

# Blood Bank V

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## I. Hematopoietic Progenitor Cell Transplantation

### A. Basics

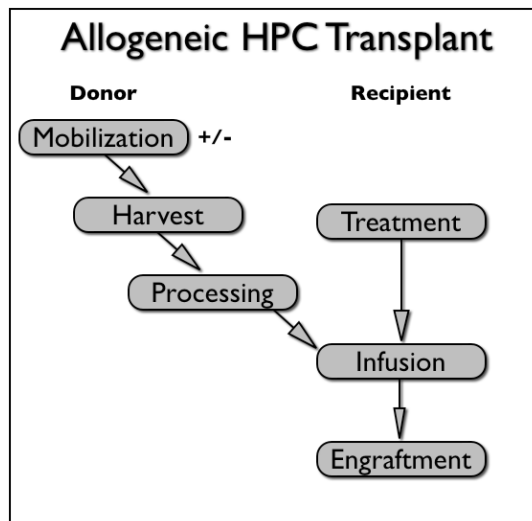
1. Hematopoietic stem cells
  - a. Present in small quantities in bone marrow (1-3%) and in tiny quantities (0.05%) in circulating blood
  - b. Definition
    - 1) “Totipotent” cells, meaning that they can differentiate into hematopoietic cells of all three cell lines (erythroid, myeloid, and megakaryoblastic).
    - 2) Terminology
      - a) **Hematopoietic Stem Cell (HSC)**: Totipotent cells as outlined above
      - b) **Hematopoietic Progenitor Cell (HPC)**: A more mature cell committed to a particular cell line
      - c) You will see these two terms used interchangeably, however, and the whole process referred to as either “HPC” or “HSC” transplantation.
  - c. Stem cells are best identified by surface expression of the **CD34** antigen
    - 1) CD34 is readily recognizable by flow cytometry; this is used to verify adequate dose of HPC products.
    - 2) Stem cells also have human leukocyte antigen (HLA) complexes on their surface, but they do NOT carry ABO blood group antigens.
2. Basic theory of HPC transplantation
  - a. Treat a disease by using very intensive therapy, either total body irradiation, chemotherapy, or combination of the two.
    - 1) This is done without the normal regard for preserving bone marrow function.
    - 2) The principle applies whether treating a primary marrow disorder (eg, leukemias) or a non-marrow disorder (eg, inborn metabolism errors).
  - b. “Rescue” the destroyed bone marrow by replenishing with HPCs.
3. HPC transplantation: Three main types
  - a. **Bone marrow transplantation (BMT); aka “HPC, marrow” or “HPC-M” transplantation**
    - 1) Approximately one liter or more of marrow harvested from a donor, usually from multiple posterior iliac aspirations
    - 2) Usually requires general anesthesia

- 3) Still done in some places but mostly replaced by HPC-A transplantation (see reasons below)
- b. **Peripheral blood progenitor cell (PBPC) transplantation; aka “HPC, apheresis” or “HPC-A” transplantation**
  - 1) Progenitor cells are harvested from the circulation of a donor using apheresis equipment
  - 2) Currently the most popular type of HPC transplantation; has mostly replaced traditional bone marrow harvesting.
  - 3) Reasons:
    - a) No general anesthesia required
    - b) Faster recovery and engraftment of neutrophils and platelets
    - c) Less acute graft-vs-host disease
    - d) Can potentially be done with patients with marrow involvement of their malignancy
    - e) Apheresis equipment is already in widespread use.
- c. **Umbilical cord blood transplantation; aka “HPC, cord blood” or “HPC-C” transplantation**
  - 1) Umbilical cord blood is rich in stem cells (as well as in progenitor cells that are cell-line committed); this blood can be harvested at the time of delivery and stored.
  - 2) Performed far less than other types (approx 6000 procedures performed since 1988 vs nearly 70K of others between 2002 and 2006 alone!)
  - 3) Potential advantages:
    - a) No risk to donor
    - b) Less susceptible to HLA mismatch problems
    - c) Lower risk of graft-vs-host disease (GVHD)

## **B. General considerations**

1. HPC transplantation is increasing in popularity and is being used as treatment for many different diseases, including:
  - a. Malignant diseases (most common indication; > 90%)
    - 1) Acute leukemia (currently the most common diagnosis treated with allogeneic transplant)
    - 2) Chronic myelogenous leukemia (less common than in the past due to new treatment regimens)
    - 3) Lymphomas (Hodgkin’s and non-Hodgkin’s)
    - 4) Breast cancer (*historical*) and other solid tumors
    - 5) Multiple myeloma
    - 6) Childhood solid tumors (Wilm’s, Ewing sarcoma, neuroblastoma, etc.)
  - b. Immune deficiencies
    - 1) Severe combined immunodeficiency disease (SCID)
    - 2) Aplastic anemia

- 3) Wiskott-Aldrich syndrome
  - c. Inborn metabolic disorders
    - 1) Mucopolysaccharidoses
    - 2) Lysosomal disorders (Niemann-Pick, Gaucher's)
    - 3) Adrenoleukodystrophy
  - d. Others
    - 1) Multiple sclerosis
    - 2) Paroxysmal nocturnal hemoglobinuria
2. Three main types of HPC transplant:
- a. **Allogeneic** HPC transplantation
    - 1) The donor and recipient are different people that are not genetically identical.
    - 2) Most commonly used in leukemias, but also in aplastic anemia, hemoglobinopathies and immune deficiencies
  - b. **Autologous** HPC transplantation
    - 1) The donor and recipient are the same person
    - 2) Most commonly used for metastatic or recurrent solid tumors and/or marrow malignancies.
  - c. **Syngeneic** HPC transplantation
    - 1) The donor and recipient are identical twins
    - 2) Less common than first two types (obviously)
3. **Allogeneic HPC transplantation**
- a. Choosing a donor
    - 1) General considerations
      - a) HLA compatibility is paramount.
      - b) A "perfect" match has compatibility at *HLA-A*, *HLA-B*, *HLA-C* (*Cw*), *HLA-DR* (*DRB1*), and *HLA-DQ* alleles ("10 of 10 match").
        - HLA-A, HLA-B, and HLA-DR considered most important
      - c) ABO compatibility is secondary.
      - d) Best chance of finding compatible donor is in siblings.
      - e) National Marrow Donor Program used if no siblings or family matches
    - 2) Most common virus transmitted via allogeneic HPC transplant: cytomegalovirus (CMV)
      - a) With today's methods, not usually significant
    - 3) Donors are screened like blood donors.
      - a) Donors should be in general good health.
      - b) Full infectious disease screening performed
      - c) Donor HIV is absolute contraindication.
      - d) Donors with some other viruses may be used if recipient is in dire need.



b. Donor processes

1) Mobilization (not traditionally done in HPC-M transplants but essential in HPC-A)

a) Process of making HPCs more available for collection

b) Cytokines dramatically increase harvest of HPCs

i) G-CSF (granulocyte colony stimulating factor)

- Most potent cytokine, typically used alone
- Peak effect on day 5 of infusion

ii) GM-CSF (granulocyte-macrophage colony stimulating factor) – less effective than G-CSF

iii) Complications: bone pain, enlarged spleen, thrombocytopenia, LFT increases, fever (with GM-CSF), headache, nausea

c) Greater harvest of HPCs = shorter time to engraftment and recovery

d) Data shows that use of mobilization in HPC-M collection also increases yield and shortens time to engraftment.

e) Measure effect by checking number of CD34+ cells by flow cytometry (best) or by counting colony-forming units (CFU) by cell culture (slower and largely replaced).

2) Harvest

a) Collection method depends on source (HPC-M vs HPC-A); see details below

b) Target a specific quantity of cells

- Typically  $3.0 \times 10^8$  nucleated cells/Kg of recipient weight OR
- $4.0 \times 10^6$  CD34+ cells/Kg

3) Processing

a) Product may be processed to:

- Reduce amount of red cells and/or plasma (especially useful in ABO incompatible allogeneic transplants).
  - Reduce T-cells to reduce risk of graft-vs-host disease (unfortunately, this has been shown to INCREASE the relapse rate).
  - Isolate and purify the CD34+ cells
- b) Product usually frozen using DMSO (dimethylsulfoxide) as cryoprotectant
- c. Recipient processes
- 1) Treatment of disease (“conditioning”)
    - a) Generally accomplished with chemotherapy with or without total body irradiation
      - Cyclophosphamide most common
    - b) Current thinking is that in many cases, marrow does not have to be completely destroyed in allogeneic HPC transplants for malignancy; allows new immune system to destroy tumor cells (see “GVT effect” below)
      - Sometimes called a “mini-transplant”
      - This contrasts with autologous HPC transplants, where the marrow is typically completely destroyed.
      - “Dose-intense” regimens are myeloablative, “mini-dose” regimens are not.
    - c) Complications of treatment
      - Mucositis, alopecia and overall GI toxicity common
      - Hepatic venous occlusion is extremely common (especially in dose-intense transplants).
      - Cardiomyopathy
      - Bone necrosis (late complication)
      - Cataract formation (late complication)
  - 2) Infusion
    - a) Liquid products may be infused right away; preferably within 24 hours.
    - b) Frozen products usually thawed at bedside and infused rapidly to minimize DMSO toxicity (still, nausea is extremely common).
  - 3) Engraftment
    - a) Evidenced by recovery of neutrophil, platelet, and red cell production (absolute neutrophil count > 500 cells/μL, platelets > 20K/μL)
    - b) In general, occurs roughly two to three weeks following infusion
    - c) With transplants with stem cells collected via apheresis (HPC-A or PBPCs), engraftment is nearly one week faster than with marrow transplants.

- d. Complications of allogeneic HPC transplantation (aside from those listed above under “Treatment”)
- 1) Infectious complications
    - a) Bacterial infections early, viral and fungal infections later
    - b) Invasive fungal infections are major cause of death in post-transplant patients.
  - 2) Host-vs-graft effect (rejection)
    - a) Leads to lack of engraftment
    - b) Factors that increase risk for rejection:
      - Increasing HLA disparity
      - T-cell purging of the infusion product
    - c) Factors that decrease risk for rejection:
      - Increased dose of HPCs in infusion
      - Dose-intensive treatment regimens
  - 3) Graft-vs-Host Disease (GVHD)
    - a) Acute and chronic forms
      - Acute (aGVHD): first 100 days, presents with skin, GI tract, and liver involvement
      - Risk increases with HLA differences and increased T-cells in transplant
      - Prevent/treat with methotrexate/steroids
      - Chronic (cGVHD): generally after day 50, have autoimmune symptoms (like Sjögren’s and systemic sclerosis) in addition to some of the acute problems above
      - HPC-A transplant patients: less risk of aGVHD, greater risk of cGVHD vs HPC-M recipients
      - Chronic GVHD seen in 40-50% of long-term survivors
    - b) Graft-vs-tumor effect (GVT); also known as “graft-vs-leukemia” effect (GVL)
      - The new stem cells are essentially giving the patient a brand-new immune system.
      - Viable newly formed lymphocytes may act to destroy residual tumor cells, leading to better survival and decreased recurrence.
      - Difficult to balance, since totally preventing GVHD also prevents GVT effect.
  - 4) Secondary cancers
    - a) Significantly increased risk for several malignancies post-transplant (both allogeneic and autologous):
      - Acute leukemias
      - Oral squamous cell cancers
      - Thyroid, brain, bone and liver cancers
      - Melanoma
  - 5) Infertility

e. Special considerations

1) ABO issues

a) **Major incompatibility**

- Introduction of an incompatible red cell antigen into a recipient, such as with a group A donor and a group O recipient
- Initial consideration: incompatible red cells infused with the HPC product may be hemolyzed by the native ABO antibody in the recipient, so process the HPCs to decrease the number of accompanying RBCs (10-20 mL residual)
- Secondary consideration: recipient ABO antibodies that may persist can hemolyze the new, incompatible red cells formed by engrafting marrow (see “Transfusion Therapy” section below).
- Until all original recipient RBCs are gone, patient may test in a chimeric pattern on routine testing (has been reported as far out as two years).

b) **Minor incompatibility**

- Introduction of an incompatible donor red cell antibody into an HPC recipient, such as with a group O donor and a group A recipient
- Initial consideration: incompatible plasma in the HPC product may hemolyze the recipient’s red cells, so process the HPCs to remove as much plasma as possible.
- Secondary consideration: engrafting lymphocytes may start making antibody that is incompatible with recipient’s red cells before all the red cells are gone from the circulation (see “Transfusion Therapy” section below).
- This antibody formation may cause severe hemolysis but is transient.

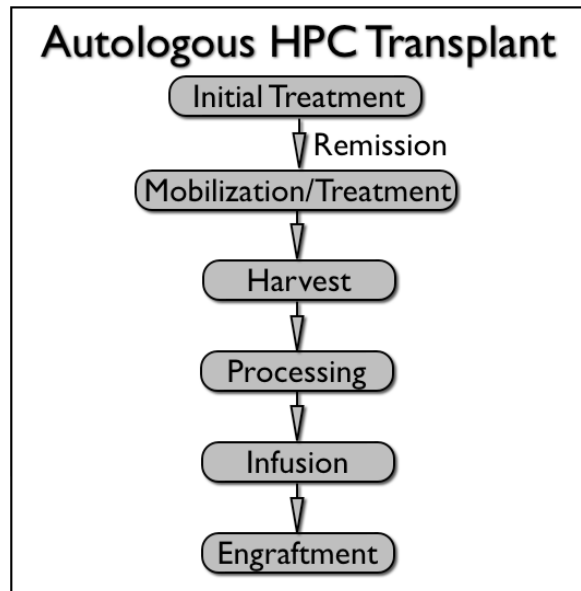
c) **Major and minor incompatibility**

- i) Introduction of both incompatible donor antigens and antibodies into a recipient; this occurs only in two situations:
  - Group A donor/group B recipient
  - Group B donor/group A recipient
- ii) Initial consideration: A combination of the above initial considerations, as hemolysis of both donor red cells and recipient red cells can occur at the same time.
- iii) See “Transfusion Therapy” section below for more information on how to transfuse these patients.

- 2) Rh issues
  - a) Rh incompatibility is not a contraindication to transplantation, though Rh antibodies may be induced when a D-negative donor engrafts in a D-positive recipient

4. **Autologous HPC transplantation:**

- a. Since the donor and recipient is the same person, the processes are similar to those outlined above, but they are occurring in one patient.
- b. Diagram below shows one way this is done



- c. Mobilization/treatment
  - 1) Mobilization of HPCs is typically done in conjunction with treatment in autologous transplants because the treatment effect helps mobilize HPCs into the circulation.
    - a) Ie, the chemotherapy agents used for treatment have the benefit of mobilizing HPCs, as well.
  - 2) “Dose-intense” regimens are typically used
    - a) Unlike allogeneic transplant, the GVT effect is not applicable in autologous transplants, so complete, intense treatment of the disease is needed.
  - 3) Ablative chemotherapy (potentially with total body irradiation) can both treat the disease and cause dramatic increases in circulating PBPCs.
    - a) The chemo also helps to “purify” the subsequent HPC product from malignant cells (“in-vivo purging”).
  - 4) Cytokines typically used in concert with chemotherapy to increase effect
  - 5) Cyclophosphamide + G-CSF quite common

- 6) Timing: chemo given, then cytokines when neutropenia begins to resolve (15-20 days).
- 7) “Tandem transplant”: under investigation for myeloma and germ cell cancers (source: NCI website)
  - a) Rather than the one course of chemo described above, these patients get a second course several weeks later.
  - b) Designed to reduce recurrence
- d. Harvest
  - 1) As with allogeneic, though the target may not be quite as high as with allogeneic donors
  - 2) 2-5 x 10<sup>6</sup> CD34+ cells/Kg is range.
- e. Processing
  - 1) Product may be processed as for allogeneic collections.
  - 2) In addition, may process using specialized methods to purge tumor cells from the product.
    - a) Not done often today
- f. Infusion
  - 1) As for allogeneic
- e. Engraftment
  - 1) See specifics under HPC-A engraftment below

### **C. HPC-M (bone marrow) transplantation**

1. No longer widely used, and almost never used for autologous HPC transplants
2. Collections typically done from posterior iliac crest under general anesthesia
  - a. Regional anesthesia (epidural, etc) may work
3. Donor considerations
  - a. Allogeneic donors
    - 1) Evaluated, in general, like blood donors and should be relatively healthy
      - a) This includes screening for infectious diseases
    - 2) Must be assessed for tolerance to anesthesia
  - b. Autologous donors
    - 1) Donor should be assessed for marrow viability, typically via bone marrow biopsy/aspiration
    - 2) Potential contraindications to using marrow as a source for autologous HPCs:
      - a) Malignant cells
        - Techniques are available for “purging” the product, but heavy infiltration by tumor cells precludes marrow use.
      - b) Prior pelvic irradiation
      - c) Marrow fibrosis
      - d) Poor anesthesia risk
4. Specific considerations:
  - a. Mobilization

- 1) Not always done for HPC-M donors
  - 2) When done, studies have shown an increase in harvested cells and resulting shortening in engraftment time.
- b. Harvest
- 1) Multiple aspirations of the posterior iliac crest on one or both sides
  - 2) A liter or more typically withdrawn
  - 3) Quantity targeted varies depending on type.
    - a)  $1 \times 10^8$  nucleated cells/Kg for autologous
    - b)  $3 \times 10^8$  nucleated cells/Kg for allogeneic
    - c) For either, a range of  $2-5 \times 10^6$  CD34+ cells/Kg
- c. Processing
- 1) Usually requires lab processing to reduce volume and red cell content and potentially enrich the CD34+ cell concentration
  - 2) May also need to process to avoid ABO issues (see above)
  - 3) More concentrated products require less DMSO, leading to less DMSO toxicity at infusion
  - 4) Product may be frozen in 10% DMSO or kept liquid for up to 72 hours before infusion.
- d. Infusion
- 1) Product may be washed or just infused directly following thaw.
  - 2) DMSO toxicity in unwashed products is common.
    - a) Nausea/vomiting
    - b) Blood pressure changes (hypertension or hypotension)
    - c) Flushing
  - 3) May need to divide infusion to limit DMSO exposure to less than 1g/Kg recipient weight/day
- e. Engraftment
- 1) Recovery to absolute neutrophil counts over 500 per microliter and platelets over 20,000 per microliter typically takes about 21 days.

**D. HPC-A (peripheral blood progenitor cell) transplantation**

1. Units collected through use of apheresis equipment
2. Vast majority of HPC transplant collections done this way today
3. Donor issues
  - a) Allogeneic
    - 1) As above, should be healthy
    - 2) Full infectious disease screening is done.
  - b) Autologous
    - 1) More difficult to manage because they may not be healthy

- 2) Ideal time for an autologous HPC transplant, though, is when patient is in remission and can tolerate the procedure better.
4. Specific considerations
  - a. Mobilization
    - 1) Must be done for HPC-A donors (insufficient HPCs are present in normal circulating blood)
    - 2) Cytokines discussed previously
    - 3) In autologous donors, chemotherapy used
  - b. Treatment/marrow conditioning
    - 1) For autologous transplants, marrow ablation as outlined above
    - 2) For allogeneic, may use “mini-transplant” (non-myeloablative therapy)
  - c. Harvest
    - 1) Standard apheresis machines used, with specific software programs for HPC collection
    - 2) Quantity targeted varies by program, but typically a minimum of  $2 \times 10^6$  CD34+ cells/Kg is targeted (up to  $5 \times 10^6$  for some programs).
    - 3) Maximizing HPC cells correlates with faster engraftment and better outcomes.
  - d. Processing
    - 1) Minimal processing usually needed (in contrast to HPC-M products)
    - 2) Volume reduction may be indicated if unit is to be cryopreserved (to save space and minimize DMSO toxicity at infusion).
    - 3) For allogeneic donations, red cell or plasma reduction may be done (see below).
    - 4) Other manipulations as outlined above (CD34 selection, etc.) may also be done.
    - 5) 10% DMSO added, frozen in liquid nitrogen
  - e. Infusion
    - 1) Product thawed at the bedside and infused rapidly
      - a) Some prefer to wash the product to remove DMSO.
    - 2) If not washed, DMSO toxicity is common.
    - 3) Avoid leukocyte reduction filters (seems obvious!)
  - f. Engraftment
    - 1) Recovery to absolute neutrophil counts over 500 per microliter and platelets over 20,000 per microliter typically takes about 11-14 days.
    - 2) Some programs use G-CSF infusion after infusion to speed engraftment (as little as 9 days).

## **E. HPC-C (cord blood) transplantation**

1. Nearly always done as allogeneic transplantation, especially with pediatric patients and adults that can't get a good HLA-matched donor

2. Cord blood is an extremely rich source of multiple progenitor cell types of varying levels of maturity and cell line commitment.
  - a. Despite this, cord blood products typically have many fewer nucleated cells than either HPC-M or HPC-A products.
3. Infectious disease testing performed on mother, and baby is evaluated for lack of infectious and inherited diseases.
4. Cord blood is collected either by OB staff before delivery of the placenta or by dedicated cord blood bank staff after delivery.
5. Units are processed to remove red cells and plasma, then product is typically frozen in 10% DMSO.
6. After thawing, most HPC-C products are washed prior to infusion.

## **F. Transfusion therapy considerations**

1. Three phases
  - a. Phase I (pre-transplant)
  - b. Phase II (peri-transplant)
    - 1) Begins at time of treatment
    - 2) Ends when full engraftment has occurred
    - 3) In ABO-incompatible transplants, phase II ends when recipient's blood type has converted to donor's type.
  - c. Phase III (post-transplant)
    - 1) After engraftment
    - 2) In ABO-incompatible, after full blood type conversion (serum and red cells)
2. **Phase I**
  - a. Two main concerns:
    - 1) Preventing alloimmunization
      - a) Generally done via use of leukocyte reduced blood products
      - b) Also, avoid family member transfusions to prevent exposure to family HLA antigens.
    - 2) Preventing transfusion-associated graft-vs-host disease
      - a) Patients are going to be profoundly immunosuppressed and so are greatly at risk.
      - b) All patients should receive irradiated blood products if they are scheduled to be transplanted.
  - b. Other concerns
    - 1) HPC transplant patients are very susceptible to CMV infection; use CMV negative or leukoreduced products.
3. **Phase II**
  - a. Continue support as above, but may have special concerns in ABO-mismatched allogeneic transfusions.

- b. See chart below for recommendations for ABO types of transfused products in this phase, remembering the principles mentioned above.
  - 1) In general:
    - a) In major mismatch, transfuse the recipient’s red cell type and donor’s FFP/platelet type (of course, you can always use AB FFP).
    - b) In minor mismatch, transfuse the donor’s red cell type and recipient’s FFP/platelet type.
    - c) For both major and minor mismatches, give O red cells and AB plasma.
  - c. Though some people wash transfused red cells to remove incompatible plasma, most do not feel it is necessary to do so.

**4. Phase III**

- a. Irradiated products should be given for the first year after allogeneic transplant (some irradiate forever).
- b. Continued CMV vigilance while recipients remain on immunosuppressive therapy
- c. ABO-incompatible transplants:
  - 1) After the patient’s original ABO antibodies have disappeared (which may take several months after engraftment), patient may be transfused with ABO-identical blood (identical to the patient’s *new* ABO type, that is).
  - 2) May utilize simple crossmatch technique to detect residual “old” ABO antibodies in a donor who has a new ABO type; ie, if the patient’s serum does not react with red cells of the new ABO type at all (a negative crossmatch), it is safe to begin using the new type.
  - 3) Until that happens, however, it is safer to use the recommendations in the table below.

Recipient Type	Donor Type	Mismatch	Red Cell Type	FFP Type
O	A	Major	O	A, AB
O	B	Major	O	B, AB
O	AB	Major	O	AB
A	AB	Major	A	AB
B	AB	Major	B	AB
A	O	Minor	O	A, AB
B	O	Minor	O	B, AB
AB	O	Minor	O	AB
AB	A	Minor	A	AB
AB	B	Minor	B	AB
A	B	Major & Minor	O	AB
B	A	Major & Minor	O	AB

(NOTE: After AABB *Technical Manual*, 14<sup>th</sup> ed)