Hematopoietic Progenitor Cell Graft Processing & Testing

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Types of "Processing"

- Minimally Manipulated
  - Preparation for Infusion
  - Plasma Removal (Minor ABO incompatibility)
  - Red Blood Cell Removal (Major ABO incompatibility)
  - Cryopreservation and thawing
  - Cell enrichment or depletion approved devices
- Extensive manipulation
  - Cell enrichment or depletion-unapproved devices or reagents
  - Ex vivo expansion of specific subsets (e.g. CTLs)
  - Gene manipulation (e.g. "Suicide genes")

Preparation for Infusion ± Processing

Sample Removal & Testing

- Cell counts & viability
- Stem cell content-Flow assessment of CD34
- Sterility cultures
- Archive sample storage (mostly cells to be frozen)

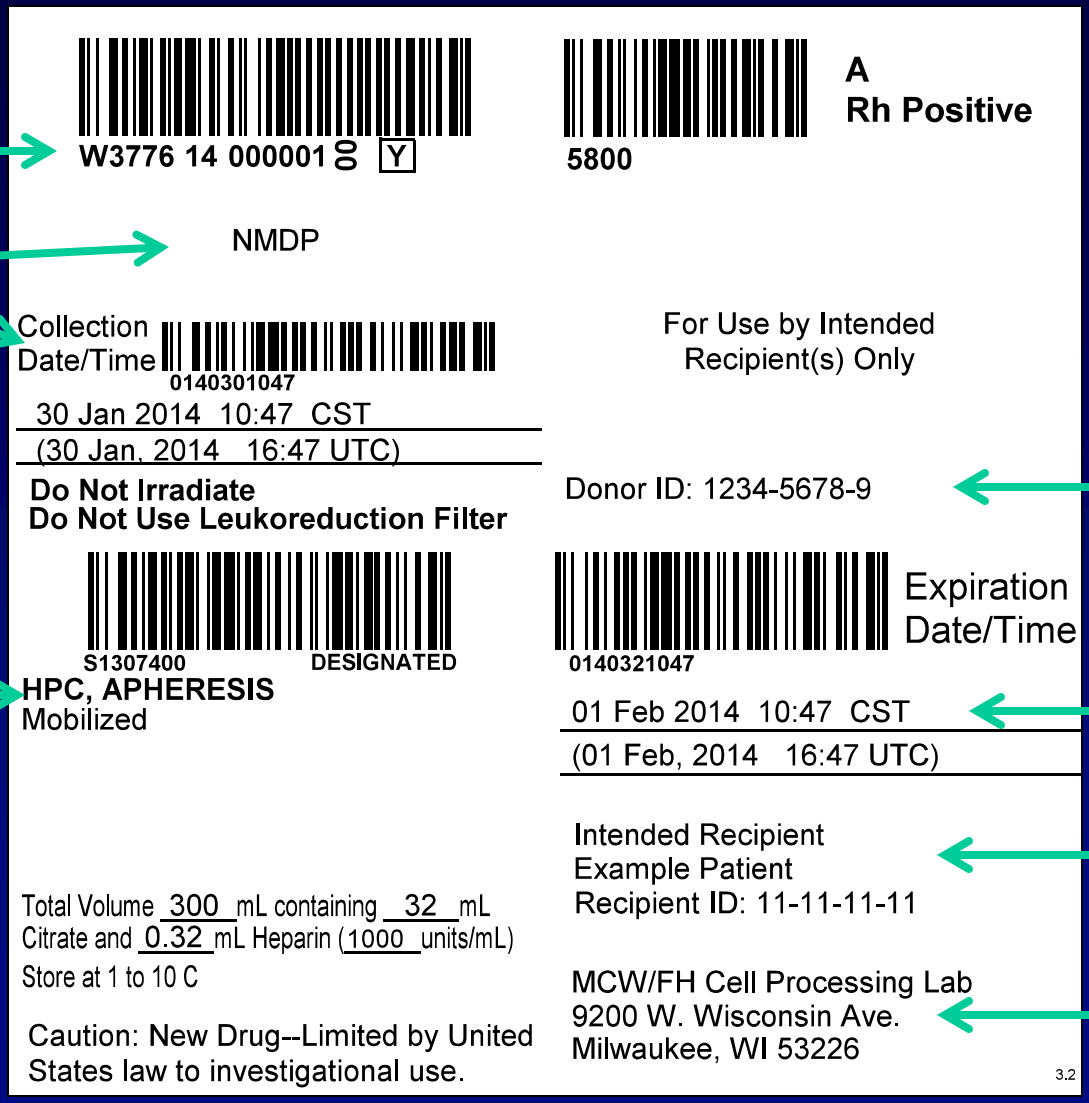
Labeling

- Composition (Cell count, volume, additives)
- Storage conditions and expiration
- Patient identification, Unit Identifier
- Collection and processing center identification
- Warnings and precautions

Documentation

- Records of all steps of product receipt, testing, processing, and infusion.

Labels



DIN

Collected by and when:

Donor ID

Product Code:

Expiration

Vol & Additives:

Patient ID

Processing Center ID

Cell Counting

Total Nucleated Cell Counts

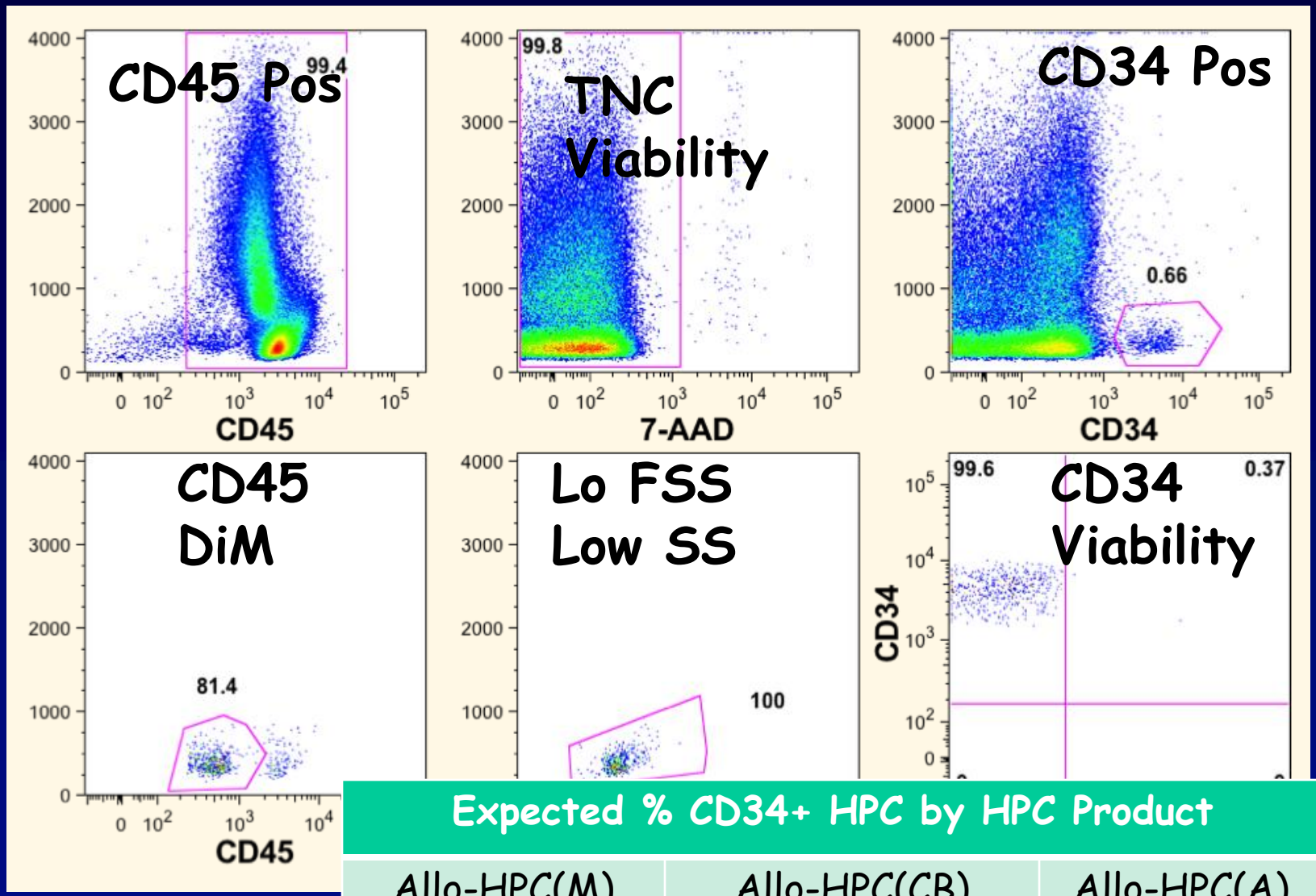
- Surrogate measure of graft quality
- Does not measure potency

Aspect	Manual Method	Electronic Method
Red Blood Cells	Manual Lyse, or Distinguish	Lysed automatically
Accuracy	Fewer events but better for marrow	More events but may ct marrow fat
Precision	Less (more manual steps)	More
Cost	Less	More (could share)
Subjectivity	More	Less

Viability Methods

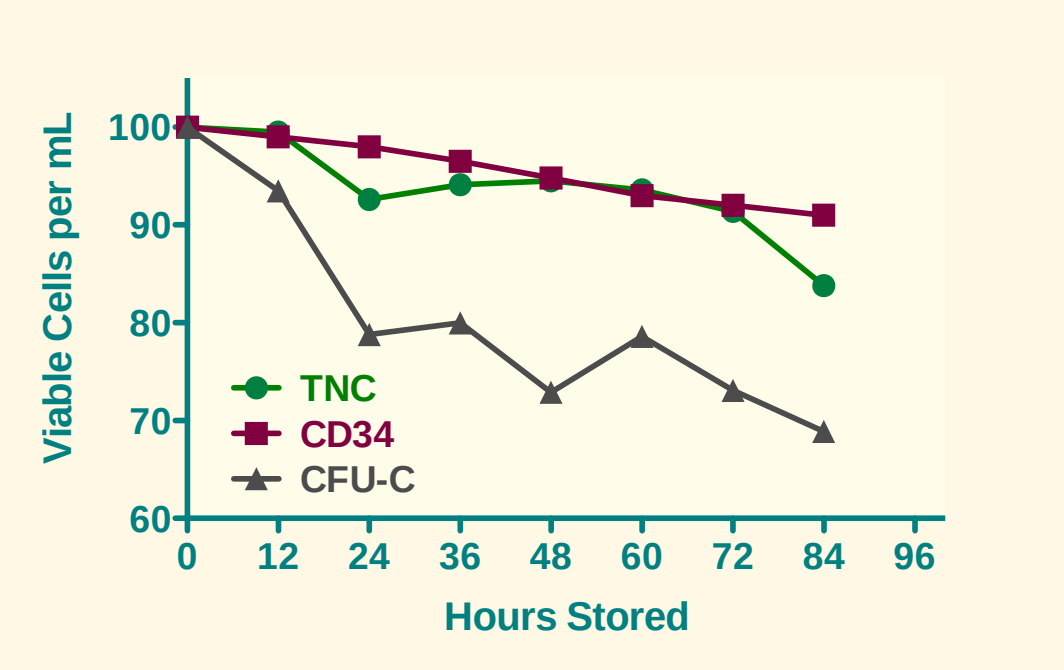
- Dye Exclusion Assays- Taken up by dead cells, excluded by cells with intact membranes
- Light or phase contrast Microscope
  - Trypan Blue-Most common in HPC laboratories
  - Erythrosin B
- Fluorescent Microscope
  - Acridine Orange with Propidium Iodide- Detects living and dead cells with two dyes
- Flow Cytometry Based Assays
  - 7-amino-actinomycin D (7-AAD)- Most common
  - Propidium Iodide (PI)

CD34+ Cell Analysis



Expected % CD34+ HPC by HPC Product		
Allo-HPC(M)	Allo-HPC(CB)	Allo-HPC(A)
1.0%±0.33%	0.93%±0.44%	0.85%±0.46%

Effect of Storage at 1-10°C



Overall viability and recovery of viable CD34+ cells was excellent over a 4 day storage period. However, there is a larger decline in colony forming cells in the same samples. N=3 experiments

While possible to store for autologous use expect a decline in engraftment potential.

Cryopreservation-Goals

- Short term or long term storage of cellular therapy products with preservation of function
- Allows for:
  - Banking of products such as HPC, Cord Blood
  - Storage while patients to undergo addition disease treatment or conditioning for transplant
  - Allogeneic donors to be collected in advance of infusion (several reasons)
  - Storage for potential or planned future use (DLI, serial infusions, etc)
  - Products to complete release testing

Cryopreservation- Basic Requirements

- Preparation of cells for freezing
- Selection and use of cryoprotectants. Mitigate freezing-induced membrane damage due to hyperosmolality, ice crystals and heat generated during the transition from liquid to solid (heat of fusion)
- A controlled slow rate of freezing to allow water to leave the cytoplasm
  - Trigger freezing and reduce heat of fusion (good but not essential)
- Storage at cold temperatures, <-80°C at minimum, Colder is better

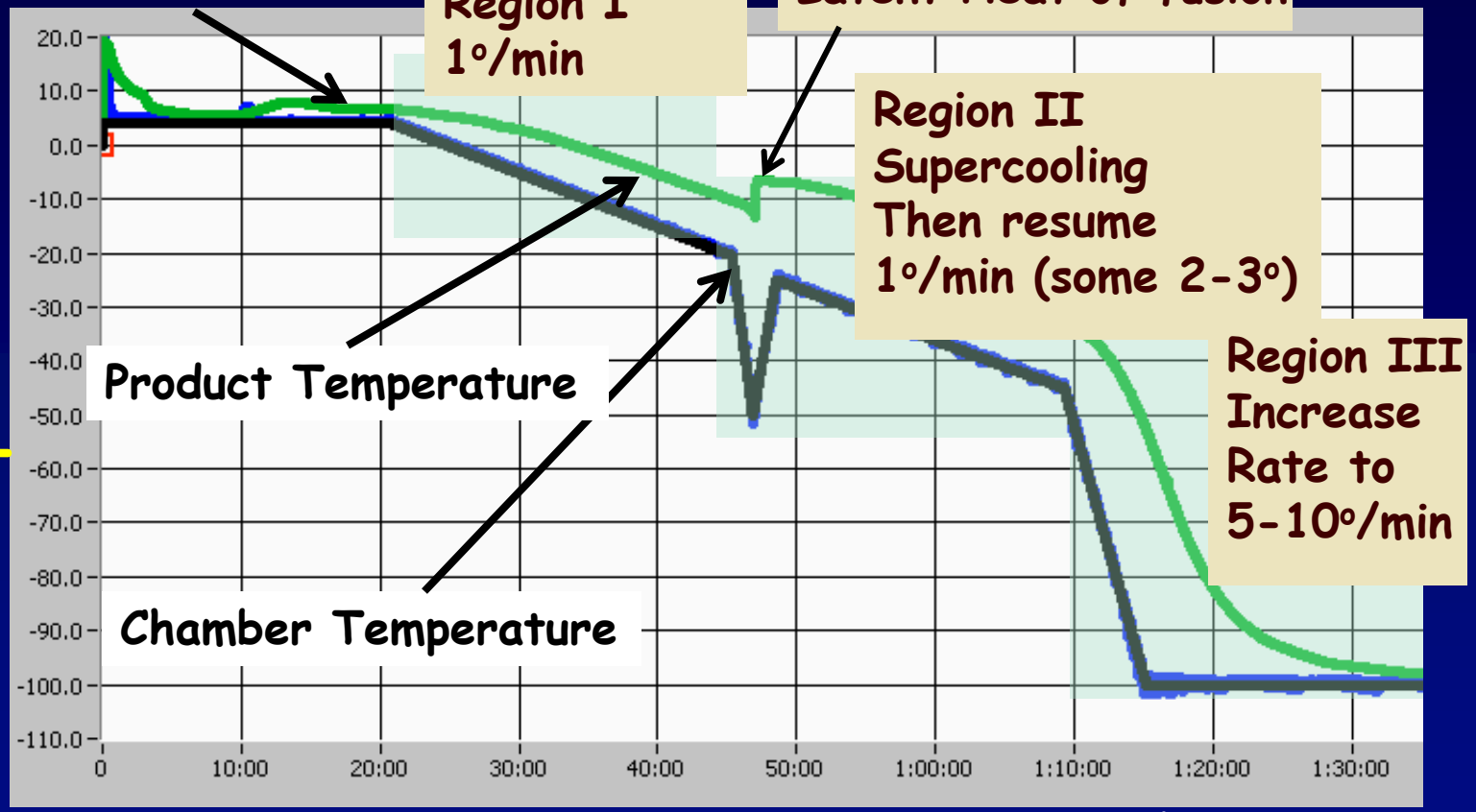
Pre-Processing

- HPC, Marrow or HPC, Cord Blood
  - RBC reduced using:
    - Buffy coat preparation
    - HES sedimentation or Density gradient separation
  - Cell concentration- 1-2 x 10^8 TNC/mL marrow
- HPC, Apheresis
  - Cell concentration- commonly 4.0 x 10^8 TNC/mL. up to 5.6 x 10^8 shown to have acceptable recovery
  - Granulocyte content- Aim for a MNC content ≥ 70% at collection
- In general low concentrations better, less clumping and lower viscosity

Computer Controlled Freezing

Let product = Chamber
Before start

Need to determine during validation



End temp may vary
from -60 to -100

Storage, Monitoring, Shipping

Storage of Products

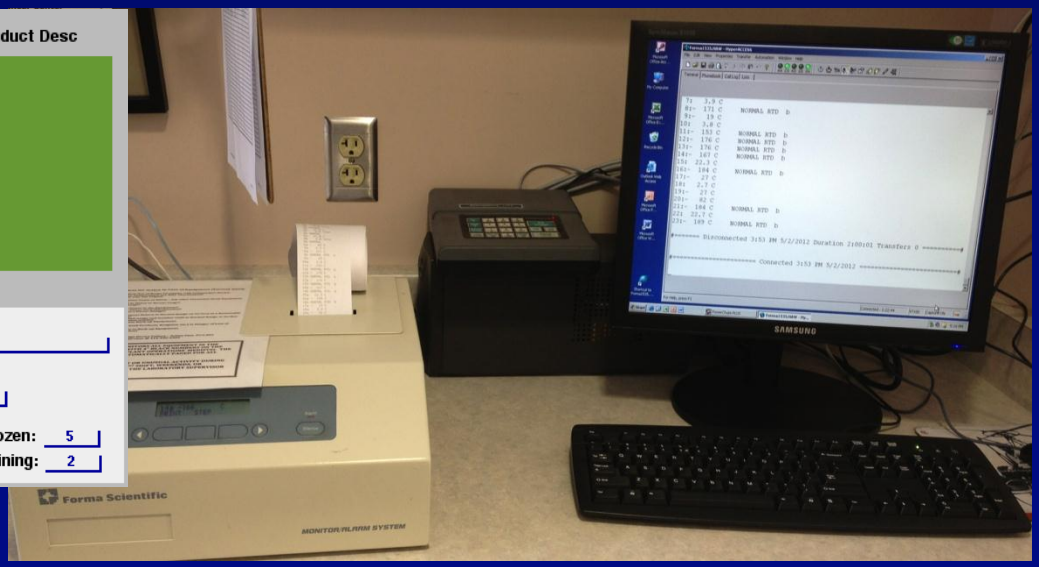
- Liquid/Vapor nitrogen tanks
  - -196 C Best for long term storage
    - Less susceptible to power interruptions
- Mechanical Freezer
  - - 80 C to -150 C
    - Need back up power supply
- Both methods need back up plan with alternate storage location

Storage of Products

- Temperature monitoring of storage location
  - Continuous recording or regular frequency
- Alarm system to notify of abnormal temperature
- Inventory system to track/locate products

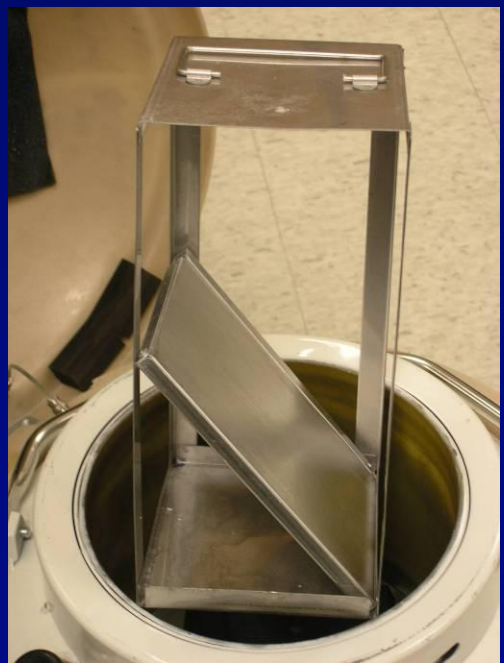
Bag #	Bag ID	Tank	Rack	Slot		Date Removed	Reason	Tech	Product Desc
1.	-1	CB	416	A	-	06/06/14	Infusion	DRM	
2.	-2	CB	416	B	-	06/06/14	Infusion	DRM	
3.	-3	CB	408	B	-	06/06/14	Infusion	KS	
4.	-4	CB	423	B	+				
5.	-5	CB	425	C	+				
6.									
7.									
8.									
9.									
10.									

Specimen Name [redacted]
Product: HPC, Apheresis-Plasma reduced
Purpose: Infusion
Product ID: W036314710140
Auto/Allo: Autologous
Patient Name [redacted]
Donor Name: Self
Cells/bag: 1.86e+10
Volume/bag: 60.0 mL
Dates: Collected: 05/21/2014
Frozen: 05/22/2014
Received @ MCW: 05/21/2014
Bags Frozen: 5
Bags Remaining: 2



Long Distance Transportation (frozen product)

- Required for cord blood from bank
  - May also ship for transfer pt
- Shipped in "dry shipper"
  - Liquid nitrogen in absorbent material
- Holds temperature for several days
  - Usually shipped without courier
- Monitor temperature during shipment



Thawing for Infusion

- Direct Thawing (At bedside)
  - Less Time
  - Fewer Manipulations
  - Higher Infusion Reaction Rate
- Dextran/Albumin Wash (In Laboratory)
  - Controlled Thawing Environment (safer!)
  - Better Cell Recovery
  - Easier Transport
  - Requires Good Communication with Infusion Team

ABO Compatibility

RBC ABO Type	Plasma Antibodies
A	Anti-B
B	Anti-A
AB	None
O	Anti-A & Anti-B

May need to remove plasma, RBC or both. RBC limit 0.3 mL/kg.

Red Blood Cell Removal

PURPOSE:

To reduce the content of donor red blood cells reactive with recipient antibody. MCW limit <20mL total or 0.3 mL/kg.

Volume reduction

METHOD:

Gel Sedimentation (Plasmagel, HES). Hct 1-2%.

Mononuclear Cell Preparation

Density Gradient Method. Hct <0.5%

Centrifugation Method. Hct 1-5%

Do not work for PBPC

Subset Depletion or Enrichment

- PURPOSE:
  - To remove undesired WBC subsets leaving behind everything else. Most often:
    - CD3+ T cells to prevent GVHD (@ transplant)
    - CD8+ T cells to prevent GVHD (DLI products)
    - CD19+ B cells to reduce chances of PTLD
  - To enrich the desired population, discarding everything else. Most often:
    - CD34+ or CD133+ hematopoietic stem cells
    - Subsets for immunotherapy, e.g. CD4+CD25+ (Treg), CD56+ (NK cells
- Performed using cell selection devices such as the Miltenyi CliniMACS

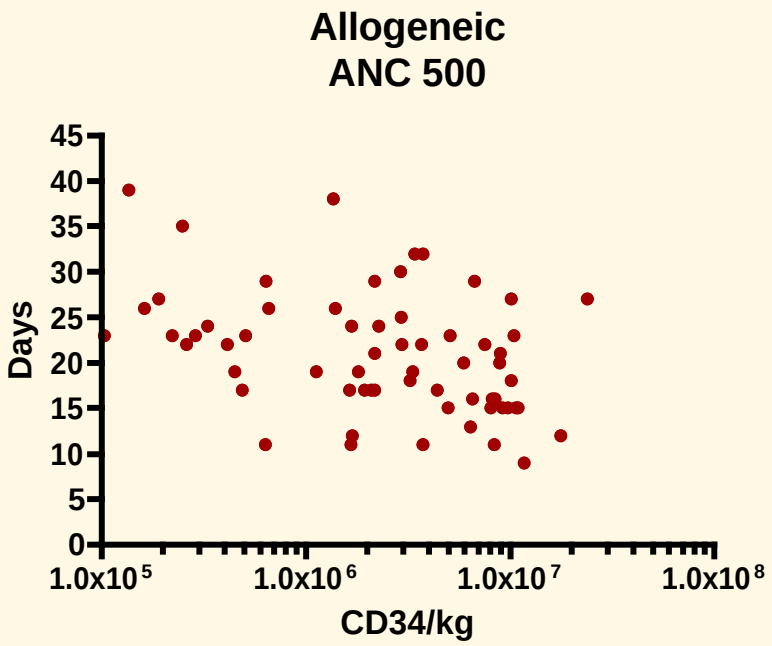
Target Infusion Cell Dose

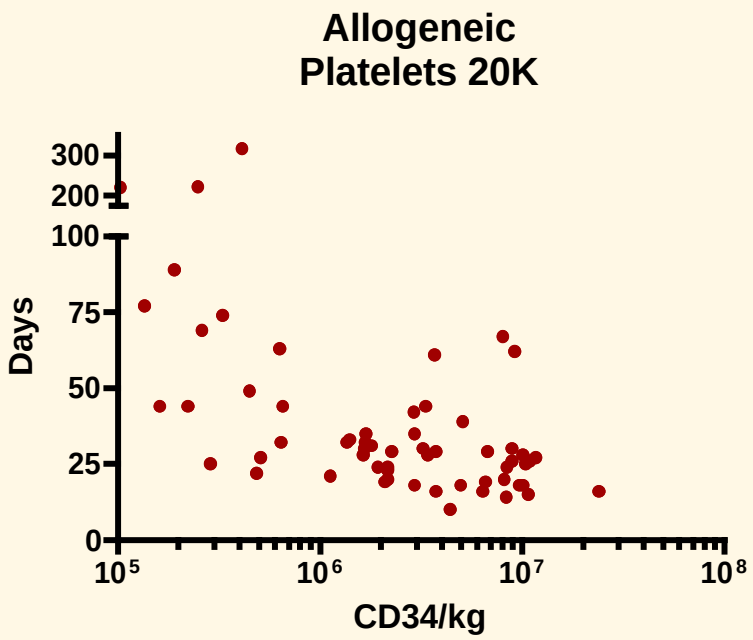
Non Manipulated HPC Products

	Nuc Cells/kg	CD34/kg
Allo Marrow	$2\text{-}4 \times 10^8$	$2\text{-}4 \times 10^6$
Auto Marrow	$1\text{-}2 \times 10^8$	$1\text{-}2 \times 10^6$
PBSC *	$2\text{-}10 \times 10^8$	$2\text{-}5 \times 10^6$
Cord Blood**	$>4 \times 10^7$	$>0.5 \times 10^5$

*Cell dose varies widely depending upon mobilization
**Required doses likely attainable only for Pediatric recipients

CD34 Dose





Day of engraftment versus CD34 dose per kg. Allogeneic patients.