STEM CELL PROCESSING METHODS AND GRAFT CHARACTERIZATION

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MAKING SOMETHING

Processing

Manufacturing

Production

CHECKING TO SEE IF YOU MADE IT CORRECTLY

Characterization
Quality Control Testing
Release Testing

EVOLVING ENVIRONMENT

HSCT is widely accepted and often considered as standard of care in many situations

Along with expanded access to HSCT services, public agencies and regulators are increasing oversight as part of their responsibility for consumer protection. The regulatory mechanism that many developed regions have adopted is to begin to equate cell product handling with pharmaceutical manufacturing. This trend is most likely to continue and expand into emerging regions of the world.

Cell therapy laboratories are increasingly required to put more stringent systems and validated processes into place that guide everyday procedures.

Types of Processing

- **Minimally Manipulated**
  - **No actions required**
  - **Plasma Removal (Minor ABO incompatibility)**
  - **Red Blood Cell Removal (Major ABO incompatibility)**
  - **Cryopreservation and thawing**
  - **Cell enrichment or depletion approved devices**
- **Extensive manipulation (“more than minimal”)**
  - **Cell enrichment or depletion-unapproved devices or reagents**
  - **Ex vivo expansion of specific subsets (e.g. CTLs)**
  - **Gene manipulation (e.g. “Suicide genes”)**

Activities Common to All Methods

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.

Process Validation

Regardless of the technical sophistication, all cell product handling should be done following a validated process.

One of the challenges for a new program is obtaining appropriate cell material for validation efforts before beginning work with patient cells.

Processing Examples

Cryopreservation
Product Storage
ABO Compatibility Management
Labeling

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)

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

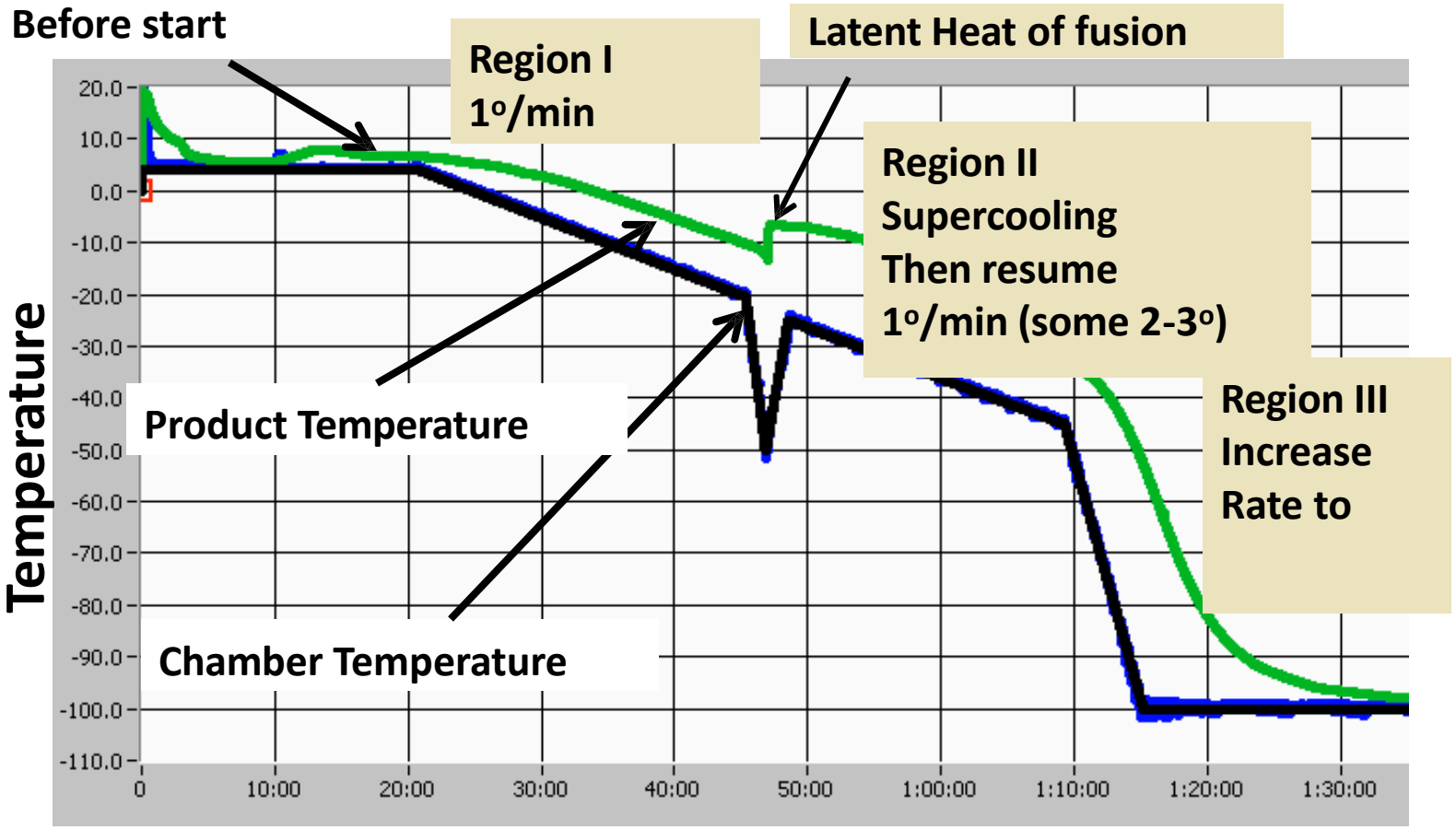
- Trigger freezing and reduce heat of fusion (good but not essential)

Storage at cold temperatures, <-80^{o}C at minimum, Colder is better

Computer Controlled Freezing

Let product = Chamber
Before start

Need to determine during validation



End temp may vary
from -60 to -100

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Non-Controlled Freezing ("Dump Freezing")

Advantages

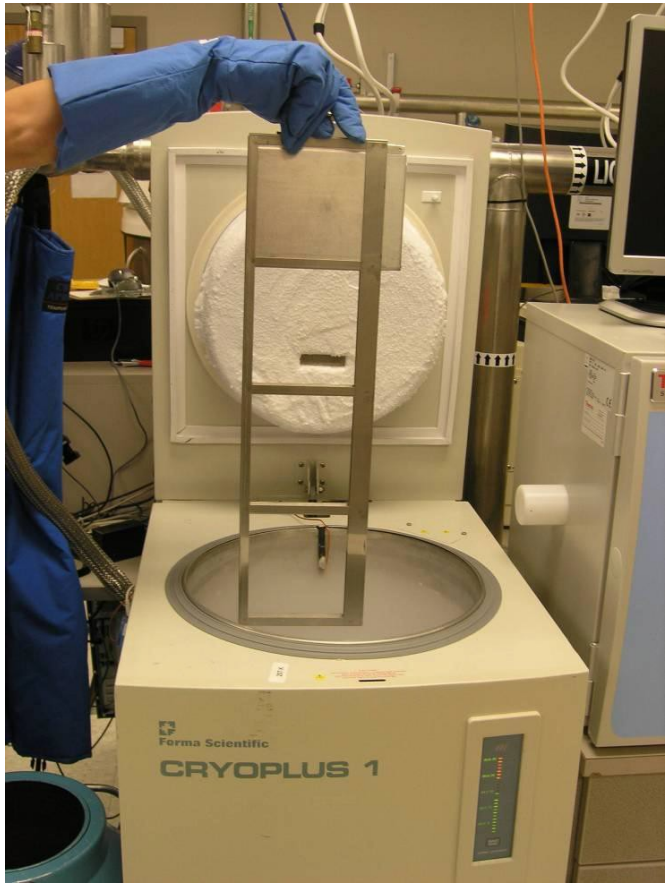
- No specialized equipment
- Less limitations of capacity
- Easier for multiple parallel processing

Disadvantages

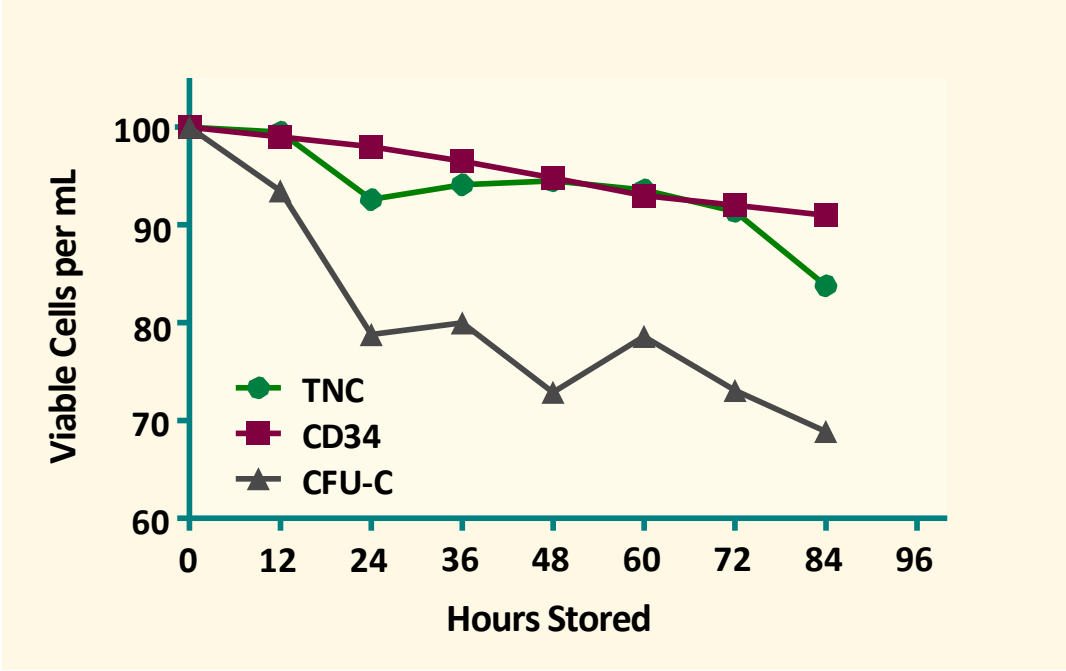
- No record of cryopreservation process
- Less control of process – potential product variability effects
- "Home made" systems require more validation efforts

Storage of Products

- Liquid/Vapor nitrogen tanks
  - Below -150 °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



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.

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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

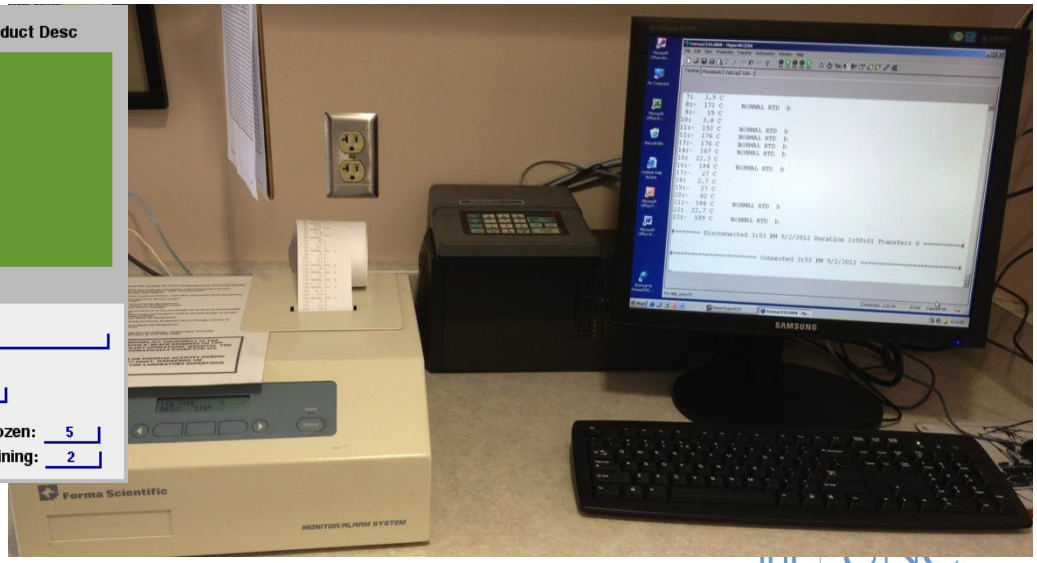
Cells/bag: 1.86e+10

Volume/bag: 60.0 mL

Dates: Collected: 05/21/2014 Frozen: 05/22/2014 Received @ MCW: 05/21/2014

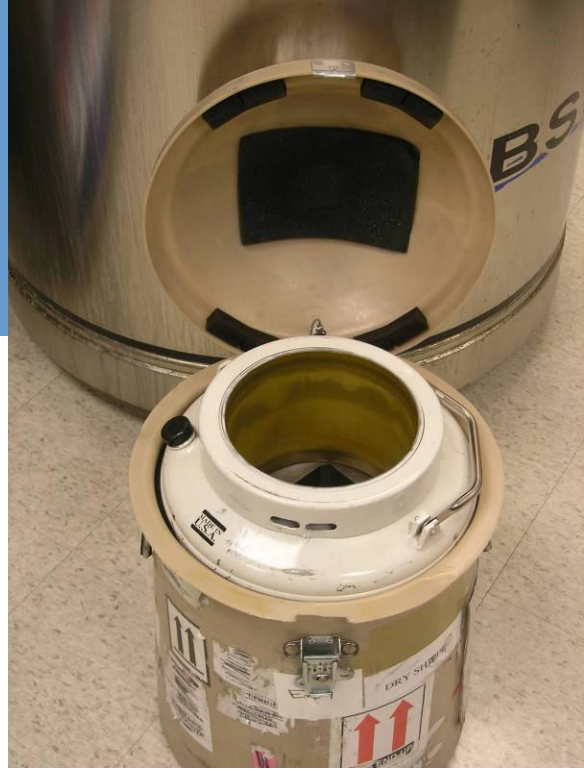
Bags Frozen: 5

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



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 should be established (e.g. 0.5 mL/kg.)

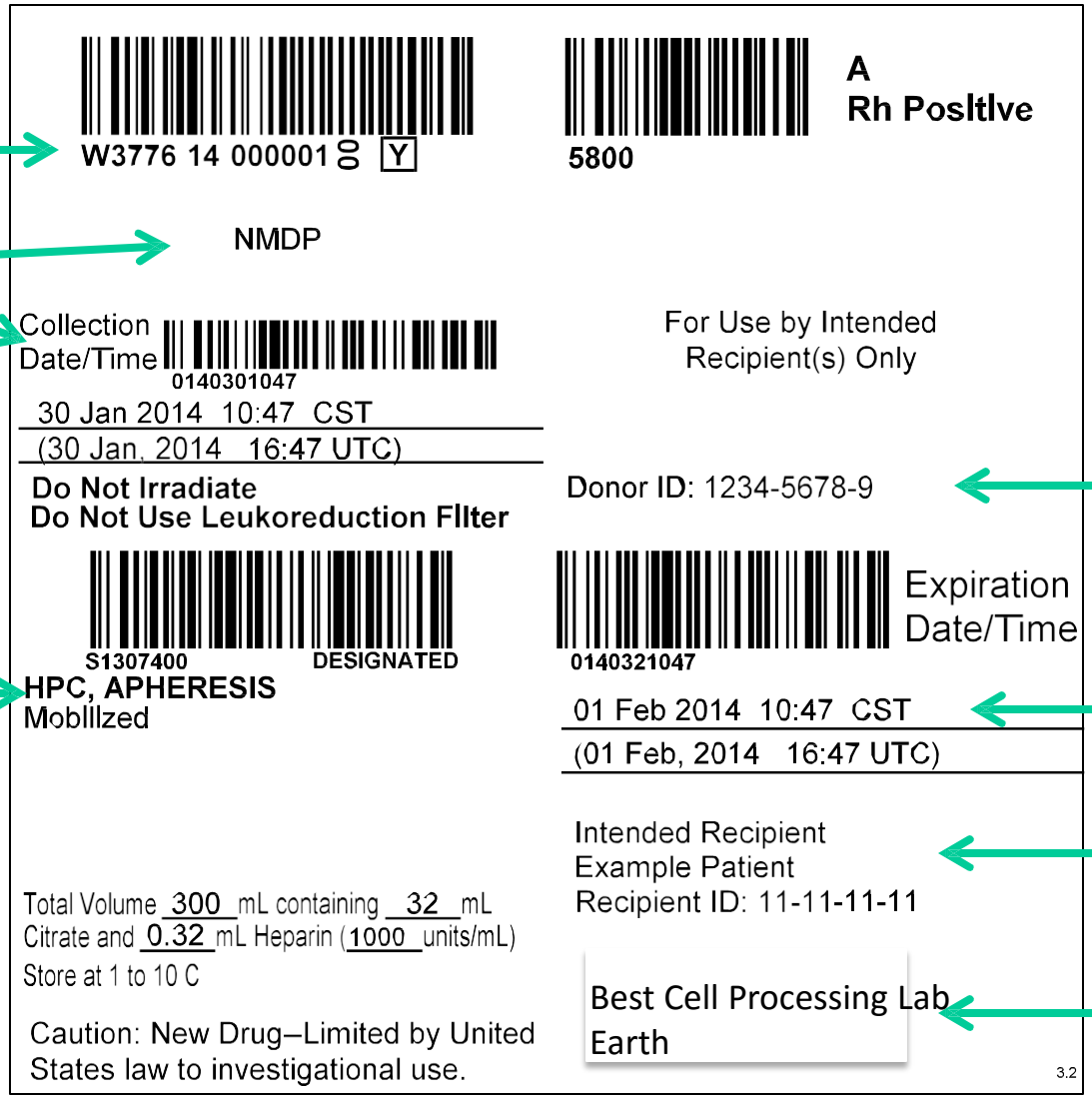
Red Blood Cell Removal

METHODS

Buffy Coat Centrifugation	**Hct 10 – 15%**
Gel Sedimentation (Plasmagel, HES).	**Hct 1-2%.**
Mononuclear Cell Preparation	
Density Gradient Method	**Hct <0.5%**
Automated Centrifugation Method	**Hct 1-5%**

Does not work effectively for PBPC

Labels


DIN
Collected by and when:
Product Code:
Vol & Additives:
W3776 14 000001 00 Y
5800
A
Rh Positive
NMDP
Collection Date/Time
0140301047
30 Jan 2014 10:47 CST
(30 Jan, 2014 16:47 UTC)
For Use by Intended Recipient(s) Only
Do Not Irradiate
Do Not Use Leukoreduction Filter
Donor ID: 1234-5678-9
Donor ID
S1307400 DESIGNATED
HPC, APHERESIS
Mobilized
0140321047
Expiration Date/Time
01 Feb 2014 10:47 CST
(01 Feb, 2014 16:47 UTC)
Expiration
Intended Recipient
Example Patient
Recipient ID: 11-11-11-11
Patient ID
Total Volume 300 mL containing 32 mL
Citrate and 0.32 mL Heparin (1000 units/mL)
Store at 1 to 10 C
Caution: New Drug–Limited by United States law to investigational use.
Best Cell Processing Lab
Earth
Processing Center ID
3.2

GRAFT CHARACTERIZATION

Identity

- Cell number
- Viability
- Cell phenotype (e.g., CD34+)

Safety

- Sterility

Potency

- Colony assays
- Surrogate assays

Cell Quantification

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 Determination

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**

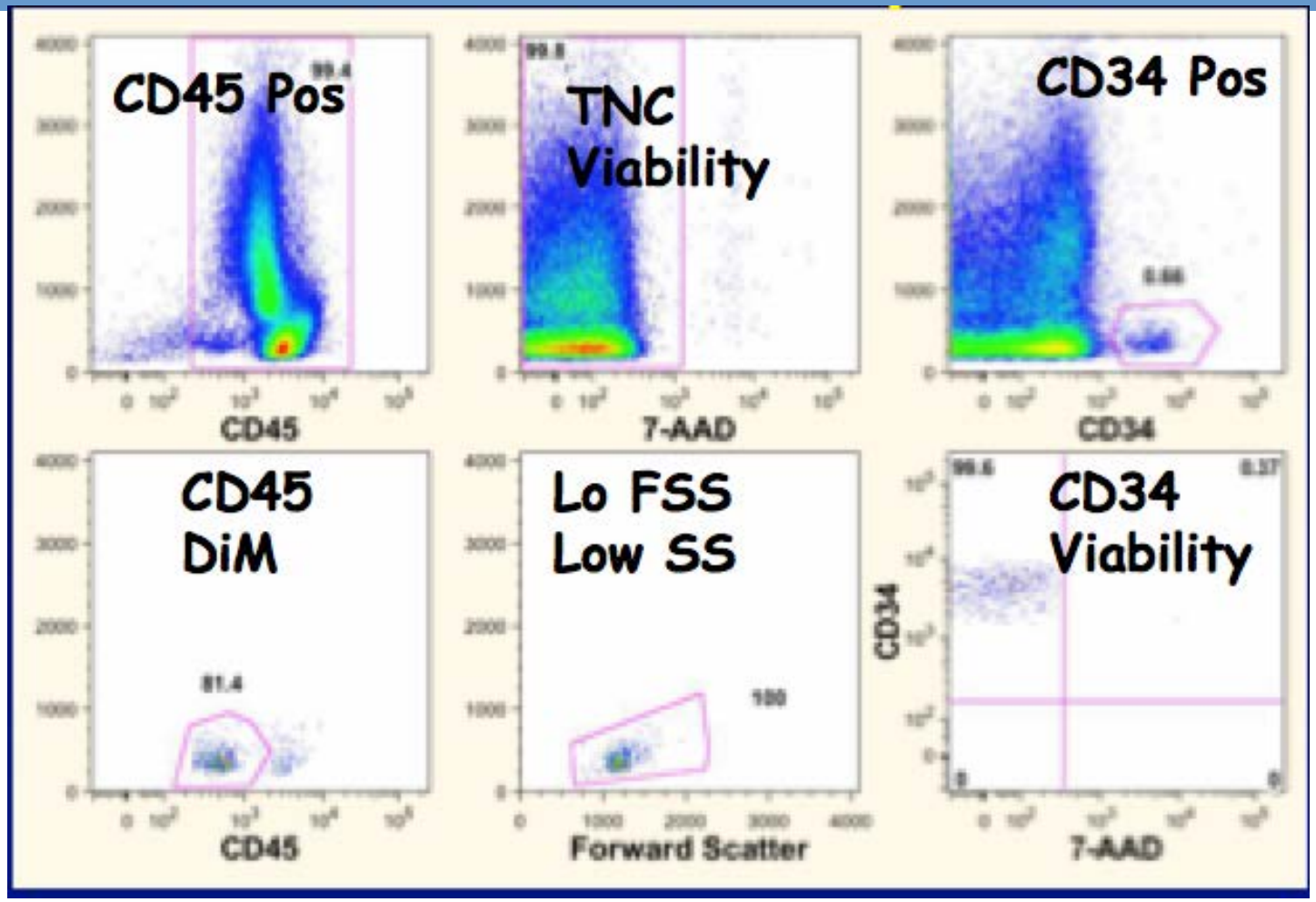
Fluorescent Microscope

- **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%

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Target Infusion Cell Dose

Non Manipulated HPC Products

	Nuc Cells/kg	CD34/kg
Allo Marrow	2-4 x 10^8	2-4 x 10^6
Auto Marrow	1-2 x 10^8	1-2 x 10^6
PBSC *	2-10 x 10^8	2-5 x 10^6
Cord Blood**	>4 x 10^7	>0.5 x 10^5

*Cell dose varies widely depending upon mobilization

**Required doses likely attainable only for Pediatric recipients

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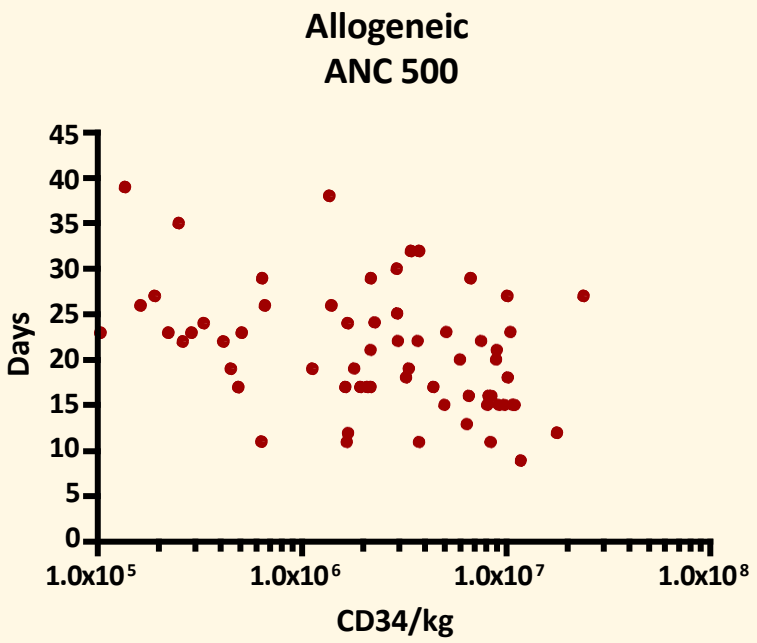
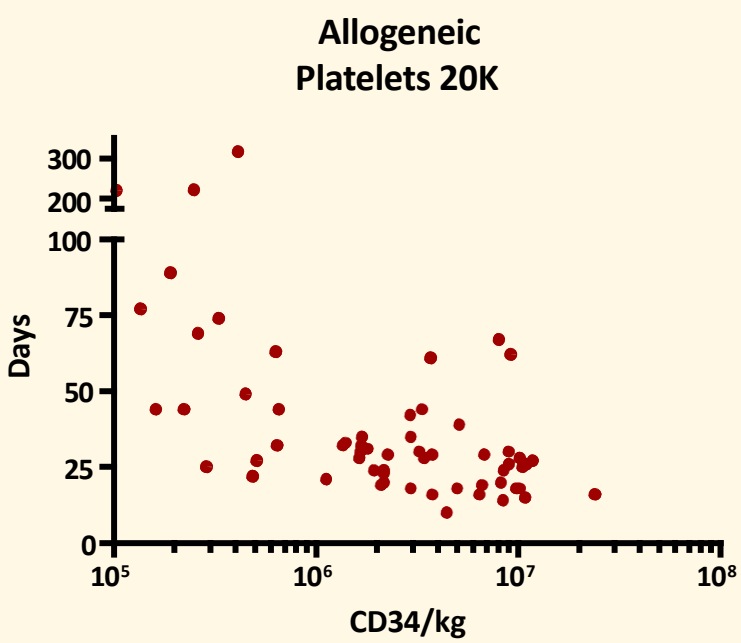
CD34 Dose





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

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Resources

Professional Organizations

- WBMT (WBMT.org)
- AHCTA (ahcta.org)
- ISCT (celltherapysociety.org)
- AABB (aabb,org)

Published methods (Books, manuals, and scientific papers)

Accrediting organizations

- FACT
- JACIE
- AABB

Peer Communication

- Small Labs Group (Google Discussion Group) (groups.google.com/forum/?hl=en#!forum/small-cell-therapy-lab)

Thank you

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