Hematopoietic Stem Cells, Stem Cell Processing, and Transplantation

Joseph (Yossi) Schwartz, MD

Director, Hemotherapy and Stem Cell Processing Facility

E-mail: js2745@columbia.edu

A 40-year old man with acute myelogenous leukemia (AML) who has a deletion of chromosome 5 is referred to your office because he has not entered remission, despite three intensive induction regimens. The patient has an identical twin, which was confirmed by HLA typing and other genetic testing. Several other siblings also registered with the National Marrow Donor Program (NMDP). One of these siblings was found to be 6-antigen match for his brother. A preliminary search of the NMDP registry found 20 potential matches. Currently the patient is not infected and has normal organ function. He has no circulating blasts, although his marrow has over 50% blasts.

Which is the best treatment option for this patient?

A) Additional chemotherapy to induce remission

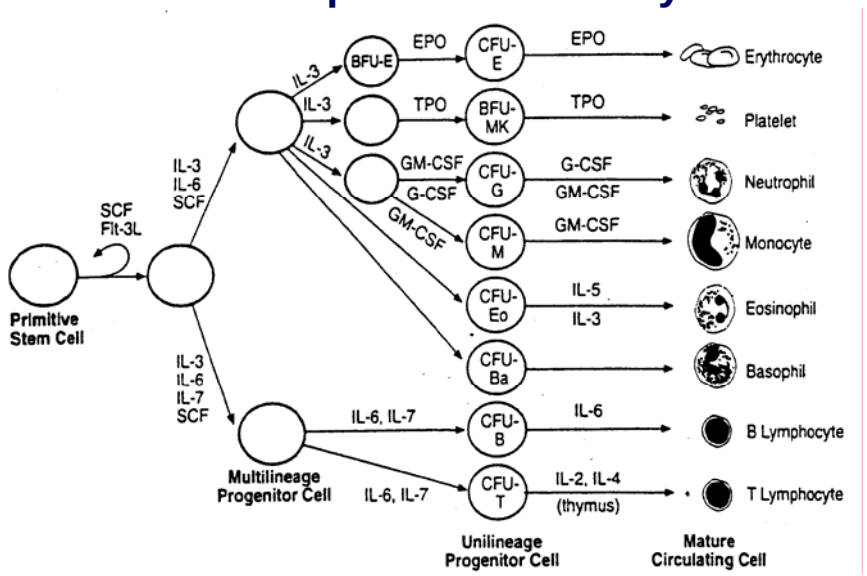
B) Syngeneic bone marrow transplantation

C) Allogeneic sibling bone marrow transplantation

D) Unrelated donor bone marrow transplantation

E) Autologous peripheral blood stem cell transplantation

Hematopoiesis Hierarchy



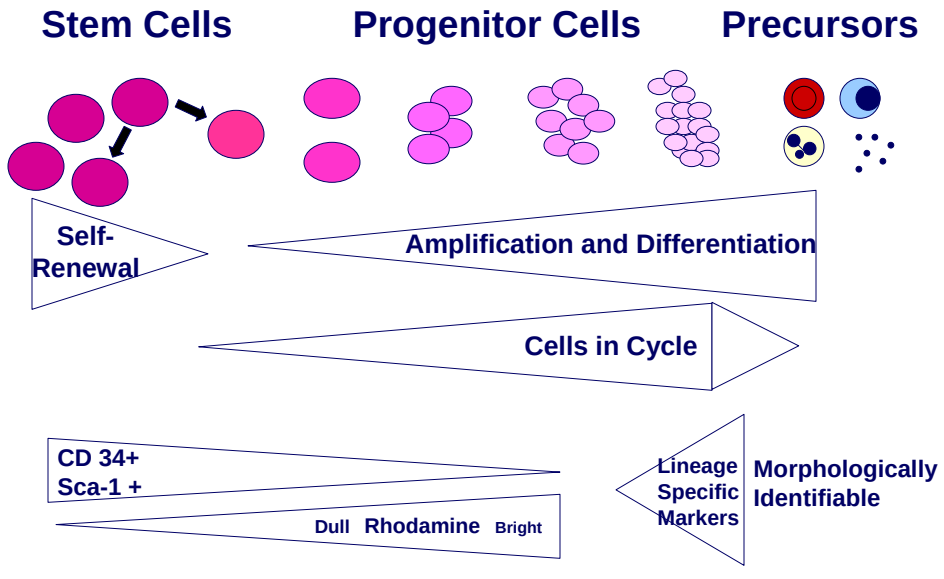
Hematopoietic Stem Cells

- Sustain hematopoiesis
- Self-renewal throughout life
- High proliferative capacity: 1 cell $\rightarrow$ millions
- Pluripotent: $\rightarrow$red cells, white cells and platelets
- Not morphologically identifiable

Maturation of Hematopoietic Cells

- Cell division
- Major amplification of cell numbers
- Progressively less capacity to proliferate
- "Mature" cells are post-mitotic when released into circulation
- Cells cannot "back-track" to less mature form
- Cells cannot "switch" from one lineage to another

Hematopoiesis Hierarchy



Hematopoietic Stem Cells

- Rare: <1 per 100,000 bone marrow cells
- Also found circulating in blood, cord blood
- Required for successful bone marrow transplantation

Discovery of Hematopoietic Stem Cells

+ whole body irradiation → death

+ irradiation + protect spleen → recovery

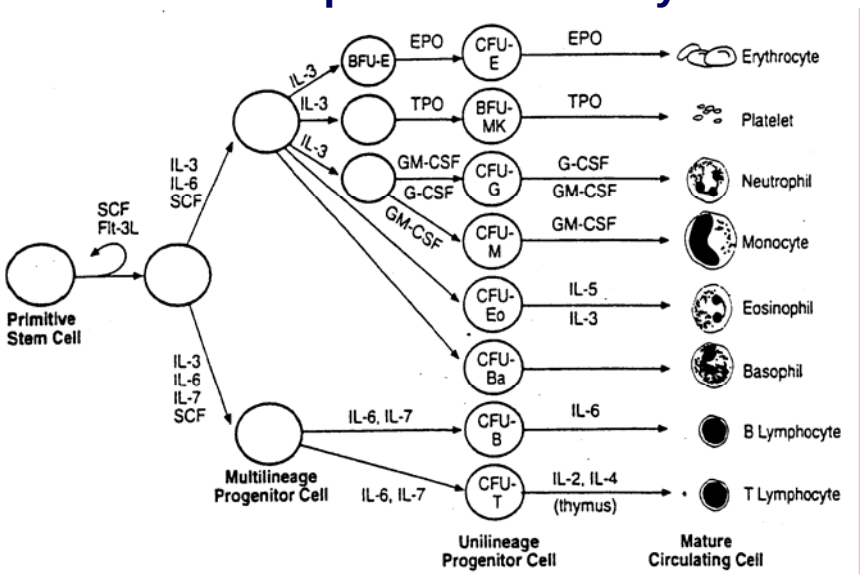
+ irradiation + spleen cells → recovery

+ irradiation + bone marrow → recovery with colony forming units spleen (CFU-S)

Identification of Hematopoietic Stem Cells

- In vivo bone marrow transplantation experiments: CFU-S and long-term reconstitution
- In vitro tissue culture assays: LTC-IC, CAFC, blast colonies
- Cell surface antigens: CD34+, thy1lo, c-kit+, rhodamine 123 lo, CD38-, lineage-

Hematopoiesis Hierarchy



Regulation of Hematopoiesis

- Hematopoietic growth factors (HGFS)
  - Glycoproteins
  - Proliferation, differentiation, and survival of hematopoietic cells
  - Act on a broad range of stem and progenitor cells: SCF, IL-3, GM-CSF
  - Hormone-like: Erythropoietin
  - Paracrine: SCF, IL's, GM-CSF
  - Biological activity in the pg to ng/ml concentration
  - Basal levels of production are very low
  - Redundancy

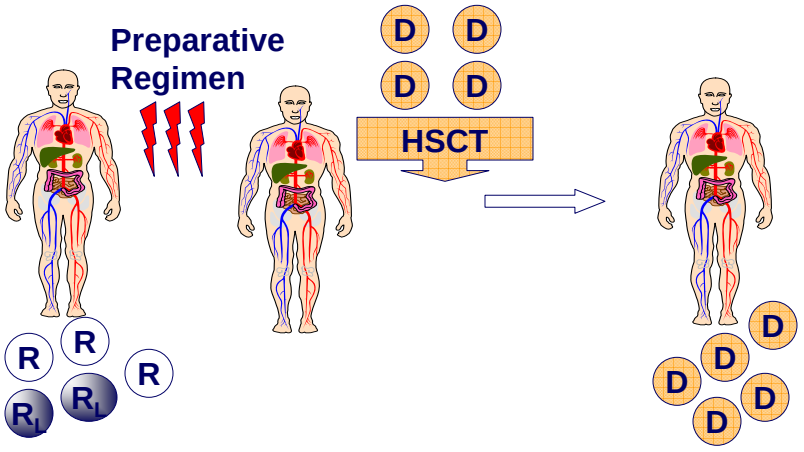
Regulation of Hematopoiesis

- Local: Microenvironment/Stroma
  - Endothelial cells, fibroblasts, macrophages, preadipocyte
  - Provides physical support/attachment for stem cells, progenitor cells, precursor cells
  - Stromal cells produce hematopoietic growth factors: ILs, GM-CSF
  - Membrane bound hematopoietic growth factors
  - Paracrine secretions of hematopoietic growth factors
  - Hematopoietic growth factors trapped in extracellular matrix

Early Bone Marrow Transplantation (BMT)

- Recipients myeloablated with chemotherapy and/or radiation
- Bone Marrow (BM) aspirated iliac crests of from normal related HLA identical donors
- BM rapidly filtered through coarse filters to remove fat and particulate matter
- Taken to bedside for immediate reinfusion
- Typical marrow for a 70 kg adult consisted of:
  - 300-400 ml of RBCs
  - 1 to 4 X 10^{8} nucleated cells/kg
  - 1.0 to 1.5 L volume

Hematopoietic Cell Transplant



Sources of Donors

Syngeneic donor

Allogeneic donor

Autologus donor

Syngeneic Transplants

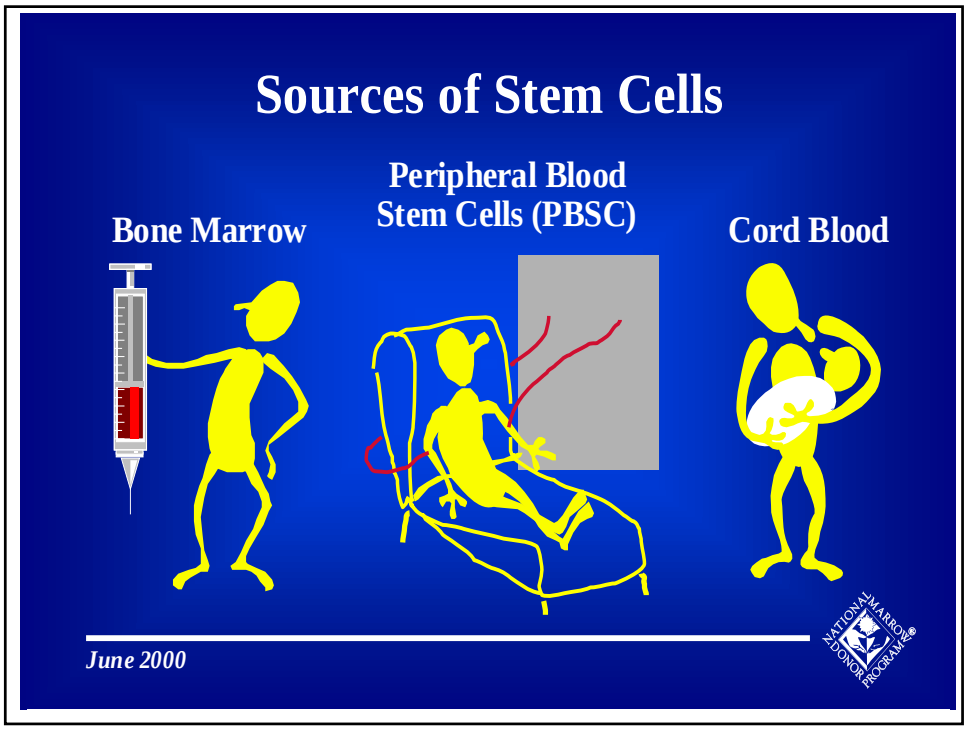
- Disadvantages:
  - Most patients don't have an identical twin
  - Infectious disease transmission
  - No Graft vs. Leukemia (GVL)
  - No Graft vs. Tumor (GVT)
- Advantages:
  - Graft free from disease
  - Reduced graft rejection
  - Reduced graft vs host disease (GVHD)

Allogenic Transplants

- Disadvantages:
  - Donor must be HLA compatible
  - Some patients don't have HLA matched family members
    - Anonymous donor registries: NMDP, Cord Blood Banks
  - Graft vs. Host Disease (GVHD)
  - Infectious disease transmission
- Advantages:
  - Graft free from disease
  - Graft vs. Leukemia (GVL)
  - Graft vs. Tumor (GVT)

Autologous Transplants

- Disadvantages:
  - Graft may contain tumor cells or other abnormal cells
  - Insufficient cells: aplastic anemia
  - No Graft vs. Leukemia (GVL)
  - No Graft vs. Tumor (GVT)
- Advantages:
  - Readily available for patients without HLA identical donors
  - No infectious disease transmission
  - Reduced peri-transplant morbidity and mortality



Cell Types for Transplantation

- (1) Bone Marrow
  - Collected from the iliac crest and/or the sternum
  - Advantages:
    - Large number of stem cells
    - Few red blood cells
    - Few lymphs
  - Disadvantages:
    - Surgical procedure
    - General anesthesia
    - Pain during recovery

Cell Types for Transplantation

- (2) Peripheral Blood Stem Cells
- Collected by apheresis following hematopoietic growth factor "mobilization" and/or chemotherapy
  - FDA approved hematopoietic growth factors: Granulocyte colony stimulating factor (G-CSF), Granulocyte/macrophage stimulating factor (GM-CSF), Erythropoietin (Ep), Interleukin-11 (IL-11)

Cell Types for Transplantation

- (2 cont.) Peripheral Blood Stem Cells
  - Advantages:
    - Easy to collect large numbers of stem cells
    - Multiple collections possible
  - Disadvantages:
    - Pre-treatment with HGF
      - risk to normal donors?
      - ↑ tumor cell proliferation
      - ↑ circulating tumor cells → ↑ graft contamination with tumor cells
    - Bone pain
    - May require central venous access

Cell Types for Transplantation

- (3) Cord Blood Stem Cells
  - Advantages:
    - Collection has no risks for mother or infant
    - Readily available, anonymous banks, family donation
  - Disadvantages:
    - Low cell dosages may limit to small recipients
    - Availability of HLA-matched donor
    - Multiple collections impossible

Problems to be overcome: All Transplants

- Myeloablative regimens very toxic
  - High peri-transplant morbidity and mortality
  - Infectious complications
  - Bleeding
- Cells had to be infused immediately
- Large volume, including donor plasma
- Too few cells
  - small donor (child, baby) to larger recipient (larger child, adult)

Problems to be overcome: Allo Transplants

- Large number of contaminating red blood cells (300-400 mL)
  - ABO/Rh incompatibility
    - Infusion of incompatible red cells with donor marrow
    - Hemolytic transfusion reation
    - Hypotension and renal failure
    - Threat of hemolysis precluded transplant across ABO barriers
- Histocompatibility
  - High risk of GVHD with mismatches

Problems to be overcome: Auto Transplants

- Insufficient cells in bone marrow failure
- Tumor cell contamination of the graft which could preclude cure
- Cryopresesrvation needed to preserve stem cells from collection to reinfusion post-myeloablative therapy.

"Mini-Transplants" = Low Dose Preparative Regimens

- Advantages:
  - Less peri-transplant morbidity and mortality
  - Increased GVL and GVT
- Disadvantages:
  - May not irradiate tumor completely
  - Increased GVHD
  - May need to be augmented with donor-derived lymphocyte infusions

Stem Cell Processing

- Volume reduction
  - Centrifugation, removal of excess plasma
- Removal of red blood cells
  - Enables transplant of ABO/Rh mismatched stem cells
  - Sedimentation

Red Cell Reduction Techniques

- Developed for all licensed blood cell separators
  - Cobe Spectra, Fresenius AS 104, Fenwall CS 3000
  - achieve 60-85% MNC recovery
  - less than 20 ml of RBC remain

RBC Reduction

- Gravity sedimentation
  - BM diluted 1:8 with 6% HES
  - RBC removal 99% in 1-3 hrs
  - MNC recovery about 75%
- Density gradient separation
  - Ficoll-metrizoate
  - CFU-GM recovery very high
  - RBC depletion less than 1%

Stem Cell Processing: "Designer Products"

- T-cell depletion
  - ◆Allows engraftment of HLA-mismatched or haploidentical matches
  - ◆Greater risk of graft rejection
- Tumor purging
  - ◆Pharmacologic agents, 4 HC

Stem Cell Processing: "Designer Products":

CD 34+ cell selection

- Effective therapeutic dose - 1 to 5 X 10^6 cells/Kg
- Higher doses result in faster engraftment
- Eliminates lymphocytes→ ↓ GVHD in allo grafts
- Eliminates tumor cells in autologous grafts

Storage

- BM, buffy coats as long as 9 d at 4 degrees
- Cryoprotectants: DMSO, HES, Glycerol
- Rate of freezing
- Cell concentration (3-7 x 10^8 cells/ml)
- Storage conditions (vapor or liquid phase)

Stem Cell Processing

Quality Control

- 1-3 x 10^8 MNC/kg correlates w/engraftment
- CFU-GM correlates with engraftment
- CD34 correlates with CFU-GM; Effective therapeutic dose - 1 to 5 X 10^6 cells/Kg
- Viability
- Sterility, Tumor cell contamination
- Engraftment, gold standard

Bone Marrow Transplantation Can Cure:

- Leukemia
- Lymphoma
- Multiple Myeloma
- Genetic Diseases: Sickle Cell Disease, Thalassemia, Fanconi's Anemia, Immunodeficiency Syndromes
- Solid Tumors: Brain tumors, ovarian cancer?, breast cancer?

Breast CA

- 180,000 new cases/yr in USA
- 44,000 deaths/yr
- Randomized trials in metastatic and high risk primary disease
- HDC alone will not completely cure breast ca
- Phase 2 trials showed benefit HDC/PBPCT

Multiple Myeloma

- Problem of tumor cell contamination in the graft
- HDC/PBPCT has a beneficial effect on response rates as well as EFS & OS
- Tandem transplant appears even better
- CD34 selection
- PBPC must be collected early

NHL, HD

- Lymphoma
  - low grade (early, higher PFS)
  - high grade (33% vs 14% at 4 yrs)
  - Hodgkin's Disease (56% vs 19% at 4 yrs)

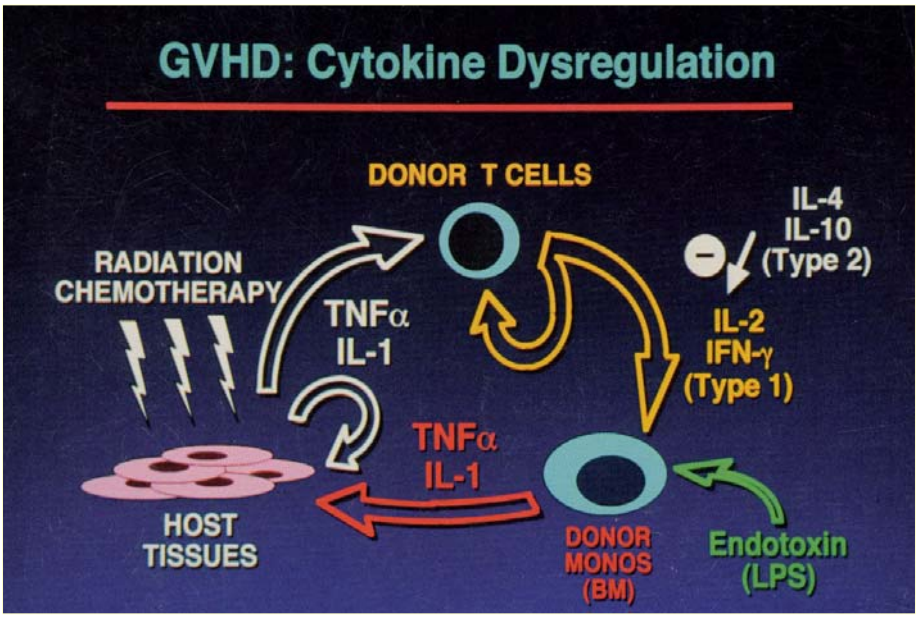
Pediatrics

- Neuroblastoma
- Brain tumors

BMT Complications

- Early
  - Tissue toxicities
    - Mucositis
    - VOD
    - Pneumonitis
  - Pancytopenia
    - Graft failure
  - Infections
    - Bacterial
    - Viral
    - Fungus
  - GVHD
- Late
  - GVHD-chronic
  - Late tissue damage
    - AVN
    - Cataracts
    - Pneumonitis
    - Growth & Development
  - 2ry Cancers
    - AML/MDS

GVHD



Evidence for Role of Cytokines in GVHD

- TNF-α
  - Produced after tissue damage
  - Induces GVHD in animal models
  - Correlates with GVHD in humans
  - GVHD can be prevented with anti-TNF antibodies.
  - Correlates with the intensity of the preparative regimen.

GVHD Prevention

- Immune-prophylaxis
  - Cyclosporine/methotrexate gold standard
  - Tacrolimus/methotrexate equivalent
  - Single agent not considered standard of care
- T Cell Depletion
  - Ex vivo-abrogates GVHD
  - In vivo-ATG and Campath 1H
  - Relapse and rejection an issue

Summary

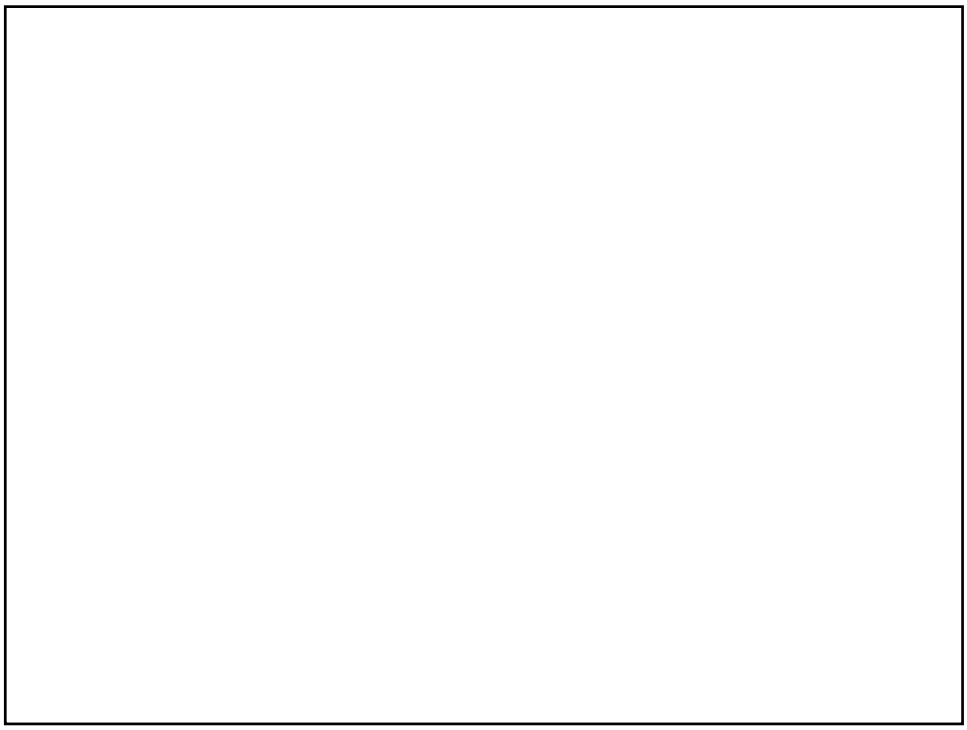
- **Blood cells have a limited life span**
- **Continuous production of blood cells achieved by proliferation of progenitor cells and precursor cells derived from stem cells**
- **Stem cells have self-renewal capacity**
- **Stem cells are identified by their ability to repopulate bone marrow in myeloablated recipients**

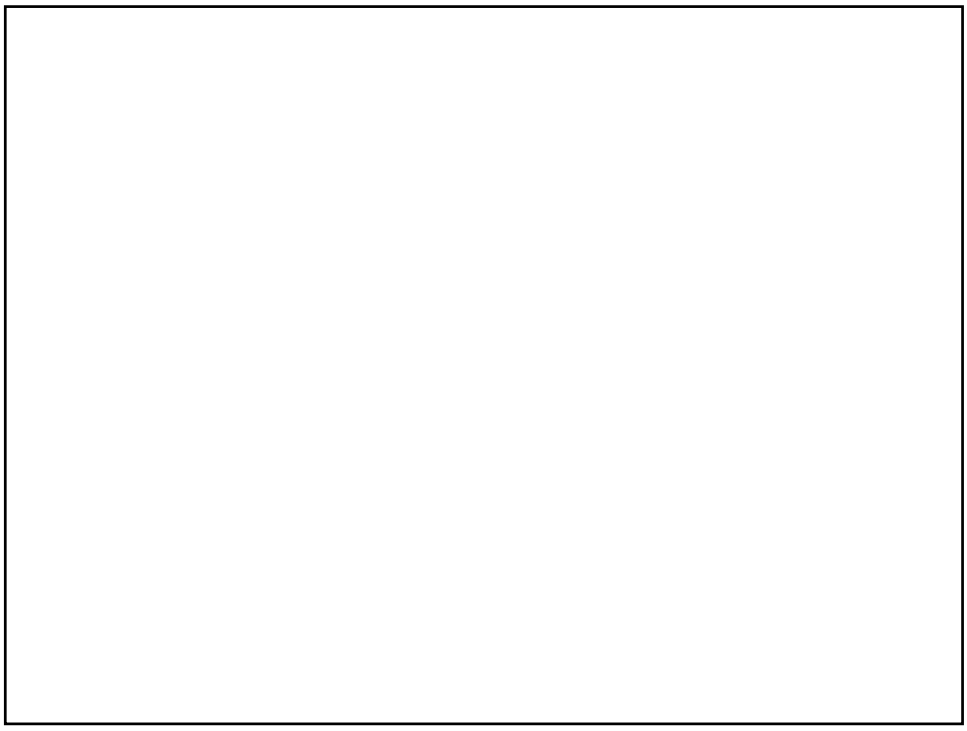
Summary

- BMT can cure leukemia, lymphoma, myeloma, solid tumors, and genetic diseases
- BMT works because stem cells removed from the donor “engraft” in the recipients bone marrow.

Summary

- Bone marrow, peripheral blood, and cord blood are all sources of transplantable hematopoietic stem cells
- Donors can be syngeneic, allogeneic or autologous
- Stem cell processing labs can customized stem cell grafts for the specific needs of the patient





Hematopoietic Stem Cells: Processing and Transplantation