CELLULAR THERAPY IN THE CLINICAL LABORATORY

Daniel Held, MHS, MT(ASCP)SBB
LSUHSC Assistant Professor/Clinical Coordinator
CLPC Continuing Education Seminar

OBJECTIVES

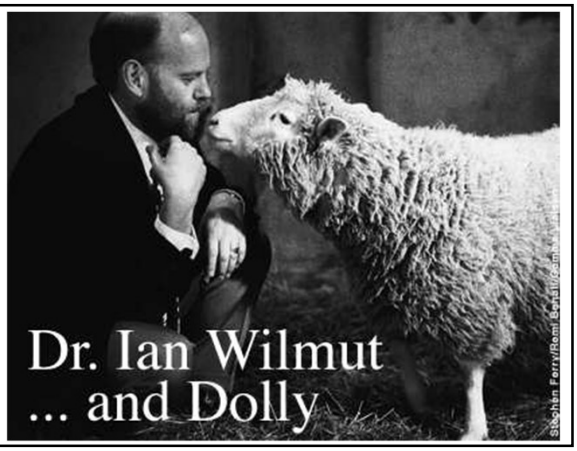
- Upon completion of this program, the participant will be able to:
  - Discuss the process of mobilization and collection of peripheral blood progenitor cells.
  - Describe CAR T-cell therapy and in which diseases it is used.
  - Define graft-versus-host disease and its classifications.

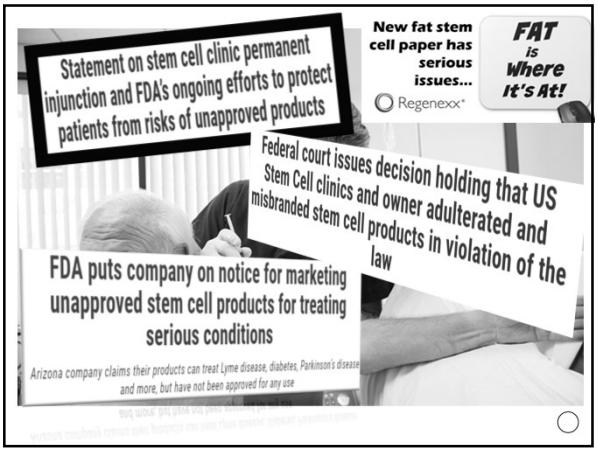
WHAT IS CELLULAR THERAPY?

- AABB Center for Cellular Therapy definition:
  - Cellular therapy (CT) is the transplantation of human cells to replace or repair damaged tissue and/or cells
- Different types of cells used as part of therapy or treatment for variety of diseases and conditions

WHAT IS CELLULAR THERAPY?

- Hematopoietic progenitor cells (HPC)
- Skeletal muscle stem cells
- Mesenchymal stem cells
- Lymphocytes (e.g. CAR T-cells)
- Dendritic cells
- Pancreatic islet cells





HISTORY

- 1950's
  - Identical twin marrow grafts successfully performed
- 1960's – Numerous significant advances in HPC transplantation
  - Animal model studies
  - Development of antibiotics
  - Platelet transfusions
  - Increasing knowledge of the HLA system

HISTORY

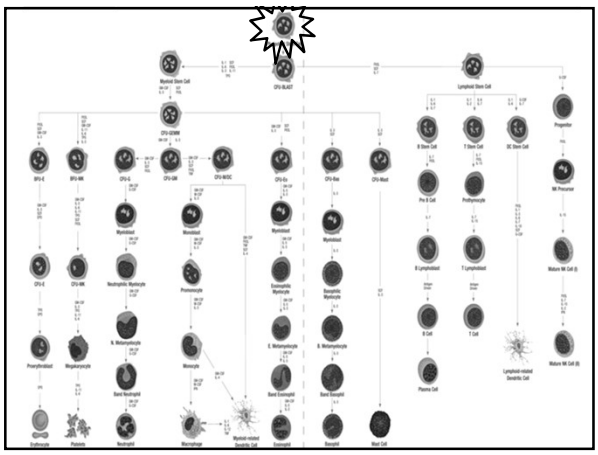
- 1968 – First successful non-twin allogenic bone marrow transplant
  - Severe combined immunodeficiency disease (SCID) treated with sibling's donation
- 1978 – HPCs discovered in human cord blood
- 1984 – CD34 marker discovered
- 1989 – First transplant using umbilical cord blood (UCB)

HISTORY

- Recent developments:
  - Immunosuppressive drugs (Cyclosporin)
  - Cytokines/colony stimulating factors
  - Conditioning regimens
  - *Ex-vivo* replication of stem cells
  - Chimeric antigen receptor T-cell therapy (CAR T-cell therapy)

WHAT IS A HEMAPOIETIC PROGENITOR CELL (HPC)?

- NIH definition:
  - An immature cell that can develop into all types of blood cells, including white blood cells, red blood cells, and platelets. Hematopoietic stem cells are found in the peripheral blood and the bone marrow. Also called blood stem cell.



WHAT IS A HPC?

- Uncommitted cell with ability to renew itself
- High N:C ratio on Wright stain smears
- Express CD34 antigen on their surface
- Contain necessary progenitors required for short- and long-term hematopoietic reconstitution
- Obtained from 3 distinct sources

DISEASES TREATED WITH HPC

- HPC transplantation has been used in:
  - Malignancies
    - Leukemias (AML, ALL, CLL, CML)
    - Lymphomas
    - Multiple myeloma
  - Solid tumors
    - Testicular
    - Neuroblastoma
    - Ewing's sarcoma

DISEASES TREATED WITH HPC

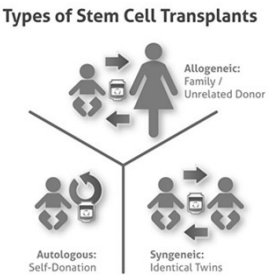
- Bone marrow disease/failure
  - Severe aplastic anemia
  - Fanconi anemia
  - Paroxysmal nocturnal hemoglobinuria (PNH)
  - Pure red cell aplasia
  - Amegakaryocytosis/congenital thrombocytopenia
- Myelodysplastic syndromes (MDS)
- Hemoglobinopathies
  - Beta thalassemia major
  - Sickle cell disease

DISEASES TREATED WITH HPC

- Inherited metabolic disorders
  - Krabbe disease (KLD)
  - Hurler syndrome
  - Adrenoleukodystrophy (ALD)
  - Metachromatic leukodystrophy (MLD)
- Chronic granulomatous disease (CGD)
- Inherited immune system disorders
  - Severe combined immunodeficiency (SCID)
  - Wiskott-Aldrich syndrome

HPC COLLECTIONS

- HPC collections derived from:
  - Self (autologous)
  - Identical twin (syngeneic)
  - Related (allogenic)
  - Unrelated (allogenic)



AUTOLOGOUS COLLECTION

- Performed during remission
- Peripheral collection (PBSC)
- HPC cryopreserved
- IDM testing not required
  - Special labeling/storage
- No HLA or ABO matching
- Rare GVHD

ALLOGENEIC COLLECTION

- Related or unrelated donor
- PBSC or BM collection
- HPC infused fresh or cryopreserved
- IDM, HLA, and ABO testing
- Donor health history required
- GVHD possible

ALLOGENEIC COLLECTION

- HLA matching
  - Two alleles inherited from parents
  - HLA-A, -B, -C, -DRB1
  - Related full match (8/8)
  - Unrelated full match (8/8)
  - Haploidentical (4/8)

Allogeneic Transplant	Donor and Recipient HLA Matching Recommendation
Matched Sibling	**6/6 HLA match** HLA-A and -B (at intermediate or higher resolution using DNA based typing) HLA-DRB1 (at high resolution using DNA based typing)
1 Antigen Mismatched Related	**7/8 HLA match** HLA-A, -B, -C (at intermediate or higher resolution using DNA based typing) HLA-DRB1 (at high resolution using DNA based typing)
Haploidentical Related	**≥4/8 HLA match** HLA-A, -B, -C (at intermediate or higher resolution using DNA based typing) HLA-DRB1 (at high resolution using DNA based typing) [fewer than 2 mismatches per locus]
8/8 Matched Unrelated Adult	**8/8 HLA match** HLA-A, -B, -C and -DRB1 (at high resolution using DNA based typing)
7/8 Unrelated Adult	**7/8 HLA match** HLA-A, -B, -C and -DRB1 (at high resolution using DNA based typing) [7/8 donor available in most situations, do not recommend ≤6/8]
Umbilical Cord Blood	**≥4/6 HLA match** HLA-A, -B (intermediate resolution or higher using DNA based typing) and -DRB1 (at high resolution using DNA based typing)

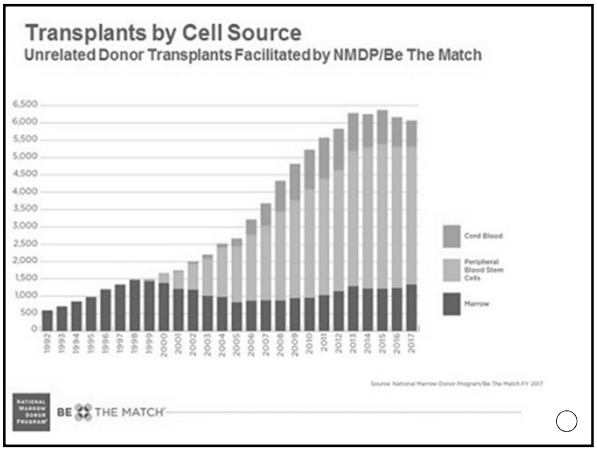
Howard A et al. Recommendations for Donor HLA Assessment and Matching for Allogeneic Stem Cell Transplantation: Consensus Opinion of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). Biol Blood Marrow Transplant. 2015 Jan: 21(1): 4-7.

AUTOLOGOUS VS. ALLOGENEIC

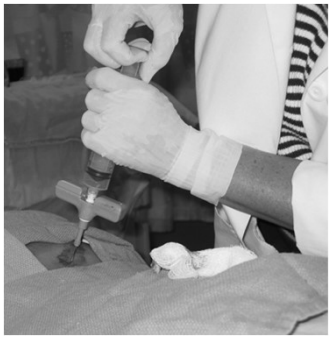
Donor	Advantages	Disadvantages
Autologous	More donors Rare GVHD No graft rejection No pre- or posttransplant immunosuppression Better tolerated by older patients	No Graft-versus-leukemia Stem cell damage (due to previous chemotherapy)
Allogeneic	Normal donor Graft-versus-leukemia	Fewer donors GVHD Graft rejection Pre- and posttransplant immunosuppression Tolerated less well by older patients

SOURCES OF HPC

- Bone marrow (BM)
  - Obtained by aspiration from iliac crests and filtering the heparinized marrow
- Umbilical cord blood (UCB)
  - Collected from umbilical vein after birth
- Peripheral blood stem cells (PBSC)
  - Colony stimulating factors mobilize HPC into the bloodstream and are collected via apheresis



BONE MARROW COLLECTION

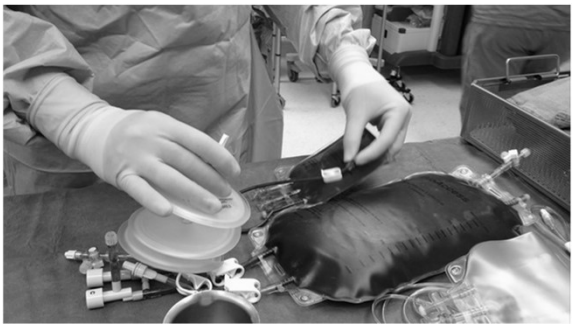


https://www.youtube.com/watch?v=UQ0wNgkVPKY&feature=youtu.be

BM HARVEST

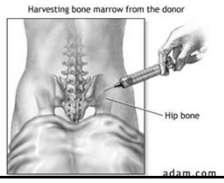
- Donor under general anesthesia
- Marrow removed from iliac crest
- Heparinized syringes
- Mid-count sample

BM HARVEST



BM HARVEST

- Advantages
  - Single collection
  - No need for special catheter
  - No drugs for mobilization
- Disadvantages
  - General anesthesia needed
  - ABO incompatibility
  - Contamination more likely
  - Slower engraftment for patient



UCB COLLECTION

By donating your **baby's cord blood,** you give **patients hope.**

BE THE MATCH

UCB COLLECTION

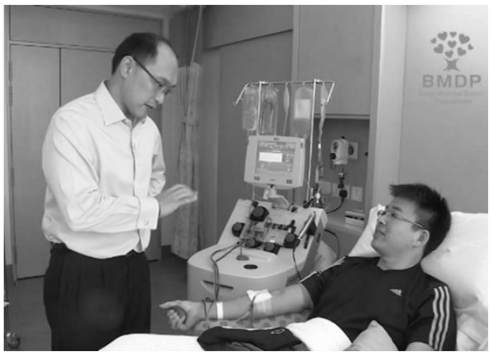
UCB COLLECTION

- Cord disinfected with iodine
- Needle inserted in vein
- Collection bag placed below placenta
- Collect until blood flow stops

UCB COLLECTION

- Advantages
  - Readily available
  - Already tested
  - No donor risk
  - Few ethical problems
- Disadvantages
  - Easily contaminated
  - ABO compatibility
  - Low cell dosage
  - Longer engraftment

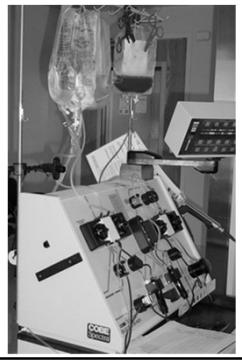
PBSC COLLECTION

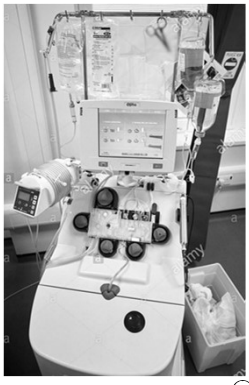


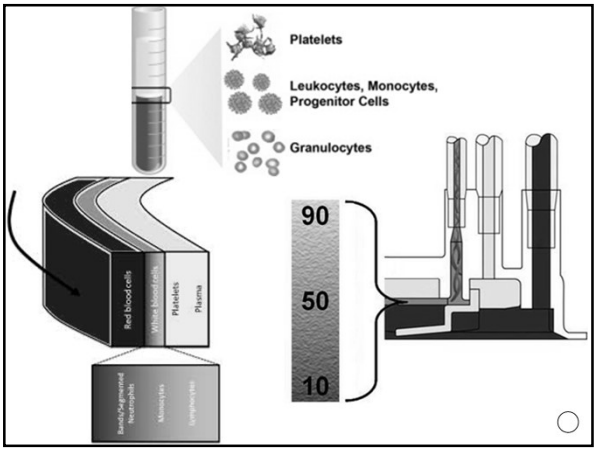
PBSC COLLECTION

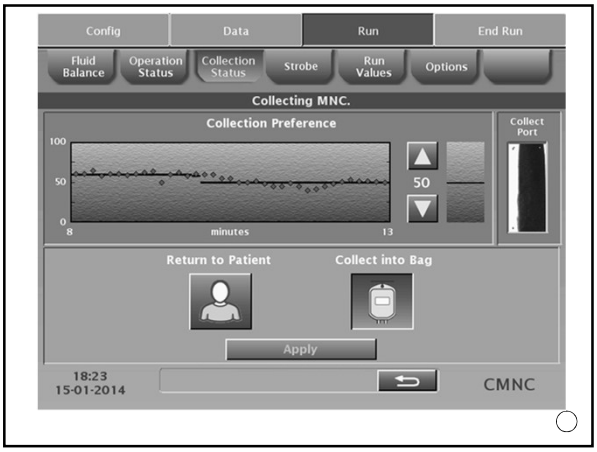
- Similar to plateletpheresis donation
- Catheter may be used
- G-CSF given to donor

PBSC COLLECTION









PBSC COLLECTION

- Advantages
  - No general anesthesia
  - Outpatient donation
  - ABO incompatibility
  - Faster engraftment
- Disadvantages
  - Multiple collection days
  - Catheter placement
  - Mobilization drugs needed
  - Higher incidence of chronic GVHD

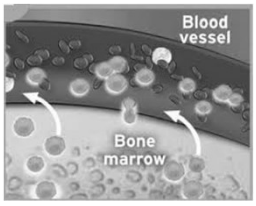
MOBILIZATION STRATEGIES

Advantages and Disadvantages of Different Mobilization Strategies

Mobilization Strategy	Advantages	Disadvantages
Growth factor mobilization	• Short time from start of mobilization to transplant. • Fewer days of GF administration • Onset of collection is more predictable	• Fewer CD34+ collected than with C+GF • High failure rate
Growth factor+CXCR4 antagonist	• Short time from start of mobilization to transplant. • Fewer days of GF administration • Onset of collection is more predictable • High success rate	• High cost
Chemotherapy+growth factor	• More CD34+ cells collected than with GF alone • Chemotherapy already used for underlying disease	• Longer stay of intravascular catheter • Higher risk of infection • Neutropenia • Thrombocytopenia • Higher risk of complications requiring hospitalization • High cost • Ideal time of apheresis is less predictable

Source: Hematopoietic Cell Transplantation for Malignant Conditions By Qaiser Bashir, Mehdi Hamadani; 2019, p. 81.

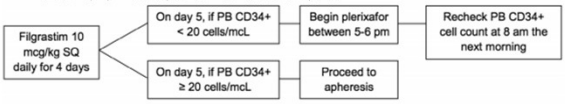
PBSC MOBILIZATION

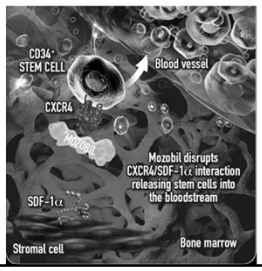


- Donor mobilization
  - Day -4 – Filgrastim
  - Day -3 – Filgrastim
  - Day -2 – Filgrastim
  - Day -1 – Filgrastim
    - Peripheral CD34
  - Day 0 – PBSC collection…maybe

*Filgrastim – G-CSF (Neupogen®)

PBSC MOBILIZATION





- Plerixafor
  - aka Mozobil
  - "PAM" for stem cells

HPC TRANSPORT/STORAGE

- Transport via validated container
  - Cooler/dewar
  - Monitor temperature during transport
- Process/infuse ASAP
  - < 48 hours of collection
  - < 72 hours if international donor
- Storage
  - 1-6°C for PBSC
  - 20-24°C for BM

HPC MANIPULATIONS

- Red cell depletion
- Plasma depletion
- CD34+ cell selection
  - FACS method
  - Immunomagnetic method
- Cryopreservation

RED CELL DEPLETION

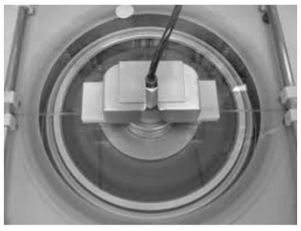
- Major ABO incompatible HPC collections
- Recipient has preformed alloantibodies to donor's RBCs
- Typically performed on BM and CBU collections

RED CELL DEPLETION

- Several different methods:
  - Sedimentation reagents (Hespan, Dextran)
  - Inverted centrifugation
  - Addition of group O RBCs

RED CELL DEPLETION

- Buffy coat concentration (COBE 2991)
- BMP on Spectra Optia

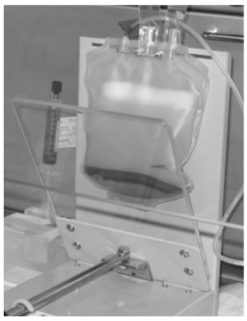


PLASMA DEPLETION

- Minimize volume transfused
  - Patients who are small, fluid-sensitive, or have preexisting fluid overload, cardiac compromise, or renal dysfunction
- Lower volume for cryostorage
- HPC collections with minor ABO incompatibility
- Donor has preformed alloantibodies to recipient's RBCs

PLASMA DEPLETION

- Main method – centrifugation, then plasma expression
- Use scale to approximate volume
  - 1.058 = specific gravity of BM
  - mL = g x 1.058

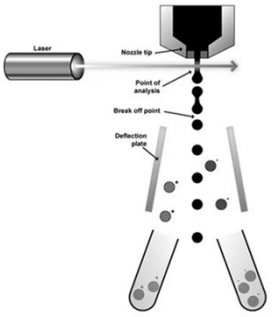


CD34+ CELL SELECTIONS

- Some patients may require positive selection of CD34+ cells from an HPC collection
  - Removal of tumor cells from HPC graft
  - Removal of donor T-cells to prevent GVHD
- CD34+ selections are intended for hematopoietic reconstitution after myeloablative therapy
  - Primarily for patients with CD34-negative tumors

FACS METHOD

- Fluorescence-activated cells sorting (FACS)
- Cells ejected one by one in stream of PBS
- Cell intercepts laser beam and computer monitors scattered light and fluorescence
- Stream is charged as cell reaches break-off point
- Charged cell passes through two high voltage plates

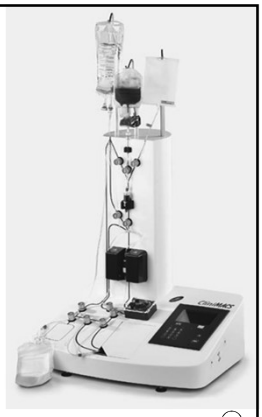


IMMUNOMAGNETIC METHOD

- HPC collection incubated with monoclonal anti-CD34 antibodies
- Magnetic beads coated with secondary antibodies are incubated with HPC collection
  - Beads form rosettes with CD34+ cells
- Mixture passed through a strong magnetic field
- CD34+ cells are captured in the magnetic field while all other cells are washed away

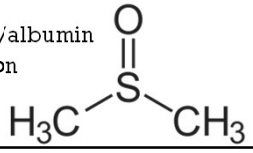
IMMUNOMAGNETIC METHOD

https://www.miltenyibiotec.com/US-en/products/cell-manufacturing-platform/clinimacs-plus.html



CRYOPRESERVATION OF HPC

- HPC preserved until transplant
- Dimethylsulfoxide (DMSO)
  - NOT FDA approved
  - Cryoprotectant
  - Reduces ice formation and cell death
  - Bi-product of Kraft process (creation of wood pulp)
  - Mixed with plasma/albumin
  - 5-10% concentration



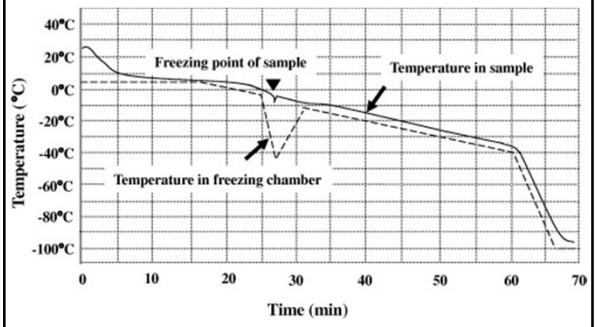
CRYOPRESERVATION

- DMSO added to plasma/albumin
  - 5-10% final concentration
  - Exothermic reaction
  - Freezing solution added to HPC
- HPC aliquoted in cryostore bags
  - Bags stored in metal canisters
- QC samples also frozen

CRYOPRESERVATION

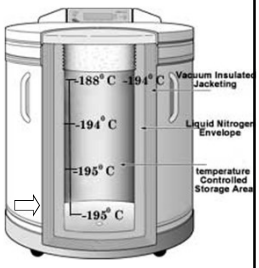
- Methodologies
  - "Dump" freezing
    - Place canister in ≤ -80°C for 24 hours
    - Transfer to storage
  - Controlled rate freezing
    - LN_2 delivered to chamber at controlled rate
    - Prevents rapid cellular dehydration and ice-crystal formation
    - Latent heat of fusion damage is minimized

CONTROLLED RATE FREEZING

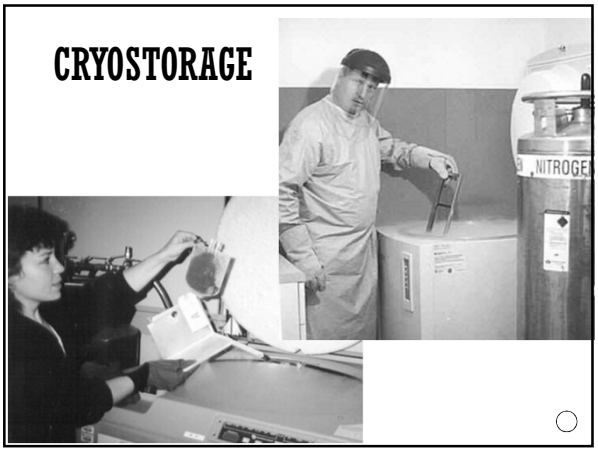


CRYOSTORAGE OF HPC

- Freezer maintained at -120°C – -196°C
- Temp/LN_2 level **must** be monitored
- Phases of storage
  - Vapor phase
  - Liquid phase
- Cells remain viable for years



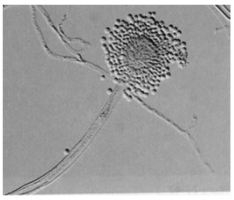
CRYOSTORAGE



HPC PRODUCT EVALUATION

- Quality of each HPC collection evaluated
  - Total nucleated cell count (TNC)
    - WBC count x product volume (mL)
  - Cell dose
    - TNC ÷ recipient's weight (kg)
  - Total CD34 count
  - CD34 dose
    - Total CD34 count ÷ recipient's weight (kg)
  - Cell viability
    - Trypan Blue stain or flow cytometry

STERILITY TESTING



- Bacterial cultures
  - 5 day incubation
- Fungal cultures
  - 10-14 day incubation
- Cultures collected at points to monitor quality
  - Post-collection
  - Post-processing
  - Post-thaw/Infusion

BACTERIAL AND FUNGAL CONTAMINATION

- Bacterial contamination
  - Usually due to improper collection technique (skin flora)
  - Occasionally due to septicemia
- Fungal contamination
  - Rarely seen
  - Minimized by collection/processing in positive-pressure room/hood
  - Primarily *Candida* spp. and aspergillosis

HPC PRODUCT EVALUATION

- Quality endpoints
  - TNC recovery
  - CD34 recovery
  - Cell viability
  - Sterility testing
- Non-conforming products
  - Lab medical director and patient's MD must be notified

AUTOLOGOUS TRANSPLANTATION

- Fewer complications
- Usually performed with diseases that do not involve the bone marrow
- Myeloablative treatment followed by transplant
- Immune system restores within 3-4 weeks
- Rare GVHD seen

ALLOGENIC TRANSPLANTATION

- First choice for leukemias and congenital/hereditary diseases
- Unrelated donors are matched through different organizations (e.g. NMDP, COBLT)
- Conditioning regimen based on HLA-matching with donor
  - Haplo vs. full-match
- GVHD – None to severe

INFUSION OF HPC

- Infused thawed or fresh
  - Autologous products are cryopreserved
  - Allogenic products are cryopreserved or fresh
- Frozen HPC placed in sterile plastic bag and thawed in a 37°C water bath at patient's besdside
- Once thawed, product can be washed with to remove DMSO before infusion (pediatrics)

INFUSION OF HPC

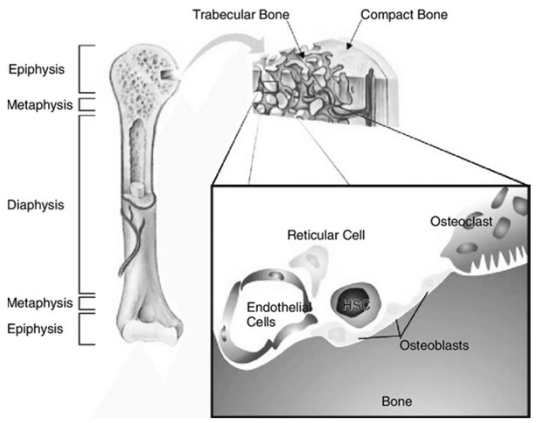
- HPC product infusion performed like a blood transfusion
- HPC bag spiked or transferred to syringe
- Connect to sterile infusion tubing leading to patient's central venous access
- Patient's vital signs are monitored entire transplant as well as afterwards

INFUSION OF HPC

- Most common symptom experienced is nausea/vomiting
  - Mostly due to DMSO
- Other symptoms include:
  - Hypotension
  - Hypertension
  - Fever
  - Chills

INFUSION OF HPC

- HPC dose should be > $2x10^6$ CD34+ cells/kg
- HPC travel immediately to lungs
  - Point of maximum circulation
  - DMSO excreted through lungs/breath
- "Homing" mechanism
  - HPC find their way back to the bone marrow
  - Endosteal niche



IMMUNOLOGIC COMPLICATIONS

- Complications from HPC infusions may include:
  - Acute or delayed hemolytic reactions
  - Febrile, non-hemolytic reactions
  - Allergic reactions
  - Transfusion-related acute lung injury (TRALI)
  - Alloimmunization to antigens
  - Graft-versus-host disease

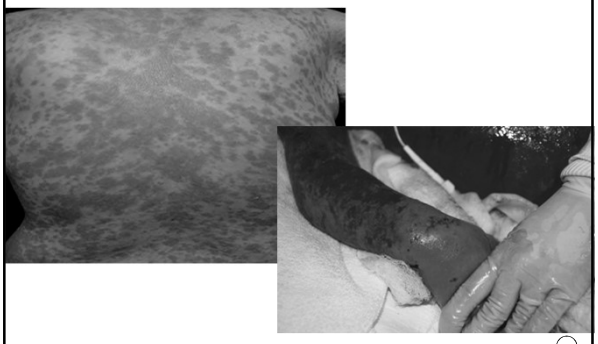
GRAFT-VERSUS-HOST DISEASE (GVHD)

- Major biological limitation of successful allogenic stem cell transplantation
- Alloimmune attack on recipient's tissues mounted by the donor's T-cells
- GVHD can be prevented by depleting T-cell lymphocytes from the stem cell graft
  - CD34+ selection

GVHD SYMPTOMS

- Include any or all areas:
  - Skin
    - Blisters, maculopapular rash, sloughing of skin
  - Liver
    - Total bilirubin levels
  - Gastrointestinal
    - Diarrhea, nausea/vomiting, pain
  - Lungs, mouth, eyes, joints, etc.

GVHD RASH/SKIN SLOUGHING



GVHD SYMPTOMS

- Acute GVHD
  - Usually <100 days following transplant
  - Skin, GI, and liver involvement typical
  - Donor T-cells respond to mismatched patient HLA
  - Graded I through IV based on number and severity of organ and system involvement
- Chronic GVHD
  - >100 days post-transplant
  - Can involve skin, mouth, hair, nails, eyes, lungs, GI, liver, etc.
  - 30% experience without preceding acute GVHD
  - Graded as mild, moderate, or severe

ACUTE GVHD GRADING

GVHD Staging			
Stage	Skin	Liver (total bilirubin)	GI tract (diarrhea output/day)
0	No GVHD rash	<2 mg/dl	Adult: <500 ml/d *Child: <10 ml/kg/d
1	Maculopapular rash <25% body surface area	2-3 mg/dl	Adult: 500-999 ml/d Child: 10-19.9 ml/kg/d -or- persistent nausea, vomiting, or anorexia with a positive upper GI biopsy
2	Maculopapular rash 25-50% BSA	3.1-6 mg/d	Adult: 1000-1500ml/d Child: 20-30 ml/kg/d
3	Maculopapular rash >50% BSA	6.1-15 mg/dl	Adult: >1500ml/d Child: >30 ml/kg/d
4	Generalized erythroderma (>50% BSA) plus bullous formation or desquamation >5% BSA	>15 mg/dl	Severe abdominal pain with or without ileus, or grossly bloody diarrhea
*Use adult values for patients ≥ 50 kg			
Overall Clinical Grade: Grade 0: No GVHD of any organ Grade 1: Stage 1-2 skin and no liver OR GI tract involvement Grade2: Stage 3 skin and/or stage 1 liver and/or stage 1 GI tract Grade 3: Stage 0-3 skin with stage 2-3 liver and/or stage 2-3 GI tract Grade 4: Stage 4 skin, liver, and/or GI tract			

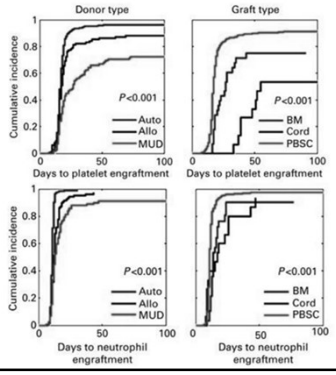
CHRONIC GVHD GRADING

Mild	• 1 or 2 organs or sites (except lung) with score 1 • Mild oral symptoms, no decrease in oral intake • Mild dry eyes, lubricant eyedrops ≤ 3x/day
Moderate	• 3 or more organs with score 1 • At least 1 organ or site with score 2 • 19-50% body surface area involved or superficial sclerosis • Moderate dry eyes, eyedrops > 3x/day or punctal plugs • Lung score 1 (FEV1 60-79% or dyspnea with stairs)
Severe	• At least 1 organ or site with score 3 • > 50% body surface area involved • Deep sclerosis, impaired mobility or ulceration • Severe oral symptoms with major limitation in oral intake • Severe dry eyes affecting ADL • Lung score 2 (FEV1 40-59% or dyspnea walking on flat ground)

ENGRAFTMENT

- Described in terms of absolute nucleated count (ANC) and platelet counts
  - ANC >500 cells/μL for 3 consecutive days
  - Plt >20K/μL with no transfusion for 7 days
- Time for engraftment depends on product type and type of transplant
  - PBPC > Bone Marrow > UCB
  - Autologous > Allogenic
- HPC labs track engraftment as reflection of quality of product infused

ENGRAFTMENT



RECENT CELLULAR THERAPIES

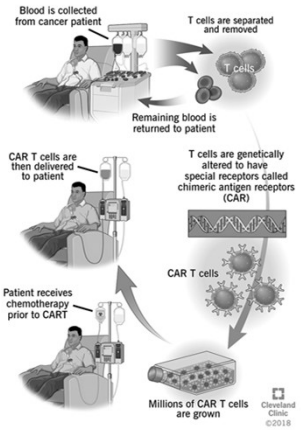
- Chimeric antigen receptor T-cell therapy (CAR T-cell)
  - Immunotherapy
  - Uses genetically-modified autologous T-cells to find and kill cancer cells
  - >80% of patients have complete or partial response
  - 2 FDA-approved therapies (so far)

CAR T-CELL THERAPY

- Kymriah™ (tisagenlecleucel)
  - Relapsed or refractory adult diffuse large-cell lymphoma (DLBCL)
  - Relapsed or refractory young adult acute lymphoblastic leukemia (ALL)
- Yescarta™ (axicabtagene ciloleucel)
  - Relapsed or refractory DLBCL
  - Primary mediastinal or high grade B-cell lymphoma

CAR T

- T-cells collected from patient via apheresis
- Retrovirus inserts DNA into T-cell to produce receptors (CARs)
- CAR T-cells are expanded *ex vivo*
- CAR T-cells infused in patient



CAR T-CELL COMPLICATIONS

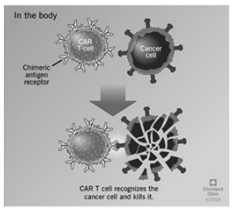
- Cytokine release syndrome (CRS)
  - Most common adverse reaction
  - 70-90% patients
  - Lasts 5-7 days
  - Mimics severe case of flu
    - High fever
    - Fatigue
    - Body aches
  - Can be reversed using tocilizumab

CAR T-CELL COMPLICATIONS

- CAR T-cell related encephalopathy syndrome (CRES)
  - Starts on day +5 and lasts 2-4 days
  - Patient becomes confused and disoriented; some unable to speak
  - Completely reversible
  - Patients recover all neurological function

FUTURE CAR T-CELL THERAPIES

- Expand CARs specificities
  - Current specificity is CD19
  - CD30 – Lymphomas
  - CD19/22 – B-cell ALL
  - CD33 – AML
  - Multiple myeloma
  - Solid tumors



SUMMARY

- Stem cells are omnipotent, pluripotent, self-replicating cells which gives rise to numerous specialized cell types
- Stem cells have the ability to cure dozens of otherwise incurable diseases
- Cellular therapy in the clinical laboratory is not limited to stem cells
- Future research in CAR T-cell therapy and *ex vivo* expansion of cells is ongoing.

RELATED WEBSITES

- AABB Center for Cellular Therapies
  - http://www.aabb.org/aabbcct/Pages/default.aspx
- National Marrow Donor Program
  - www.bethematch.org
- National Institute of Health
  - https://www.cancer.gov/about-cancer/treatment/research/car-t-cells
- FDA-approved therapies
  - https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

QUESTIONS?