bridging procedure that allows for high dose chemotherapy for the treatment of malignancies
HPC Transplantation Based on Donor Type

- Autologous
  - Donor = Patient

- Allogeneic
  - Selected based on HLA-match, ABO match desirable but not required
  - Can be related to recipient or unrelated
HPC Transplantation

- Bone Marrow HPC
- Cord Blood HPC
- Peripheral Blood HPC
  - Most commonly used source
  - Donor stimulated with G-CSF/GM-CSF, with or without chemotherapy
  - Collection via apheresis technology
HPC Transplantation

- **Bone Marrow HPC**
  - Requires general anesthesia, invasive
  - >1L of marrow collected from donor
  - Often requires transfusion / preoperative autologous blood donation

- **Cord Blood HPC**
  - Contains large number of stem cells
  - Harvest at time of delivery - little risk to donor
  - Some HLA mismatch okay, little GVHD
  - Dose may be insufficient for many adult recipients
PB-HSCT vs. BMT: Pros and Cons

- **Pros**
  - Outpatient
  - Viable option for pt with BM disease involvement
  - Less invasive
  - Lower cost
  - No general anesthesia
  - Less discomfort
  - Less transfusions
  - Faster engraftment

- **Cons**
  - Donor may experience side effects of growth factors
  - Procedure is longer, (3-4h/procedure), may take several procedures
  - Increased incidence of GVHD
Indications for PB-HSCT

- **Malignancies**
  - Solid tumors: breast
  - Childhood cancers: Ewings, Wilm’s, neuroblastoma
  - Hematological: acute leukemias, CML, multiple myeloma, lymphomas, myelodysplastic and myeloproliferative diseases

- **Immunodeficiencies:** SCID, Wiskott-Aldrich

- **Hemoglobinopathies:** sickle cell, thalassemias

- **Metabolic disorders:** mucopolysaccharidoses

- **Autoimmune diseases**
Phases of PB HPC-T

- Mobilization of PB-HSC in the donor
- Collection of PB-HSC from the donor by apheresis
- Myeloablative/myelosuppressive conditioning of the recipient with chemotherapy and/or total body irradiation
- Infusion of PB-HSC product into the recipient
- Engraftment (~3 weeks following HPC-T)
Chemotherapy: increases PB-HSC count, usually done for autologous donors

Cytokines: given at ~10 micrograms/kg, once or twice daily as subcutaneous injections
- G-CSF (Filgrastin)
- GM-CSF (Sargamostrim)
  - Both are myeloid growth factors
  - How do they work?
G-CSF Mechanism

- Mobilization of Hematopoietic Progenitor Cells into Peripheral Blood is Associated with V-CAM-1 Proteolytic Cleavage in the Bone Marrow. --- Levesque JP et al
- Proposed that G-CSF-> neutrophil maturation and margination-> accumulation of neutrophil proteases, which disrupt the VLA-4/VCA-1 adhesive interactions with HPCs in the Bone marrow-> CD34+ cells break free and circulate in peripheral blood
- Can increase peripheral blood CD34 count by 30-50 fold
- Combined with chemotherapy: >100 fold
AMD3100 is a reversible inhibitor of CXCR4/SDF1a binding. CXCR4 is expressed on CD34+ hematopoietic progenitor cells,

- AMD3100 (day 5) significantly increases G-CSF-stimulated CD34+ cell mobilization. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone.
- Apheresis CD34+ cell collection is comparable between individuals mobilized by single dose AMD3100 (240 μg/kg) or 5 day G-CSF regimen.

**Neulasta:** A new derivative of G-CSF, pegylated G-CSF I

- Plasma half-life of filgrastim versus pegylated filgrastim is 3–4 h versus 33H
- Stable drug level-> donor convenience, better mobilization
Monitor for G-CSF side effects:
- “Neupogen fever”
- Headaches (75%), bone pain (63%), myalgia, fatigue
- Spleen enlargement, rupture
- Increased WBC count -> risk of thrombosis

Determining when to collect:
- Generally after 5 days of G-CSF for allogeneic donors, 10+ days for autologous donors who also received chemotherapy
- Could obtain peripheral blood CD\textsubscript{34}+ count: >10 CD\textsubscript{34}+>microliter is desirable
CD₃₄⁺ peripheral counts vs. product collected

**FIG. 1.** Correlation between the number of CD₃₄⁺ cells in peripheral blood and in the leukapheresis product on first leukapheresis day.

PB-HSC Apheresis Collection

- Collected by apheresis
- Access
  - Peripheral line may be okay for allogeneic donor as typically 1-2 collections are adequate
  - Central line needed for autologous donors as collection may require 5-10 procedures
- CD34+ cells upon centrifugation, stay near the the mononuclear cells layer, away from the RBC layer
- Use the PBSC/MNC protocol setting of the instrument
- Collect/output line hct should be 2-3%
Processing of Product

- CD34+ cell counted enumerated, usually by flow cytometry
- If ABO incompatible, RBC content of the product may be reduced
- May be frozen and infused at a later date using DMSO (dimethylsulfoxide) as a cryoprotectant. (Always done for autologous HPC-T)
- Thawed at bedside at the time of infusion
- Further manipulations:
  - Depletion of T cells to reduce GVHD
  - Positive selection of CD34+ cells
  - Negative selection of tumor cells
Number of CD34+ cells transplanted correlates with speed of engraftment

1-2x10^6/kg: sufficient/minimum amount for transplant

5x10^6/L gives fast engraftment

More is not always better -> higher dose may lead to “hyper-engraftment syndrome”:

- Slower platelet engraftment
- Fever, discomfort
- Increased GVHD
Healthy allogeneic donors: 1-2 procedures to reach target.

Autologous donor may take several procedures to reach target.

Large volume collections can be done to reduce the number of procedures

- 15-35L of donor blood processed
- The increase of PBPC recoveries was explained by the mobilization PBSC from the BM during apheresis
- Accomplished primarily by increasing flow rate -> more anticoagulant infused/min-> citrate toxicity
**Infusion**

- Performed at the bedside.
- Product is generally used fresh or thawed.
- Infused through a central vein under 30 minutes.
- The hematopoietic stem cells engraft within the bone marrow cavity by a homing-like mechanisms that have not yet been fully elucidated.
- Dimethylsulfoxide (DMSO) used for cryopreservation of stem cells may give rise to facial flushing, headache, tickling sensation in the throat, and strong taste in the mouth (the taste of garlic). Rarely, it could cause bradycardia, abdominal pain, encephalopathy/seizures, and renal failure.
- Stem cell infusions exceeding 500 mL are infused over 2 days and the rate of infusion is limited to 20 mL/min.