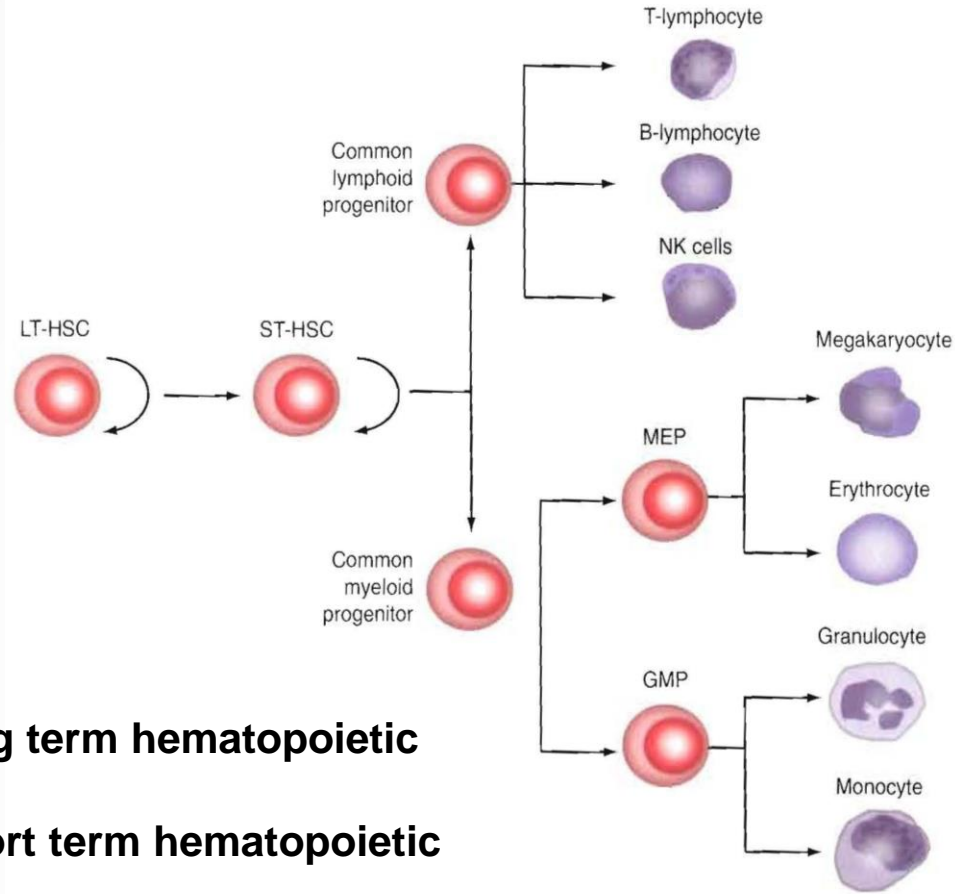


Hematopoietic Stem Cells: Cell Processing, and Transplantation

Rona Singer Weinberg, PhD
Director, Cellular Therapy Laboratory
New York Blood Center
Phone: (212) 570-3488
E-mail:
rweinberg@nybloodcenter.org

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

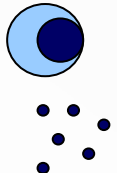
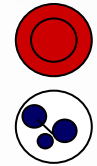
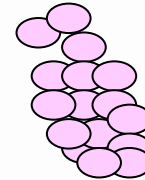
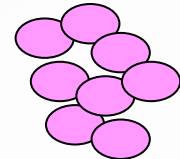
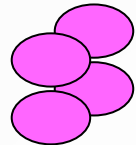
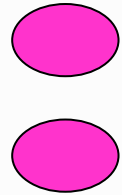
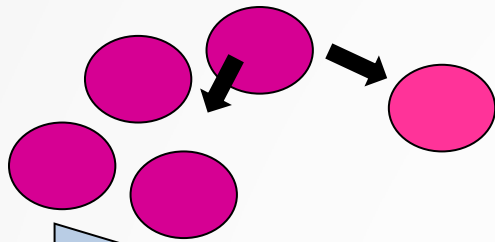
From: Hematology Basic Principles and Practice, Hoffman, Editor, Chapter 19, page 191

Hematopoiesis Hierarchy

Stem Cells

Progenitor Cells

Precursors



Self-Renewal

Amplification and Differentiation

Cells in Cycle

CD 34+
Sca-1 +

Dull Rhodamine Bright

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.

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

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



Hematopoietic Progenitor Cell (HPC) Products

HPC, Bone Marrow	HPC, Apheresis	HPC, Cord Blood
<ul style="list-style-type: none">• Volume: 1000-1500 mL• Hct: 20-30%• 10-15 mL of marrow/Kg of recipient body weight• Donor CBC predictive of cellular yield of product• TNC: 2-6 fold higher than PB TNC• Granulocytes and lymphocytes predominate• CD 34+ cells ~ 1 log lower than in mobilized HPC, apheresis products	<ul style="list-style-type: none">• Volume: 300-600 mL• Hct: <5%• Most common• Ease of collection• Circulating CD 34+ cell count correlates with HPC, apheresis yield• TNC of HPC, apheresis is 10 X HPC, marrow, 2X un-stimulated blood• Lymphocytes 40-50%• Granulocytes 30-50%• G-CSF mobilization yields 10 to 20 fold higher CD 34+ cells than marrow and 40-50 fold higher than unstimulated blood	<ul style="list-style-type: none">• Major limitation: cell dose• Unrelated CB dose $>3.7 \times 10^7$/Kg predicts higher probability of myeloid engraftment• Availability—public banks• ~ 30% collected enter inventory• Discard: donor deferrals, low volume, low cell counts, microbial contamination

Unique Processing Considerations

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

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

New York Blood Center
 Cellular Therapy Laboratory, 310 East 67th St. Room 2-45 New York, New York 10065 Phone: 212-578-3484 FAX: 212-578-3485

HEMOPOIETIC STEM CELL PROCESSING LABORATORY STEM CELL TRANSPORTATION REQUEST

DONOR: _____ **MR #:** _____
 Risk Bone marrow Cord Blood Other, Specify: _____

PICK UP AT: _____ **DEPT/ROOM #:** _____

DATE: _____ **TIME:** _____
PHONE#: _____ **CONTACT:** _____
DELIVER TO: _____ **DEPT/ROOM #:** _____

DATE: _____ **TIME:** _____
PHONE#: _____ **CONTACT:** _____

SPECIAL INSTRUCTIONS: _____

RECEIVING INSTITUTION: PLEASE INSPECT IMMEDIATELY UPON ARRIVAL AND COMPLETE THE APPROPRIATE SECTION BELOW

DRY ICE DRY ICE IS PRESENT IN THE CONTAINER <input type="checkbox"/> Yes <input type="checkbox"/> No CITY/COUNTRY ORIGIN: <input type="checkbox"/> Green (In Range) <input type="checkbox"/> Red (Out of Range) * If out of range immediately notify NYBC Clinical Services and hold product for further instructions.
WET ICE PRODUCT/SAMPLE INSPECTION UPON RECEIPT <input type="checkbox"/> Yes <input type="checkbox"/> No ICE STILL SOLID <input type="checkbox"/> Yes <input type="checkbox"/> No
AMBIENT TEMPERATURE DELIVERY PRODUCT/SAMPLE INSPECTION UPON RECEIPT <input type="checkbox"/> Yes <input type="checkbox"/> No
ABSORBED LIQUID NITROGEN ABSORBED LIQUID NITROGEN IS PRESENT IN THE CONTAINER <input type="checkbox"/> Yes <input type="checkbox"/> No CITY/COUNTRY ORIGIN: <input type="checkbox"/> Green (In Range) <input type="checkbox"/> Red (Out of Range) * If out of range immediately notify NYBC Clinical Services and hold product for further instructions.

PERSON RELEASING PRODUCT
 Name (Print) _____ Signature _____ Date _____ Time _____

DRIVER
 Name (Print) _____ Signature _____ Date _____ Time _____

PERSON RECEIVING PRODUCT
 Name (Print) _____ Signature _____ Date _____ Time _____

Transportation confirmed: yes no

J:\InternalForms\CTL_accession\0213\transportation request form 2 08 4/13/14 1:58

New York Blood Center
 Cellular Therapy Laboratory, 310 East 67th Street, Room 2-45 New York, New York 10065
 Phone: 212-578-3484 FAX: 212-578-3485

HPC Accessioning Form

Date	Time	LUPN
Donor	MR #	
Hospital		
Allogeneic Recipient (if applicable)	Allogeneic Recipient MR # (if applicable)	
Delivered on time	<input type="checkbox"/> Yes <input type="checkbox"/> No	
If no, explain		
Outer shipping container ok	<input type="checkbox"/> Yes <input type="checkbox"/> No	
If no, explain		
Shipping Conditions		
<input type="checkbox"/> wet ice <input type="checkbox"/> ambient temperature		
If wet ice, ice still in container	<input type="checkbox"/> Yes <input type="checkbox"/> No	
If no, explain		
Condition of primary collection container:		
Collection bag labeled and information complete:	<input type="checkbox"/> Yes <input type="checkbox"/> No	
If no, explain		
Collection bag leaking	<input type="checkbox"/> Yes <input type="checkbox"/> No	
If yes, explain		
Collection bag or HPC product discolored	<input type="checkbox"/> Yes <input type="checkbox"/> No	
If yes, explain		
Comments:		
Inspected by:		

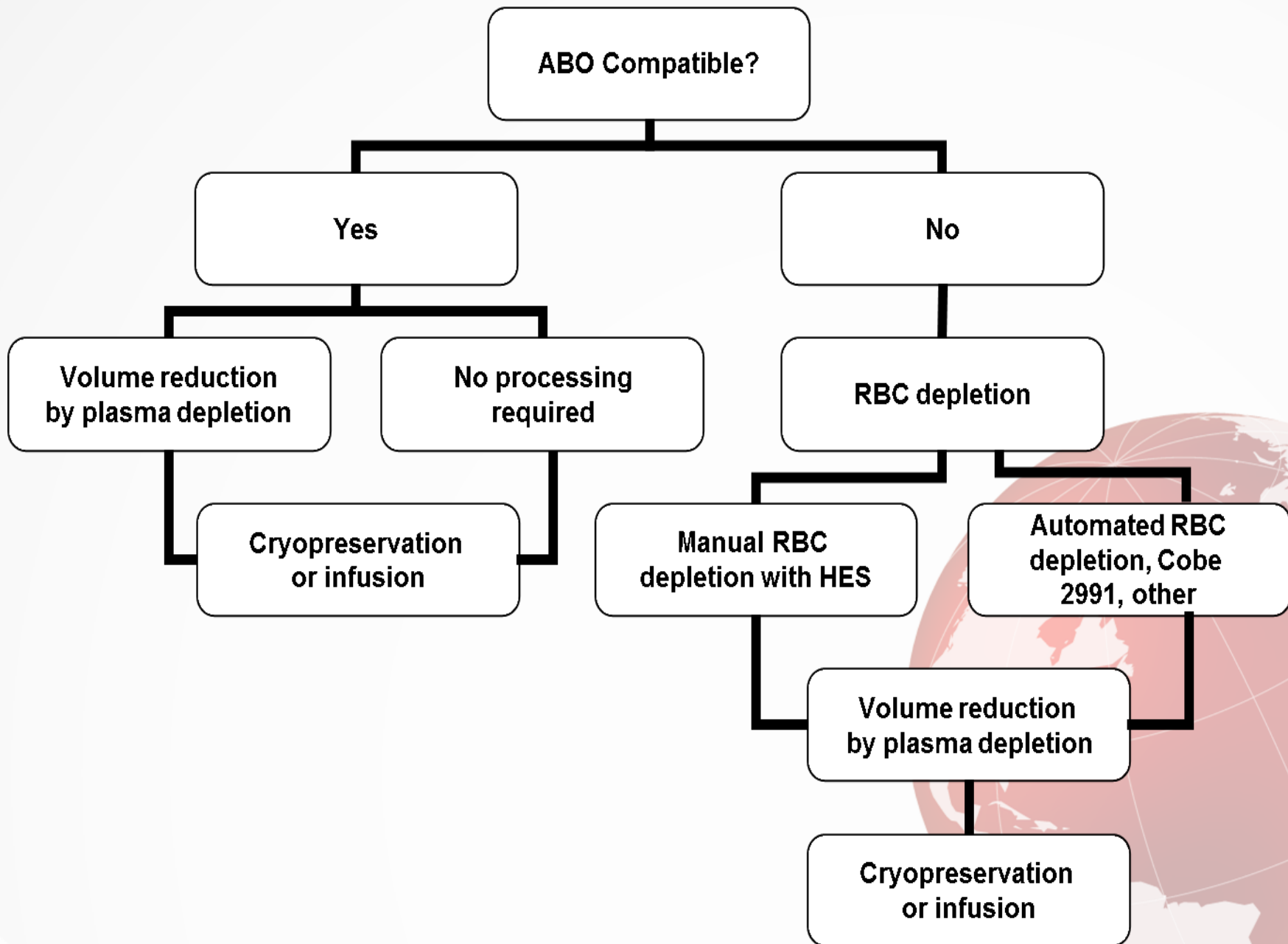
Processing HPC Products: Minimal Manipulation ABO/Rh Incompatibility

• 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



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 MNC recovery about 75%

• Automated

- COBE 2991 cell washing device
- Buffy coat collected into a collection bag
- 60-85% MNC recovery; <20 ml of RBC remain
- Good de-bulking large volumes, large number 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 survival
 - Liquid storage: optimize 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 $< 1 \text{ gm/Kg/24 hours}$ to reduce adverse reactions. $1\text{mL DMSO} = 1 \text{ gm DMSO}$.

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
- 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 1×10^4 /Kg
 - MNC: $1-3 \times 10^8$ /Kg
- CD 34+ cell enumeration: monoclonal antibodies and flow cytometry
 - 2×10^6 /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
 - Microbial 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 10⁶ 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:

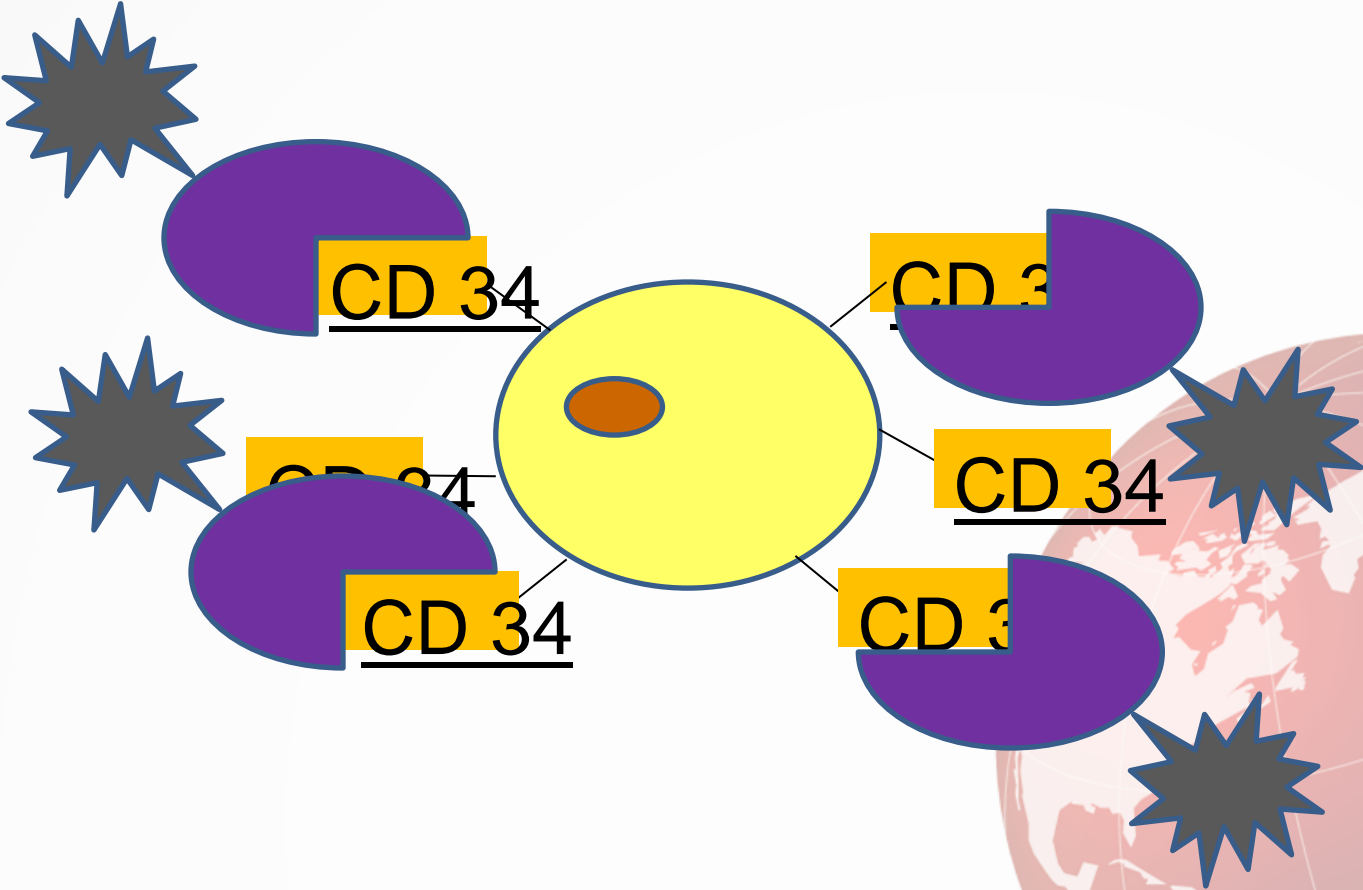
- Some HSCs may be CD34 neg, Hoechst-33342+
- Eliminates lymphocytes → ↓ GVT, ID risk, graft failure

CD 34+ cell selection: Methods

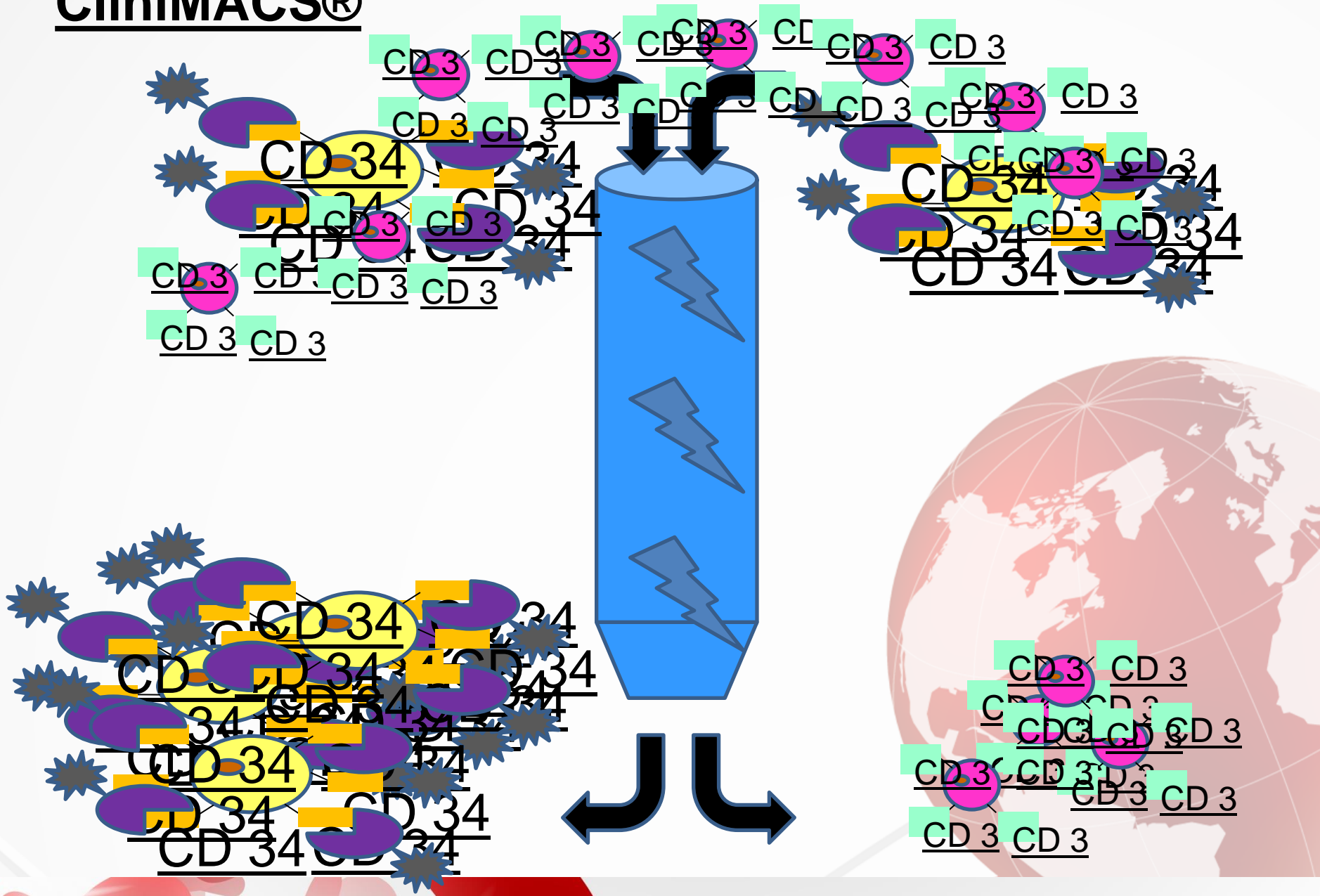
- CliniMACS™
 - Anti-CD34 nanoparticles
 - Biocompatible nanoparticles remain on HPC
 - FDA approved for humanitarian use for AML
 - Regulatory approval in Europe



Hematopoietic Stem Cells



CliniMACS®



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
- Genetic Engineering
 - T cells: T Cell Receptor (TCR), Chimeric Antigen Receptor (CAR-T cells)

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

- Method

- HPCs +hematopoietic growth factors, cytokines, drugs
- Infuse before, with, or after non-expanded portion or other HPC product

- Concerns

- Failure to engraft

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

