# Hematopoietic Stem Cells, Stem Cell Processing, and Transplantation

**Joseph (Yossi) Schwartz, MD**

**Director, Hemotherapy and Stem Cell Processing Facility**

**Bone Marrow Transplantation Can Cure:**

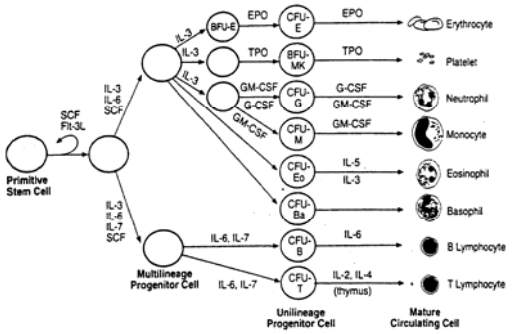
- Leukemia
- Lymphoma
- Multiple Myeloma
- Genetic Diseases: Sickle Cell Disease, Thalassemia, Fanconi's Anemia, Immunodeficiency Syndromes
- Solid Tumors: Brain tumors, ovarian cancer?, breast cancer?

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**



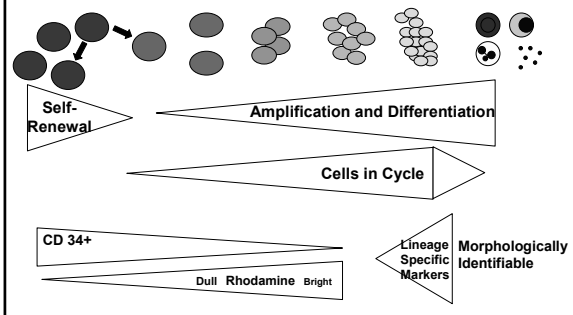

**Stem Cells** **Progenitor Cells** **Precursors**

## Hematopoietic Stem Cells (HSCs)

- **Sustain hematopoiesis**
- **Self-renewal throughout life: produce daughter cells that retain stem cell properties, do not become "committed"**
- **High proliferative capacity: 1 cell → millions**
- **Pluripotent: →red cells, white cells and platelets**

**Hematopoiesis Hierarchy**

**Stem Cells** **Progenitor Cells** **Precursors**



## 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

## Hematopoietic Stem Cells

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

## Discovery of the role of HSCs

+ whole body irradiation → death

+ irradiation + protect spleen → recovery

+ irradiation + spleen cells → recovery

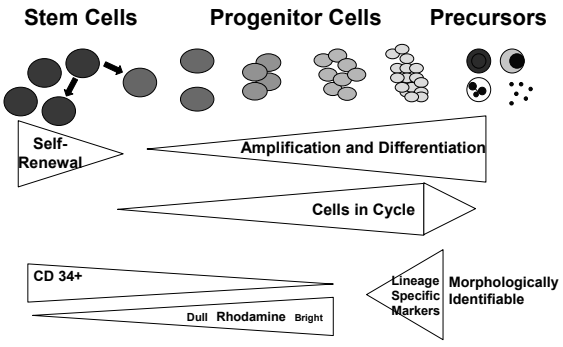
+ irradiation + bone marrow → recovery with colony forming units spleen (CFU-S)

## Identification of HSCs

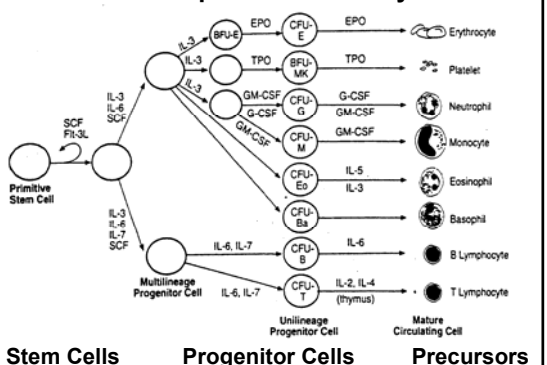
- Not morphologically identifiable
- In vitro tissue culture assays (at different level of maturation):
  - *CFU-GM, CFU-E,
  - **Less mature: LTC-IC, Cobble area forming cells
  - ◆Cell surface antigens: CD34

  A close correlation exists between the number of CD34+ cells and CFU-GM in peripheral blood stem cell collections.

## Hematopoiesis Hierarchy




## Hematopoiesis Hierarchy




Hematopoietic Stem Cells: Processing and Transplantation

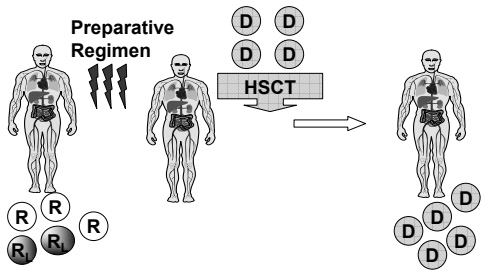
## Regulation of Hematopoiesis

- 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

## Hematopoietic Stem Cells Transplant




Preparative Regimen: TBI, Chemo
Role: eradicate cancer, immunosuppression to allow engraftment (allotransplant)

## Sources of Donors

Syngeneic donor

Allogeneic donor

Autologus donor

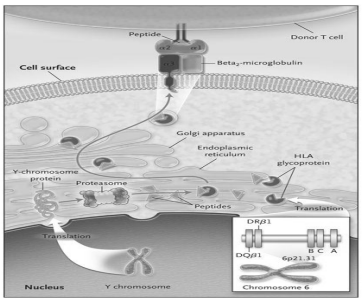
## Human Leukocyte Antigen (HLA)

- The major hisotcompatability complex
- Genes for HLA – on chromosome 6
- Inherited as haplotypes→ two siblings have 1:4 chance of being HLA identical
- HLA identical = HLA matched → recipient will not reject the allograft

## The story has two sides…

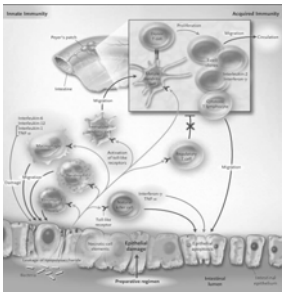
- Recipient T-cells recognize foreign donor antigens and can reject graft
- Donor T-cells recognize recipient antigens and can cause GVHD
- Donor T-cells recognize recipient antigens (minor histocompatabilty) and can cause graft-versus-tumor

### Graft Versus Tumor Effect

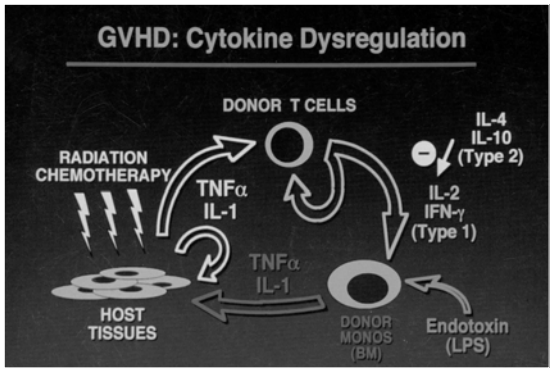



### Mechanism of GVHD

- Accentuated immune response , stimulated by injury from the preparative regimen prior to transplant
- Primarily confined to the GI tract
- Cytokines are critical to the development of GVHD



### GVHD




### Syngeneic Transplants

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

### Allogeneic 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. Tumor (GVT)

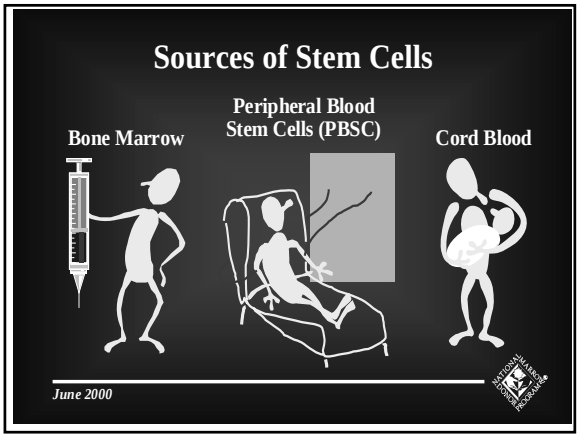
### Autologous Transplants

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

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




## 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

## Cell Types for Transplantation

- **(1) Bone Marrow**
  - **Collected from the posterior iliac crest**
  - **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
- Marrow stem cells detach continuously, enter the circulation, and return to marrow
- Collected by apheresis following "mobilization" by hematopoietic growth factor and/or chemotherapy
- FDA approved hematopoietic growth factors: Granulocyte colony stimulating factor (G-CSF), Granulocyte/macrophage stimulating factor (GM-CSF),

## 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
- Blood from the umbilical cord and the placenta is rich in HSC
  - Advantages:
    - Collected immediately after birth
    - 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: 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.

## 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 reaction
    - Hypotension and renal failure
    - Threat of hemolysis precluded transplant across ABO barriers
- Histocompatibility
  - High risk of GVHD with mismatches

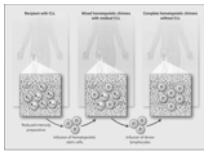
## "Mini-Transplants" = Low Dose Preparative Regimens

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

**Reduced-intensity Preparative Regimen Transplant:**

**primarily immunosuppressive**

**depend on the graft to eradicate the cancer**



## BMT Complications

- Early
  - Tissue toxicities
    - Mucositis – oropharyngeal, GI
    - VOD
    - Pneumonitis
  - Pancytopenia
  - Infections (mucositis, catheters, ↓WBC)
    - Bacterial, viral, fungal
  - GVHD- acute
- Late
  - GVHD-chronic
  - Late tissue damage
    - AVN
    - Cataracts
    - Pneumonitis
    - Growth & Development
  - 2ry Cancers
    - AML/MDS

## HSC Transplantation Outcome

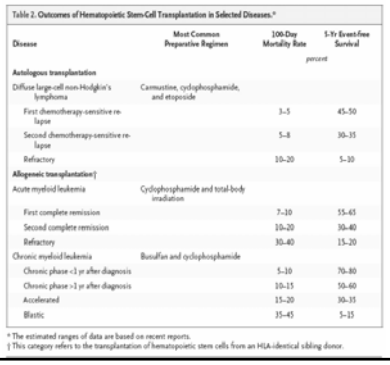


Table 2. Outcomes of Hematopoietic Stem-Cell Transplantation in Selected Diseases.*

| Disease | Most Common Preparative Regimen | 100-Day Mortality Rate | 5-Yr Event-free Survival |
|---|---|---|---|
| | | percent | |
| **Autologous transplantation** | | | |
| Diffuse large-cell non-Hodgkin's lymphoma | Carmustine, cyclophosphamide, and etoposide | | |
| First chemotherapy-sensitive relapse | | 3–5 | 45–50 |
| Second chemotherapy-sensitive relapse | | 5–8 | 30–35 |
| Refractory | | 10–20 | 5–10 |
| **Allogeneic transplantation†** | | | |
| Acute myeloid leukemia | Cyclophosphamide and total-body irradiation | | |
| First complete remission | | 7–10 | 55–65 |
| Second complete remission | | 10–20 | 30–40 |
| Refractory | | 30–40 | 15–20 |
| Chronic myeloid leukemia | Busulfan and cyclophosphamide | | |
| Chronic phase <1 yr after diagnosis | | 5–10 | 70–80 |
| Chronic phase >1 yr after diagnosis | | 10–15 | 50–60 |
| Accelerated | | 15–20 | 30–35 |
| Blastic | | 35–45 | 5–15 |

* The estimated ranges of data are based on recent reports.
† This category refers to the transplantation of hematopoietic stem cells from an HLA-identical sibling donor.

## Stem Cell Processing

- Volume reduction
  - Centrifugation, removal of excess plasma
- Removal of red blood cells (after peripheral stem cell collection <20ml)
  - Enables transplant of ABO/Rh mismatched stem cells
  - Methods:
  - Gravity sedimentation W/ HES
  - Density gradient separation W/Ficoll-metrizoate

## 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

## 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

## 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