

# Product Characterization

## *Overview and Practical Considerations*

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# Topics

- Review various product types and applications
  - ◆ 351 and 361 products
- What does product characterization include, why do we need to do it
- Process control/validation
- Analytical method validation
- How does the phase of clinical trial factor in?

# Topics (cont.)

- How to deal with manufacturing changes
  - ◆ What is Comparability?
- What to include in your IND (CMC section)?
- Appropriate product release specifications
- Operational approaches
  - ◆ How does this apply to the cell processing laboratory?
- Challenges, obstacles and strategies specific to cell therapies

# Diversity of Products and Applications

<b>Immune, hematopoietic replacement</b>	Cancer, autoimmune diseases, immunodeficiencies	Blood, bone marrow HPC/HSC, MSC
<b>Immune effector cell therapy</b>	Cancer, autoimmune diseases, infectious diseases	Dendritic cells, NK cells, lymphocytes, macrophages
<b>Tissue repair, regeneration</b>	Cardiovascular disorders	Cardiomyocytes, bone marrow
	Neurologic disorders, injury	Neural cells, stem cells, macrophages
	Orthopedic disorders	Chondrocytes, MSC
	Wound healing	Keratinocytes, dermal fibroblasts
<b>Metabolic replacement, support</b>	Diabetes	Islet cells, stem cells
	Liver failure, metabolic disorders	Bioartificial liver, hepatocytes, stem cells
	Renal failure	Bioartificial kidney
<b>Gene replacement, modification</b>	Immunodeficiencies	Gene replacement therapy
	Cancer	Inducible lethal “suicide” gene therapy
	Cardiovascular disorders	Angiogenesis gene therapy
	Genetic disorders	Gene therapy - multiple cell types

# Regulatory Pathway

## ■ 361 products

- GTP regulations apply (CFR 1271)
- Focus on safety; minimizing communicable disease risk
- Full characterization not usually performed

## ■ 351 products

- GMP regulations apply (CFR 210, 211)
- Safety and product characterization important
- Developed through clinical trials

# Product Characterization

*“ Characterization of a biological product (includes determination of physiochemical properties, biological activity, immunochemical properties, purity and impurities) by appropriate techniques is necessary to allow relevant specifications to be established.”*

*ICH Q6B, Guidance on Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*

# Product Characterization – Why?

- Specifications help to ensure product quality
- Measurements can assess product integrity and stability
- Product parameters can help anticipate possible adverse events
- Demonstrate control of manufacturing process and ensure lot-to-lot consistency

# Product Characterization – Elements

## ■ Safety

- ✓ Freedom from adventitious agents: bacteria, fungi, mycoplasma, viruses

## ■ Purity

- ✓ Freedom from extraneous material

## ■ Identity

- ✓ Specific to the product in a manner as to adequately identify it on containers and labels

## ■ Potency

- ✓ Measure of the product's relevant biological function

# Safety Testing

## ■ Sterility Testing – microbial cultures

- Different methods used: Bactec, BacT-Alert, 14-day culture
- 21 CFR 610.12, USP sterility, or validated equivalent by phase III
- Should there be different validation requirements for GTP products?

## ■ Mycoplasma (21 CFR 610.30)

- Consider validating PCR-based alternative assay

## ■ Adventitious agents

- Commonly follows practice for blood donor testing, other agents tested as appropriate (xenogeneic exposure)

# Safety Testing Strategies

- Cryopreserved products - preferred
  - Thaw and administer product when release testing complete
  - Release testing performed on pre-cryopreservation product
    - ◆ Clinical product thawed and administered ***without further testing (!)***
- Products administered fresh
  - Culture 24-48 hr pre-harvest, repeat prior to infusion
  - Stat Gram stain and endotoxin, prior to infusion
  - Release based on negative 48-hr culture, Gram stain, endotoxin, final results pending
- Specific to stem cell products where patients are lethally myeloablated
  - May release product with release testing results pending
  - May administer despite failure to meet release criteria (!)
  - Release under exception, report as deviation, clinical release by patient's physician and lab medical director, should inform the patient of risks

# Purity, Identity Testing I

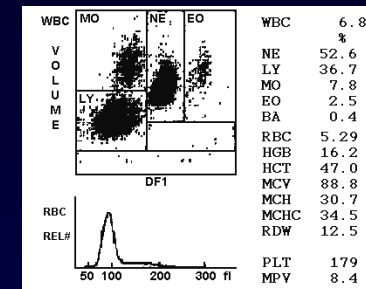
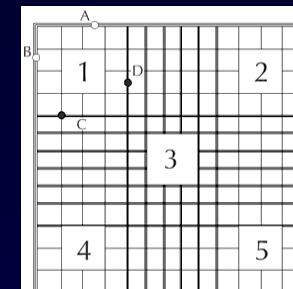
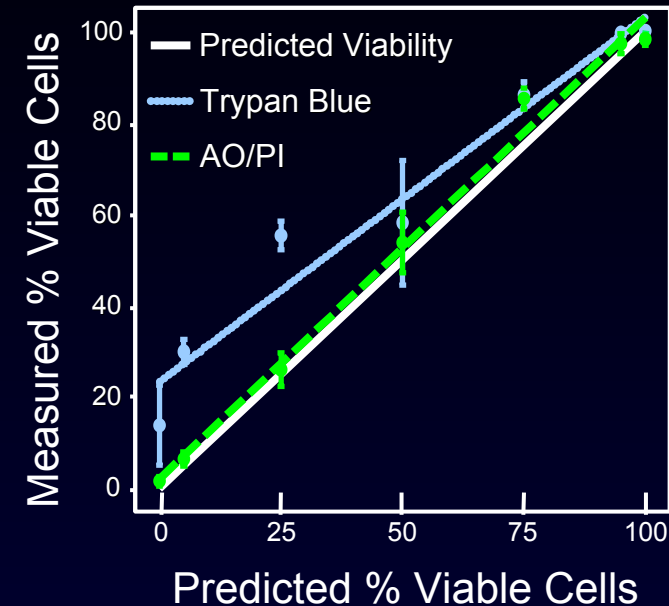
## Cell Viability, Morphology, Cell Counts

### ■ Cell viability

- Trypan blue, acridine orange-propidium iodide, 7-AAD

### ■ Technology

- Manual hemacytometer
- Automated impedance-based cell counters
- Image analysis instruments
- Controls in place
- Limitations and variability

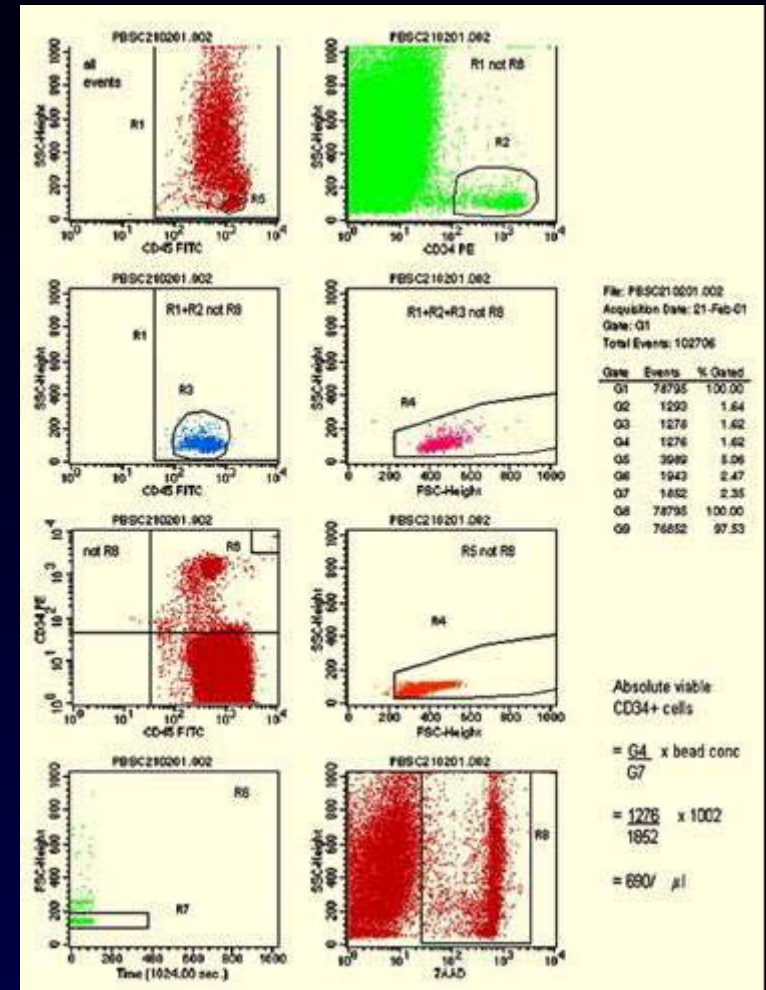


WBC	MO	NE	EO	WBC	6.8
V				NE	52.6
O				LY	36.7
L				MO	7.8
M				EO	2.5
E				BA	0.4
				RBC	5.29
				HGB	16.2
				HCT	47.0
				MCV	88.8
				MCH	30.7
				MCHC	34.5
				RDW	12.5
				PLT	179
				MPV	8.4

# Purity, Identity Testing II

## Immunophenotype Determination – Flow Cytometry

- Flow cytometry - an essential element of product definition for most cell therapies although certain tissue-based products not easily analyzed
- Multiple cell surface antigens may be tested to develop product specifications
- Potential sources of error including variability and subjective interpretation
- Requires optimization, rigorous control, **validation** - yet full IQ/OQ/PQ rarely performed on flow cytometers



# Purity Testing – Impurities

- Detection of reagents used in manufacturing
  - Residual peptides
  - Proteins
  - Cytokines
  - Antibodies
  - Sera
- Pyrogen testing
  - Endotoxin (21 CFR 610.13)
    - ◆ Dose < 5 EU/Kg/hr
- Detection of product-related impurities
  - Tumor cells

# Potency

## ■ Potency

- Quantitative measurement(s) of a relevant biological function or activity
- Examples:
  - ◆ Antigen presentation
  - ◆ Cytotoxicity
  - ◆ Cytokine release
  - ◆ Clonogenicity

# Validation

*“Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.”*

*FDA, Guidelines on General Principles of Process Validation, 1987*

- Facility
- Equipment
- Processes
  - All manufacturing processes
  - Significant manufacturing process changes
- Analytical methods
  - Raw material
  - In-process product
  - Final product
- Sterility assurance

# Qualification/Validation - IQ, OQ, PQ

1. Objective
2. Scope
3. Responsibilities
4. Definitions
5. Reference Materials
6. Documentation Procedures
7. System Description
8. ***Installation, Operational, Process Qualification***
9. Deviations
10. Attachments
11. Final Report

# Performance Qualification

- PQ study verifies performance, under real-life conditions, across anticipated working range
  - Helps to develop consistency of process, evidence of control, performance effects
- Clinical cellular raw material rarely available for PQ; often use mock critical raw material (normal donor cells, other)
- PQ outcome specifications - examples
  - Sterility cultures negative
  - MNC viability >90% post-cryopreservation/thaw
  - CD34<sup>+</sup> cell purity > 90% and recovery > 80%, CFU-GM recovery > 50%
  - >X log<sub>10</sub> removal of spiked tumor cells
  - Engraftment indices - days to ANC > 500, days to platelet count

# Analytical Method Validation Parameters (USP and ICH)

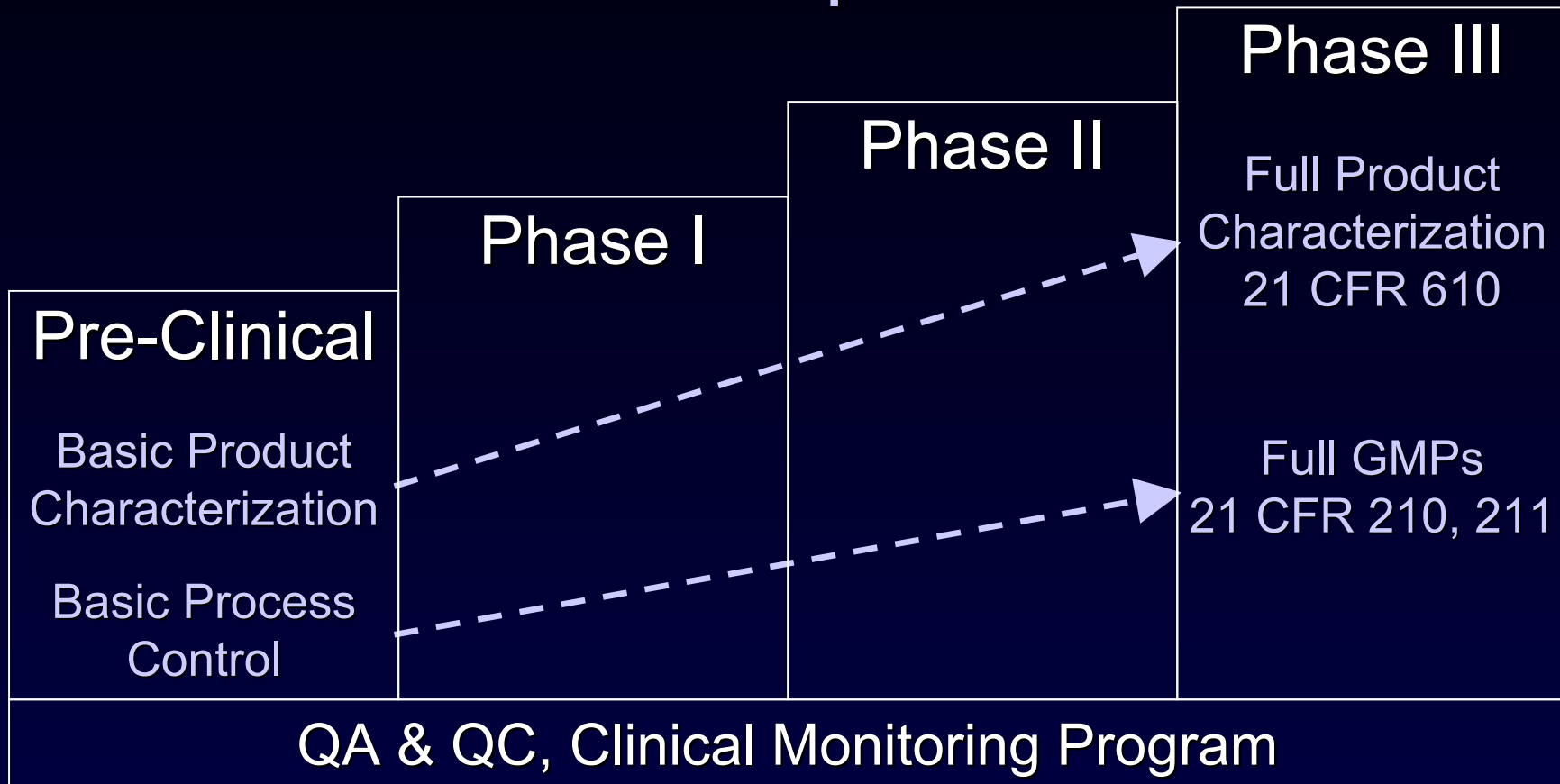
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantitation
- Specificity
- Linearity and Range
- Ruggedness, Robustness
- Suitability

# Analytical Method Validation Issues

- Determine analytical method's suitability for providing useful data
  - Quantify assay performance characteristics
  - Identify potential sources of error
  - Quantify potential errors
- Realize that validation more than simply repetitive performance of assay

# FDA “Sliding Scale”

## Requirements Increase With Product Development



# FDA Regulatory Requirements

- Progressive implementation of compliance with cGMPs throughout clinical trials
  - “Spirit of GMP” vs. Total GMP compliance
  - Degree of product characterization
    - ◆ Safety and basic process control early on, with appropriate release specifications ensuring safety)
    - ◆ then refine and tighten later, develop potency assay
  - Degree of process and analytical validation
  - Degree of QC/QA

# What to include in your IND

- How product will be characterized – safety, identity, purity, potency, viability, cell dose/number
- Production procedures (in-process testing)
- Process controls to minimize cross-contamination
- Final product release
- Stability (in-process, final)
- Validation and qualification of manufacturing process (QA/QC program)

# Product Release Specifications

- Chosen to confirm quality rather than characterize the product
- Provide assurance that quality of the product maintained
- Linked to the manufacturing process
- Linked to preclinical and clinical studies
- Linked to analytical procedures

## Include in Batch Record

- ☑ Appearance and Description
- ☑ Identity
- ☑ Purity and Impurities
- ☑ Potency
- ☑ Quantity

# Product Release Specifications - Example

Cell viability	$\geq 70\%$
Percent CD3 <sup>+</sup> cells (Post CD3 depletion, Pre IL-2 activation)	<5% CD3 <sup>+</sup> cells
Microbial cultures (48 hr pre-harvest)	Preliminary results negative
Endotoxin (LAL)	<0.5 EU/mL
Gram stain	No organisms observed
Mycoplasma	Negative

# What happens if you change your Process during clinical trials?

- Examples:
  - Change from open to closed methods resulting in improvements in yield, better aseptic processing
  - Other changes in process control, test methods
  - Facility changes, addition of manufacturing sites
- Assess improvements/changes to manufacturing process, do these changes effect safety, purity, potency?
- Should demonstrate product comparability

# Comparability - Bridging the Changes

- Demonstrate comparability using:
  - Analytical testing
  - Biological assays
  - Preclinical animal studies
  - Clinical testing - as needed
- Compare “old” versus “new”
- Detail in a written comparability protocol
- Describe changes in your IND
  - Changes may require new IND
- Best to contact your FDA reviewer with questions
  - Justify your position with data

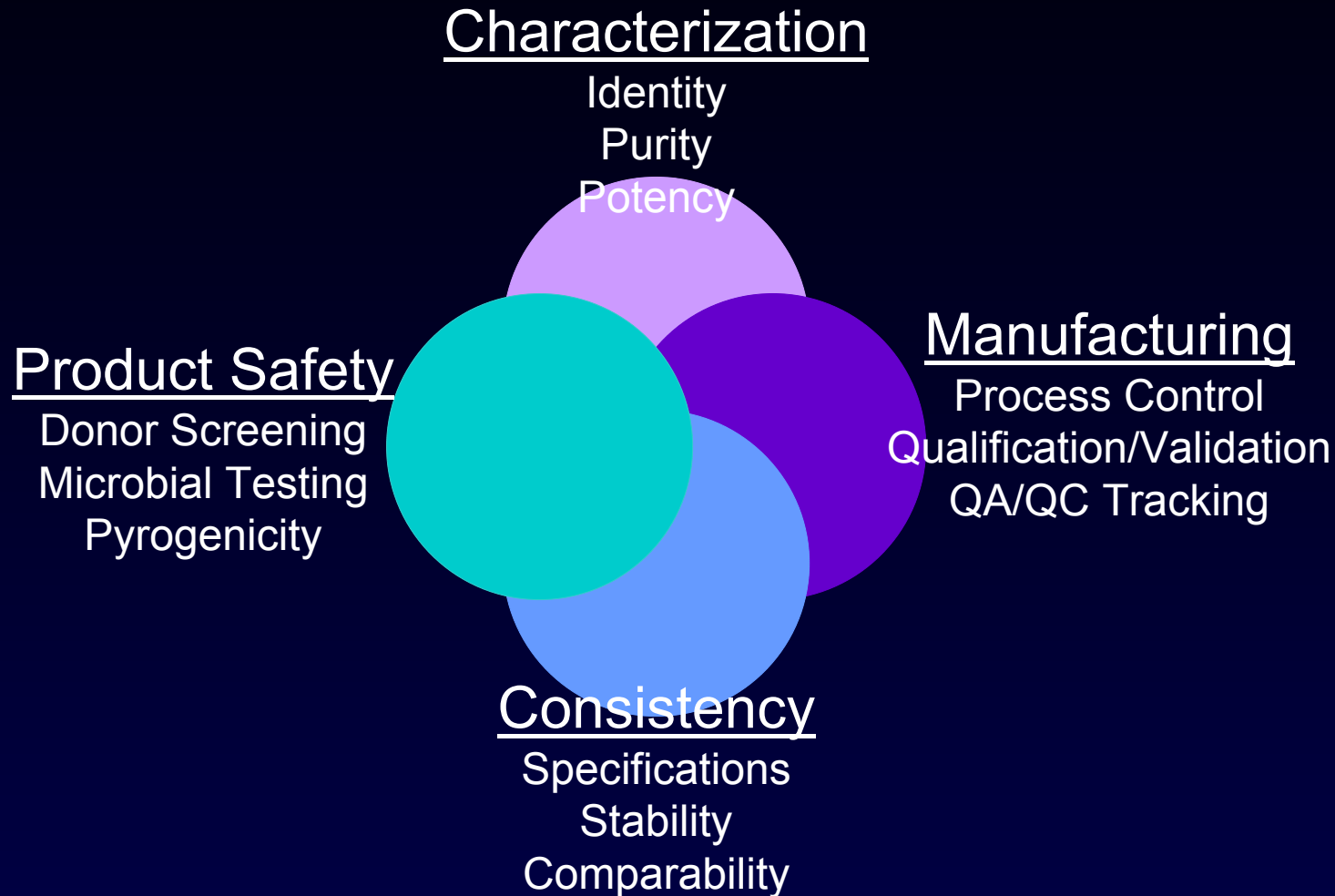
# Practical Considerations

- Significant differences in performing work in research lab vs. GMP environment
- Adequate time and staff needed to perform necessary analytical and process qualification/validations, media fills, etc.
- Who does what in the cell processing laboratory?
  - Investigator/sponsor knows the product best
  - Translational group needed to bridge pre-clinical and help develop GMP manufacturing
  - QA/QC group knows principles of validation, perform or at least review QC testing, and ensure appropriate product release
  - Outside laboratories with particular expertise may be needed

# Practical Considerations (cont.)

- Identify the appropriate team with the right expertise
  - Principal Investigator
  - Research Tech
  - Clinical Med Tech/Manufacturing Tech
  - QA/QC
  - Lab Director
- Have a good project plan and timeline in place
- Teamwork is critical to product development, product quality and patient safety

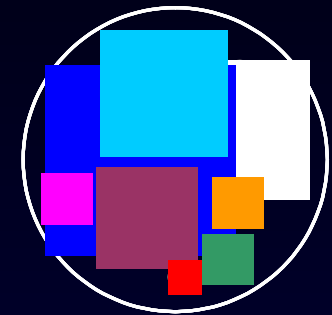
# Regulatory Interdependence



# Challenges Specific to Cell Therapies

- Living cells have significant biologic variability
  - Autologous and allogeneic donor variability
- Heterogeneous composition - even when highly purified
  - Known and unknown cell subpopulations
- Products are generally patient specific
  - 1 donor = 1 product = 1 patient
  - Some exceptions – e.g. allogeneic MSC
- Limited stability
  - Short shelf-life
- Complete product characterization is likely unattainable

“>95% CD34<sup>+</sup> cells”



# Product Characterization - Obstacles

- How much information is needed for your IND? For a specific stage of development?
- Appropriate stringency of GMPs for Phase I?
- Trying to live up to GMP/GTP in a hospital environment
- “... potentially lifesaving products” failing release specs
- Dealing with manufacturing changes, comparability
- Potency testing
  - Establishing “relevant biological function”
  - Establishing/optimizing analytical tools

# Summary

- Product characterization is fundamental to well-controlled production of cellular therapies
- Central Concepts
  - Product safety, purity, identity and potency
  - Process and analytical validation, process control
  - Novel release testing strategies for cellular therapies
- QC testing alone cannot assure product safety, quality
  - Cell therapy products cannot be fully characterized
- ***The manufacturing process must protect product, patient***
  - Controlled, consistent processes  $\Rightarrow$  controlled, consistent products

# References

- 21 CFR Parts 210, 211, 600, 610, 1271
- Briefing Document, Cellular, Tissue, and Gene Therapies Advisory Committee, Meeting #41, February 9, 2006.
- ICH Q6B, Guidance on Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.
- Draft Guidance for Reviewers: Instructions and Template for CMC Reviewers on Human Somatic Cell Therapy IND, August 2003.
- FDA Guidance Concerning Demonstration of Comparability of Human Biological Product, Including Therapeutic Biotechnology-derived Products, April 1996.

Thank you!