Hematopoietic Hierarchy

LT-HSC: long term hematopoietic stem cell
ST-HSC: short term hematopoietic stem cell
GMP: granulocyte-monocyte precursors
MEP: megakaryocyte-erythrocyte precursors
NK: natural killer

From: Hematology Basic Principles and Practice, Hoffman, Editor, Chapter 19, page 191
Hematopoiesis Hierarchy

Stem Cells
- Self-Renewal

Progenitor Cells
- Amplification and Differentiation
- Cells in Cycle
- CD 34+
- Sca-1+
- Dull Rhodamine Bright

Precursors
- Lineage Specific Markers
- Morphologically Identifiable
Cellular Therapy Laboratory Functions

- Accession HPC products
- Process
- Cryopreserve
- Store
- Distribute
- Outcome
- Quality indicators
- Maintain records 10 years post-infusion
**Cellular Therapy Labs are Highly Regulated, FDA**

PHS Act Section 361, 21 CFR 1271

HCT/P must meet all 4 criteria:

1. Minimally manipulated
2. Intended for homologous use only
3. Not combined with a device or drug, except for sterilizing, preserving, or storage agents that do not raise clinical safety concerns
4. Does not have a systematic effect and is not dependent on metabolic activity of living cells for primary function, or
   - Has a systematic effect and is dependent on metabolic activity of living cells for primary function and is for: autologous use, or allogeneic use by a first or second degree relative, or reproductive use.

**PHS Act Section 351, Biological Products, Premarket Approval**

HCT/P meets any of the following:

1. Manipulated altering relevant functional characteristics of cells or tissues
2. Genetically modified
3. Expanded ex vivo
4. Non-homologous use
5. Combined with drug, device, or biological product that may raise clinical safety concerns
6. Active systemically or dependent on metabolic activity of living cells for primary function unless minimally manipulated for: autologous use, use in first or second degree blood relative or reproductive use.

*New York Blood Center*
## FDA Regulation of Minimally Manipulated HPC Products

<table>
<thead>
<tr>
<th>Donor</th>
<th>Marrow</th>
<th>Peripheral Blood (Aphereis)</th>
<th>Cord Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autologous</strong></td>
<td>No federal regulation</td>
<td>21 CFR 1271, except Subpart C, donor eligibility, recommended but not required</td>
<td>21 CFR 1271, except Subpart C, donor eligibility, recommended but not required</td>
</tr>
<tr>
<td><strong>Related Allogeneic (1st or 2nd degree blood relative)</strong></td>
<td>No federal regulation</td>
<td>21 CFR 1271</td>
<td>21 CFR 1271</td>
</tr>
<tr>
<td><strong>Unrelated Allogeneic</strong></td>
<td>Health Resources and Services Administration (HRSA) oversight, contract with NMDP</td>
<td>21 CFR 1271, FDA requested product and establishment standards, eventual licensure as biological product</td>
<td>21 CFR 1271, FDA licensure or IND required</td>
</tr>
</tbody>
</table>

CFR = Code of Federal Regulations  
Adapted from “Cellular Therapy: Principles, Methods and Regulation, AABB, 2009
Cellular Therapy Labs are Highly Regulated

- FDA: Code of Federal Regulations (CFR) Title 23—Food and Drugs, Part 1271 Human Cells Tissues, and Cellular and Tissue Based Products. **Minimally manipulated products** ("361" products)
- Regulates **Good Tissue Practices (GTP)** to prevent the transmission of communicable disease
  - Facility Registration: Recovery/collection, screen, test, package, process, store, label, distribute
  - Facility requirement: Clean, uncluttered, ample size, lighting, ventilation, temperature control.
  - Handle products individually to prevent mix-ups
  - Use closed systems when available
  - All supplies must be sterile and appropriate for use
  - Reagents: qualify and release for use
  - Prevent transmission of communicable disease
    - Donors: Infectious disease testing, review health history
    - Products: Microbiology testing

New York Blood Center
Cellular Therapy Labs are Highly Regulated

• FDA Investigational New Drug (IND)
  • More than minimally manipulated products: cell depletion, ex vivo expansion, donor leukocyte infusions, gene therapy (“351” products)

• States: Example NYS DOH
  • Licensure of Cellular Therapy/Stem Cell Labs
  • Qualifications for Director, Medical Director
  • Donor infectious disease testing requirements
  • Laboratory requirements
  • Collection to processing time requirements

• Accreditation
  • FACT
  • AABB

New York Blood Center
### Hematopoietic Progenitor Cell (HPC) Products

<table>
<thead>
<tr>
<th>HPC, Bone Marrow</th>
<th>HPC, Apheresis</th>
<th>HPC, Cord Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Volume: 1000-1500 mL</td>
<td>• Volume: 300-600 mL</td>
<td>• Major limitation: cell dose</td>
</tr>
<tr>
<td>• Hct: 20-30%</td>
<td>• Hct: &lt;5%</td>
<td>• Unrelated CB dose &gt;3.7 X 10^7/Kg predicts higher probability of myeloid engraftment</td>
</tr>
<tr>
<td>• 10-15 mL of marrow/Kg of recipient body weight</td>
<td>• Most common</td>
<td>• Availability—public banks</td>
</tr>
<tr>
<td>• Donor CBC predictive of cellular yield of product</td>
<td>• Ease of collection</td>
<td>• ~ 30% collected enter inventory</td>
</tr>
<tr>
<td>• TNC: 2-6 fold higher than PB TNC</td>
<td>• Circulating CD 34+ cell count correlates with HPC, apheresis yield</td>
<td>• Discard: donor deferrals, low volume, low cell counts, microbial contamination</td>
</tr>
<tr>
<td>• Granulocytes and lymphocytes predominate</td>
<td>• TNC of HPC, apheresis is 10 X HPC, marrow, 2X un-stimulated blood</td>
<td></td>
</tr>
<tr>
<td>• Healthy donor ~ 2% of leukocytes = CD 34+ cells. (~ 1 log lower than in mobilized HPC, apheresis products)</td>
<td>• Lymphocytes 40-50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Granulocytes 30-50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• G-CSF mobilization yields 10 to 20 fold higher CD 34+ cells than marrow and 40-50 fold higher then unstimulated blood</td>
<td></td>
</tr>
</tbody>
</table>
## Unique Processing Considerations

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>Peripheral Blood</th>
<th>Cord Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large volume, &gt; 1000 mL</td>
<td>Volume 300-600. Plasma reduction: reduce volume, minor ABO incompatibility</td>
<td>Small collection volume, 75-150 mL</td>
</tr>
<tr>
<td>Bone spicules, fat, clots, anticoagulants</td>
<td>Plasma removal to provide plasma for processing procedures</td>
<td>Small number of cells. Engraftment correlated with cell dosage</td>
</tr>
<tr>
<td>Filtration may be necessary during collection or prior to processing</td>
<td>Multiple collections large volumes of anticoagulants (DMSO)</td>
<td>Care to prevent cell loss</td>
</tr>
<tr>
<td>Sedimentation to remove fat</td>
<td>Hct ≤ 5%, ABO incompatibility, Remove RBC??</td>
<td>Unknown recipient, question of ABO incompatibility, remove RBC</td>
</tr>
<tr>
<td>Hct: 20-30%, ABO incompatibility, Remove RBC</td>
<td></td>
<td>Long term storage, possibly 15 or more years</td>
</tr>
<tr>
<td>Washing to remove large volume of anticoagulants, pediatric patients</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

New York Blood Center
HPC Product Quality

- Establish parameters/indicators to evaluate quality of product at each step of production/manufacture
- Donor status: healthy normal or patient following chemo
- Mobilization: G-CSF, chemo + G-CSF, AMD 3100 (mozobil, plerixafor)
- Collection procedure: Marrow vs apheresis
  - volume, sterility
- Processing: aseptic technique, clinical grade reagents, sterile materials, skill
- Cryopreservation: freezing rate, cryoprotectant
- Storage temperature:
  - liquid or vapor LN2, mechanical freezers
- Thawing: temperature, time from thawing to infusion
- Infusion: adverse events
- Engraftment
Accessioning HPC products

- Visual inspection
- Condition of container
  - Color of product
- Free of contamination
- Labeling
- Volume/weight
- Review of transport conditions and records
  - Temperature maintained appropriately
- Chain of custody
Processing HPC Products: Minimal Manipulation
ABO/Rh Incompatibility

• **Minor**
  - Donor alloantibodies in plasma against red cells in recipient’s circulation
  - Example: O donor, A or B or AB recipient, product plasma antibodies react against recipient RBCs
  - Donor antibody titer used to determine need for plasma reduction
  - Plasma depletion reduces level of donor alloantibodies

• **Major**
  - Recipient has circulating alloantibodies to antigens on donor’s RBCs
  - Red cell depletion of product
  - Patient immunosuppression may prevent reaction with infused RBCs

New York Blood Center
ABO Compatible?

Yes
- Volume reduction by plasma depletion
  - Cryopreservation or infusion

No
- No processing required
  - RBC depletion
    - Automated RBC depletion, Cobe 2991, other
      - Volume reduction by plasma depletion
      - Cryopreservation or infusion

Manual RBC depletion with HES
Processing HPC Products: Minimal Manipulation
RBC Depletion

- **Manual**
  - Hydroxyethyl starch (HES, hespan, ~ 20% product volume) facilitates sedimentation
  - Sediment by gravity or centrifugation
  - RBC removal 99% in 1-3 hrs
  - Desirable HPCs also sediment
    - Cannot eliminate all RBCs without loss of HPC
    - HPC loss can be significant—problem if low cell number in product
  - MNC recovery about 75%
Processing HPC Products: Minimal Manipulation
RBC Depletion

- **Automated**
  - COBE 2991 cell washing devise
  - Buffy coat collected into a collection bag
  - 60-85% MNC recovery; <20 ml of RBC remain
  - Good de-bulking for large volumes, but large number of RBCs remain
  - May require additional manual RBC depletion
Processing HPC Products: Minimal Manipulation
Plasma Reduction

- HPC, apheresis products similar to blood bank products, similar techniques adapted
  - Simple centrifugation method of choice
  - Automated e.g., Cobe 2991 can be employed
- Reduces final volume of product
  - Pediatric recipients
  - Reduces amount of cryoprotectant
- Reduces plasma antibodies
  - ABO incompatibility
Cryopreservation

- Handling prior to cryopreservation influences ability of cells to survive cryopreservation
  - Liquid storage should be optimized to maintain viability: time, temperature, duration
  - RBC and plasma concentrations and depletions
  - Ex vivo culture
  - Cell enrichments
- Cryopreservation media
  - Balanced salt solution (or culture media),
  - Cryoprotective agents: dimethyl sulfoxide (DMSO), glycerol
Cryopreservation

**DMSO**
- Alters fluidity and permeability of the cell membrane
- Influences biological cell and water within the cell
- Hypotonic: Addition → efflux of water from cell, can → cell lysis
- Equilibration occurs
- Thawing + transfer to isotonic solution → influx of water → lysis
- Cold temperatures reduce lysis
- Pre-freeze and post-thaw exposure should be limited
- Freezing within 15 minutes of adding
- Care during thawing
- Infusion immediately post-thawing
- Limit infusion dose to < 1 gm/Kg/24 hours to reduce adverse reactions. 1mL DMSO = 1 gm DMSO.
Cryopreservation

- Cooling rate strongly influences post-thaw survival
- Cooling rate of -1 to -2 degrees Celsius optimal
- “passive freezing” vs. controlled rate freezing
- Ice formation occurs in extracellular solution
  - Pulls out water
  - Raises temperature
- The lower the temp at which extracellular solution freezes the more intracellular ice formation and decrease in post-thaw viability
- Controlled rate freezers have a warming phase to “seed” freezing at a specific temp
Thawing

- Thaw at bedside for rapid infusion
- Removal of DMSO
  - Thaw must be in lab
  - Manual vs. Automated (Cobe 2991)
  - Centrifugation and removal of supernatant
  - Add fresh wash solution such as dextran/albumin solutions time consuming
  - Labor intensive
  - Significant cell loss due to physical stress and osmotic stress of added wash solutions
Assessment of HPC Product Quality: Surrogate Markers

- TNC and MNC: Suggested effective doses vary widely:
  - TNC: 0.1 to 1 $\times 10^4$ /Kg
  - MNC: 1-3 $\times 10^8$ /Kg
- CD 34+ cell enumeration: monoclonal antibodies and flow cytometry
  - 2 $\times 10^6$/Kg for engraftment within 14 to 21 days w/out growth factor support
- Colony assay
  - 14 day CFU-GM
  - Limited utility because of time to know answer
- Viability
  - Trypan blue
  - Flow cytometry: 7 AAD
Assessment of HPC Product Quality

- Viability
  - Trypan blue exclusion:
    - Vital dye: enters cells if membrane damaged
    - Negative cells = viable,
    - Positive cells = dead.
  - Non-specific: Stem cells < 1% of cells → inaccurate
  - 7AAD flow cytometry 7-amino-actinomycin D
    - excluded by viable cells.
    - Gating on CD 34+ cells+ 7AAD → viable CD 34+ cells

- Microbiological evaluation
  - Thioglycollate
    - 14 day, infusion may occur before results available
  - Automated: BacT and Versa Trek
    - Quick, must be validated with challenge experiments
Storage

• Liquid
  • Short term
  • Pre-processing and Post-Thaw
  • Ambient temperature
  • Refrigeration: 2-8 °C
• Cryopreserved
  • LN2 vapor: -130° to -196° C
  • LN2 liquid: -196° C
  • Mechanical freezers: -130° C
Cellular Therapy Laboratory Functions: Distribution

- Labeling
  - Recipient
  - Product name
  - Unique identifiers
    - Unique product number, MR #, DOB
  - Additives
  - Date
  - Storage temperature

- Donor Qualification
  - Donor health history
  - Infectious disease testing: HIV, Hepatitis A, B, C, WNV, HTLV I/II, Syph, CMV
  - Biohazard labels

- Transportation
  - Temperature
  - Chain of custody
Cellular Therapy Laboratory Functions: Outcome

Outcome

• Adverse events
  • Minor: nausea, vomiting
  • Major: hypertension, tachycardia, LFTs, breathing
    • Must be investigated
    • May be reportable: FDA, state

• Engraftment
  • Gold standard for quality
More than Minimally Manipulated: CD 34+ Cell Enrichment/T Cell Depletion

- **Goal**
  - Remove GVHD causing T cells
  - Preserve GVT causing T cells

- **Immunomagnetic separation**
  - Select CD 34+ cells
    - Removes cells that are not CD 34+
    - Non-specific
      - GVHD vs GVT T-cells
      - Engraftment enhancing cells (stroma, endothelial cells)

- **Select T cells, subsets**
  - Challenges: identifying target population
  - Possible selections: CD3, CD4, CD8, CD 56
More than Minimally Manipulated: CD 34+ Cell Enrichment/T Cell Depletion

**Advantages:**
- Most HSCs CD34+, c-kit+, thy1+, CD38-
- Effective dose = 1 to 5 X 10^6 cells/Kg
- Higher doses result in faster engraftment
- Long-term engraftment (> 10 years)
- May eliminate tumor cells in autologous grafts
- Eliminates lymphocytes → ↓ GVHD
  - Haploidentical transplants

**Concerns:**
- Some HSCs may be CD34 neg, Hoechst-33342+
- Eliminates lymphocytes → ↓ GVT, ID risk, graft failure
CD 34+ cell selection: Methods

- CliniMACS™
  - Anti-CD34 nanoparticles
  - Biocompatible nanoparticles remain on HPC
- FDA approved for humanitarian use for AML
- Regulatory approval in Europe
More than Minimally Manipulated: Graft Engineering

- Donor Leukocyte Infusion (DLI)
  - Anti-tumor effect
  - Leukemia, lymphoma, Hodgkin lymphoma, multiple myeloma
  - unmodified donor lymphocytes
  - High risk of GVHD
- Natural Killer Cells
  - CD 56+ cell selection or CD 3+ cell depletion
More than Minimally Manipulated: Ex-Vivo Expansion

• Rationale
  • Increase HPC for transplant
  • HPC, apheresis: fewer collections, smaller volume, “poor mobilizers”
  • HPC, cord blood: small collections, larger donors, shorten time to engraftment
  • Allogeneic: shorten time to engraftment
  • ? Decrease tumor contamination/tumor cell purging?

• Method
  • HPCs + hematopoietic growth factors, cytokines, drugs
  • Infuse before, with, or after non-expanded portion or other HPC product
More than Minimally Manipulated: Ex-Vivo Expansion

- **Problems/Controversial issues:**
  - Which cells provide short- and long-term recovery?
  - Is there loss of stem cells in ex vivo expansion?
  - Do committed progenitors (CFUGM, BFU-E, CFU-mega) contribute to early recovery?
  - Homing: adhesion receptors? cells contributing to microenvironment? effect of engraftment?
  - Enormous cellular expansion -- lineage committed cells or stem cells?
  - Does telomere loss occur in culture, proliferation? Senescence?

- **Current Status**
  - No significant toxicity
  - Failure to demonstrate clear impact on engraftment
Summary

- Cellular Therapy Labs “manufacture” HPC products
- Labs are highly regulated: FDA, NYDOH, FACT, AABB
  - FDA: “361” products, minimally manipulated, 21 CFR 1271
    - RBC depletion
    - Plasma depletion
  - FDA” “351” products, more than minimally manipulated, IND
    - CD 34+ cell enrichment
    - T-cell depletion
    - Donor lymphocyte infusion
Summary

Many factors influence the quality of the final product

- Collection
- Handling (temp, transport)
- Processing methods
- Cryopreservation methods
- Thawing
- Quality: purity and potency must be evaluated
  - TNC
  - CD34+ cells
  - Viability
  - Adverse events
  - Gold standard = engraftment
Comprehensive Cell Solutions ™

• Partnering for Success
• We offer a comprehensive menu of services and products to support and manage the full spectrum of transfusion medicine, regenerative medicine and cellular-therapy related research projects, from basic science to full scale clinical trials.
Comprehensive Cell Solutions ™

- Clinical Trials Management
- Innovative Research Services
- Research Products
- Cell Therapy and Tissue Banking