

# **CQAs for C&GT Products to Enable Comparability Assessment: Case Studies**

ISCT Cell Therapy Liaison Meeting

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# Objectives

- Demonstrate the value of defining CQAs early in product development for...
  - Managing analytical changes and maintaining comparability of analytical results
  - Managing process changes and defining formal *in vitro* comparability studies
- Describe key learnings and challenges experienced in CMC development of *ex vivo* gene therapy product
- Generate discussion and learn best practices from others

Disclaimer: Scenarios presented are hypothetical for illustration only.

# Critical Quality Attributes

## **CQAs are:**

- Product attributes with potential to impact safety or efficacy
- The foundation for managing product quality through all stages of the product lifecycle

## **CQAs are NOT:**

- Analytical methods
- Specifications

# Product Profile

- *Ex vivo* Gene Therapy – genetically modified autologous CD34<sup>+</sup> cells
- Target: metabolic disorder
- Data
  - *In vitro* characterisation
    - 20 batches of product for patient treatment
    - 5 batches of product generated from healthy donors
  - *In vivo* biodistribution study
    - Compare transduced with mock-transduced cells in NSG mice – no difference noted
    - inter-animal variation in engraftment & VCN
  - Clinical
    - 20 patients treated
    - Follow-up to 6 years (mean = 3 yr)
    - No treatment-related SAEs and clear signs of efficacy in majority of patients

# Step 1: Define the CQAs

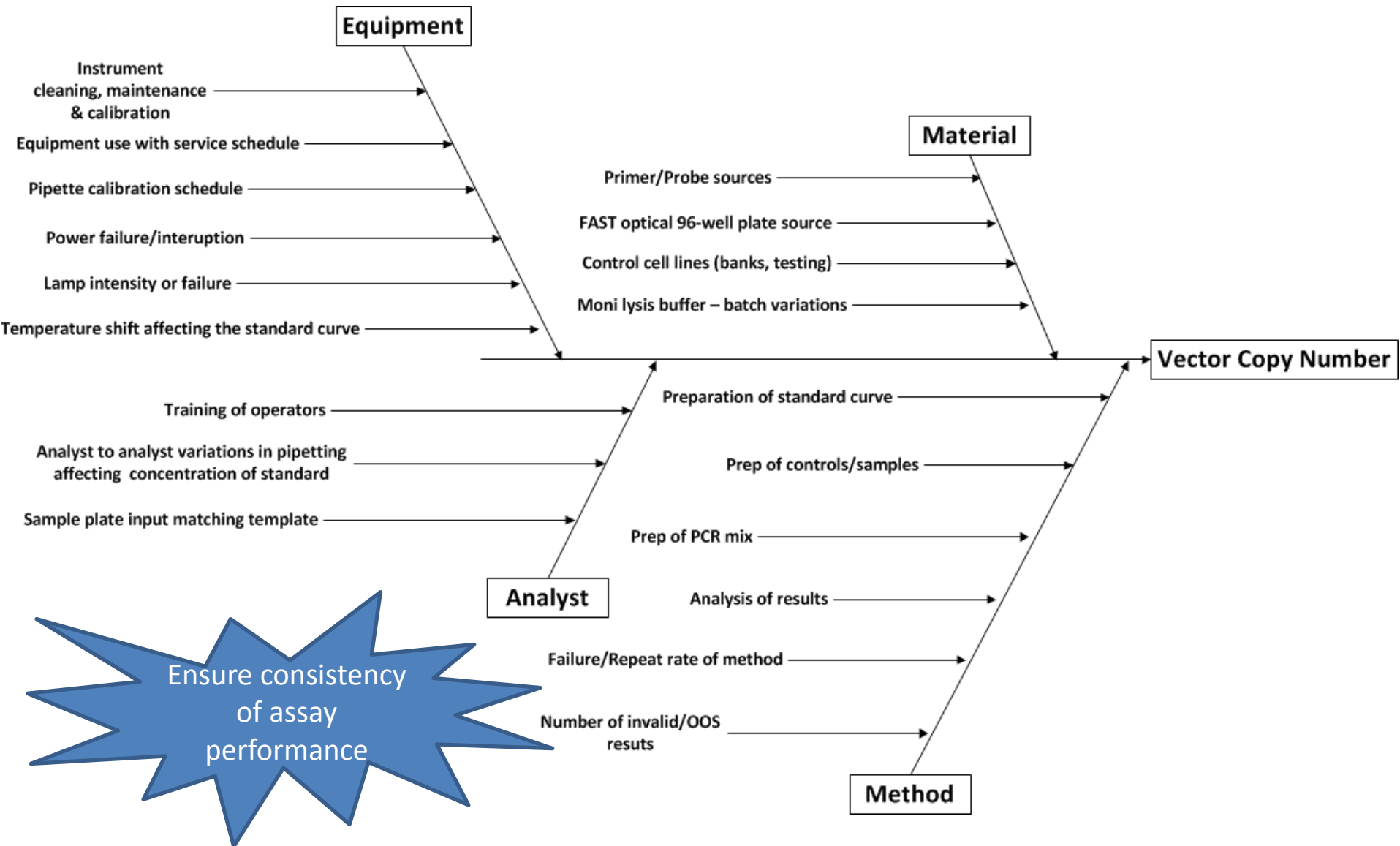
Cell Product CQAs	
Identity	Percent CD34+
	Vector copy number
Potency / Purity	CD34+ Stem Cell Potential
	Enzyme Activity
	Cell Viability (%)
	Transduction efficiency
	Vector copy number (VCN)
Safety	Endotoxin
	Mycoplasma
	Microbiological Control
	RCL
	Adventitious virus
Process Related Impurity	Host Cell Protein
	Plasmid DNA
	Host Cell DNA
	Residual infectious particles
	Residual cytokines

Vector CQAs	
Potency	Infectious viral titer
	Infectivity
Identity	Transgene sequence
	Vector Integrity
Purity	Vector infectivity
Process related impurity	Host Cell Protein
	BSA
	Host Cell DNA
	Benzonase
Safety	Microbiological Control
	mycoplasma
	endotoxin
	Adventitious virus
	Plasmid DNA
	RCL

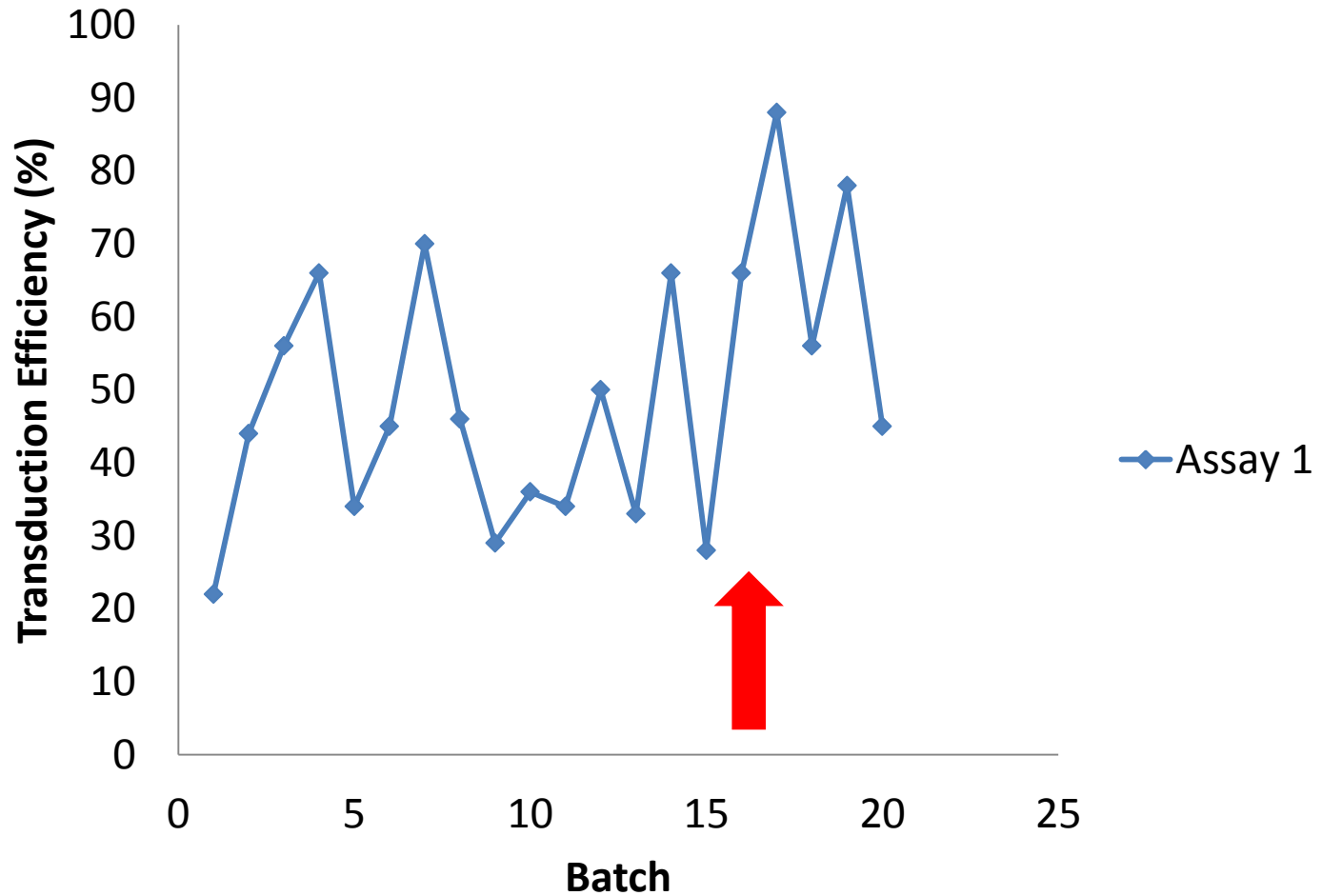
# Step 2: Identify Analytical Methods

Cell Product CQAs	Analytical Method
Percent CD34+	Flow cytometry
Vector copy number	qPCR assay
CD34+ Stem Cell Potential	Clonogenic capacity assay
Enzyme Activity	Enzyme assay using HPLC to detect activity
Cell Viability (%)	Trypan blue
Endotoxin	LAL
Mycoplasma	qPCR
Microbiological Control	BAC T Alert
HCP	ELISA

# Step 3: Assess Robustness of Analytical Method

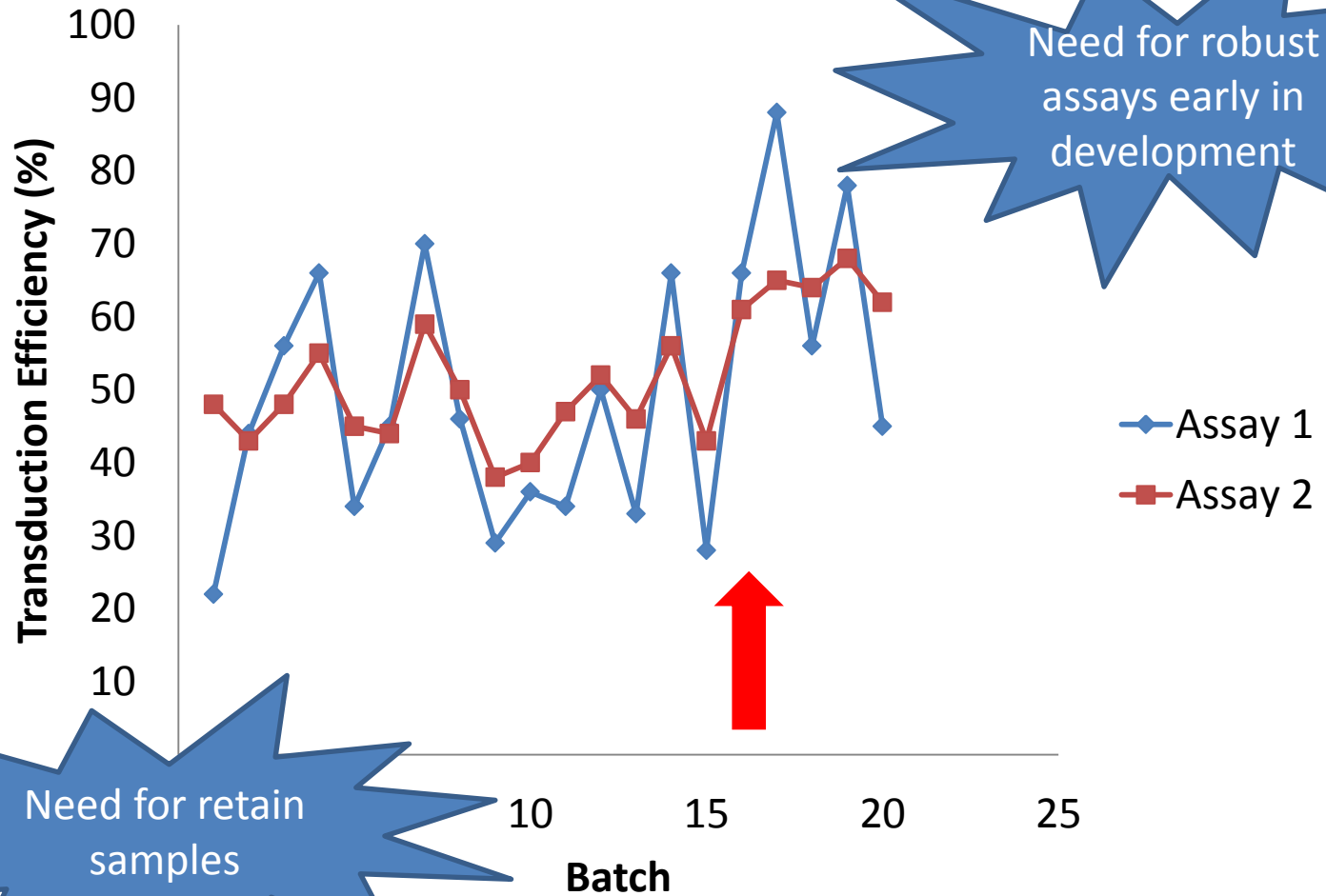


# Impact of Assay Robustness





# Impact of Assay Robustness



Need for retain samples

Need for robust assays early in development

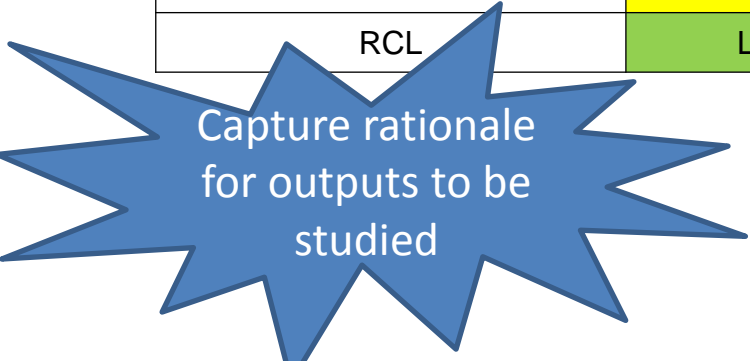
# Proposed Changes

Manufacturing Process Component		Process v 1.0	Proposed Process v 2.0	Rationale for Change
Vector Process Case 1	Cell expansion	Adherent	Suspension	<ul style="list-style-type: none"> <li>• Enable treatment of larger population of patients including some older patients</li> <li>• Improve supply chain robustness</li> </ul>
Cell Process Case 2	Cell manipulation	Manual production	Implementation of automation	
	Final product formulation	Fresh product with 4 hour shelf life	Cryopreserved product.	

# Assess Impact of Vector Process Change

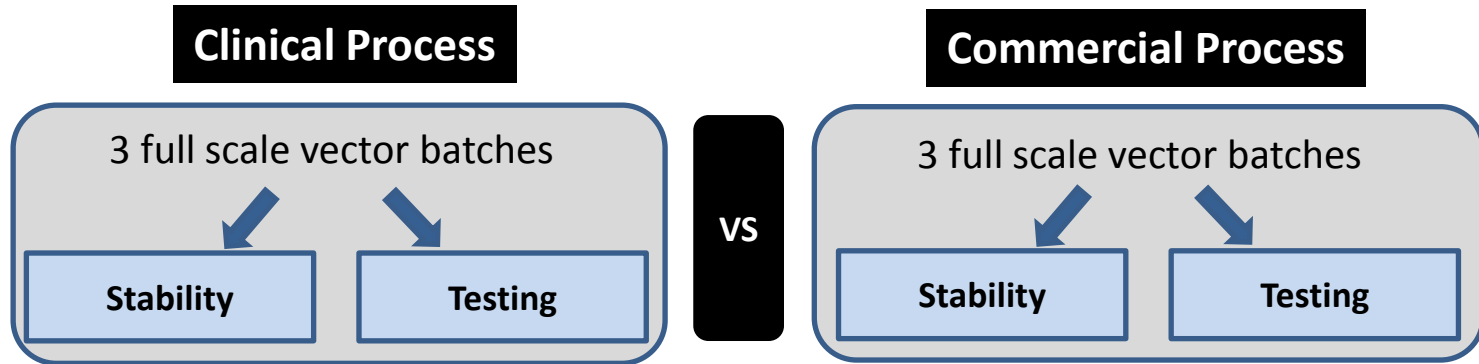
Vector CQAs	Potential Impact
Infectious viral titer	H
Infectivity	H
Transgene sequence	L
Vector Integrity	L
HCP	H
HC DNA	H
Benzonase	L
Microbiological Control	L
mycoplasma	L
endotoxin	L
Adventitious virus	L
Plasmid DNA	M
RCL	L

Cell Product CQAs	Potential Impact
Percent CD34+	L
Vector copy number	H
CD34+ Stem Cell Potential	L
Enzyme Activity	H
Cell Viability (%)	L
Transduction efficiency	H
Endotoxin	L
Mycoplasma	L
Microbiological Control	L
RCL	L
Adventitious virus	L
HCP	H
Plasmid DNA	M
Host Cell DNA	H
Residual cytokines	L

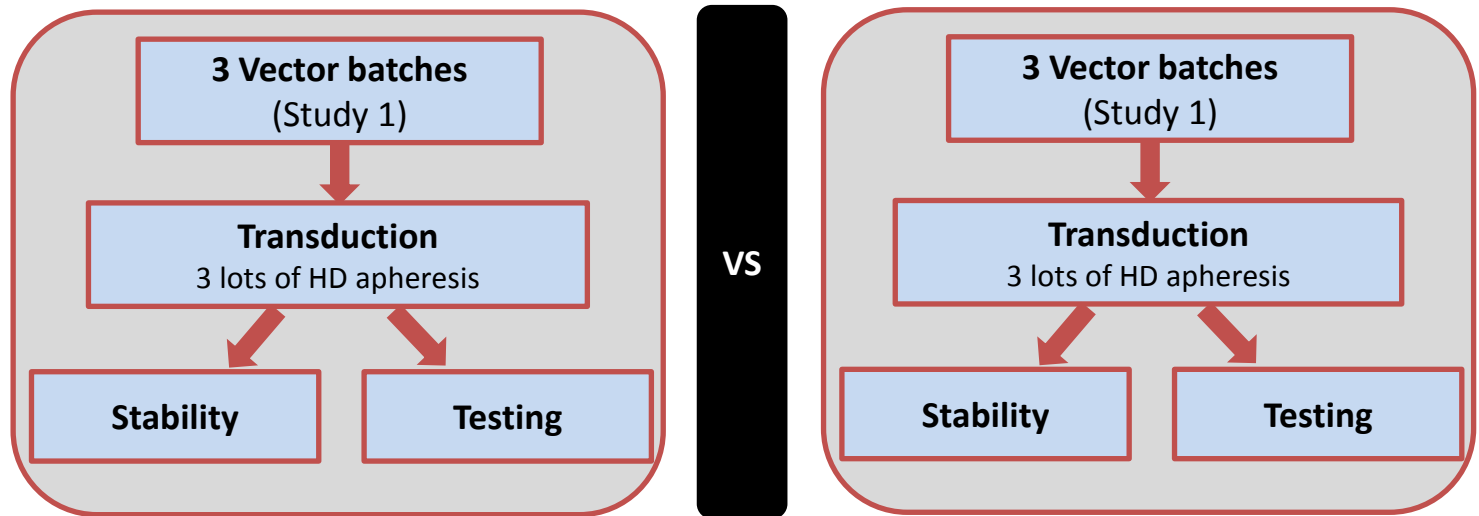


Capture rationale  
for outputs to be  
studied

# Comparability Study Design



## Vector characterisation based on impact assessment



## Cell characterisation based on impact assessment

# Define the Model System

- Use of healthy donor apheresis
- Prospectively assess impact of use of healthy donor material
  - No expected difference between healthy donor and patient material for safety or impurity attributes.

CQAs	Impact of HD as Surrogate for Patient Cells
Vector copy number	No expected difference
Transduction Efficiency	No expected difference
CD34+ Cell Growth	Lower growth rate in patient samples compared to HD cells
Cell viability	No expected difference
Percent CD34+	No expected difference
Clonogenic potential	Higher clonogenic potential expected in healthy donor
Sequence	No expected difference
Enzyme activity	Expected normal levels in healthy donor material compared to patient cells

# Proposed Changes

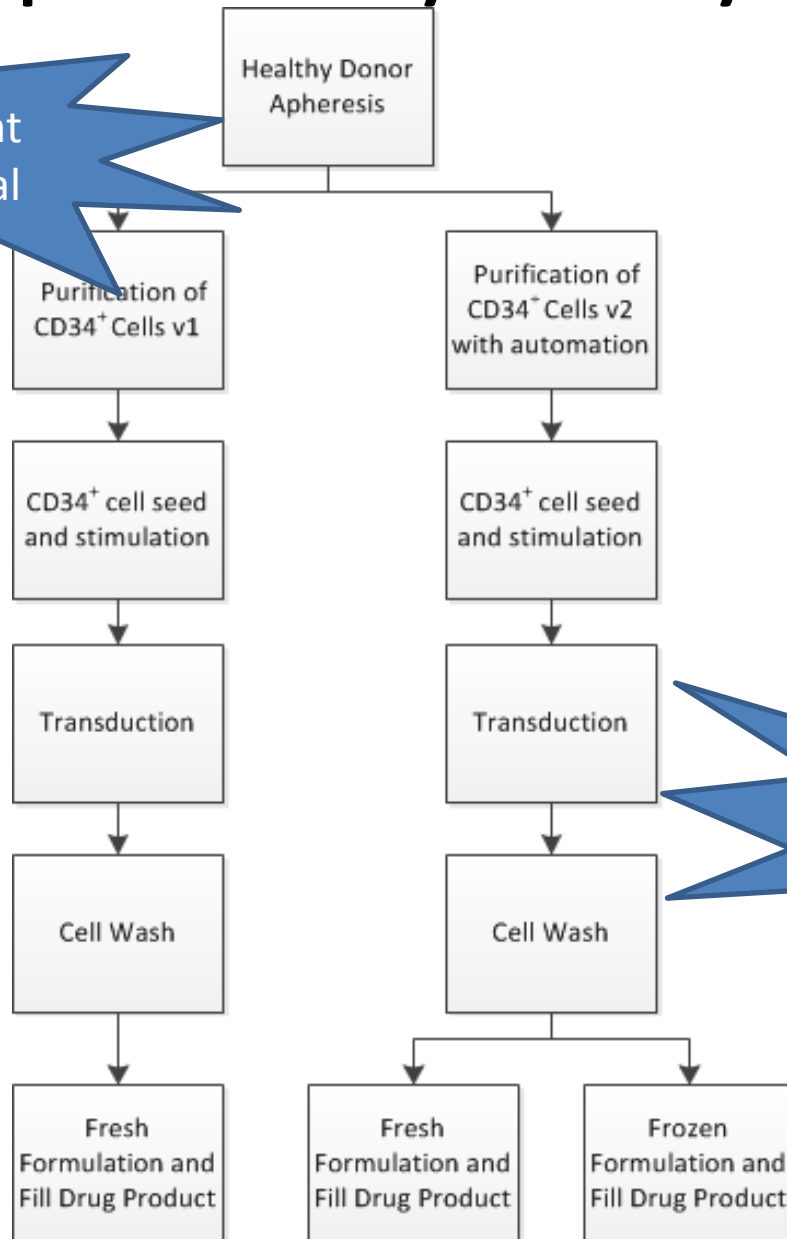
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Cell Process Case 2	Cell manipulation	Manual production	Implementation of automation	
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# Assess Impact of Cell Process Change

Cell Product CQAs	Potential Impact of automation	Potential Impact of cryopreservation
Percent CD34+	H	H
Vector copy number	H	M
CD34+ Stem Cell Potential	H	H
Enzyme Activity	H	M
Cell Viability (%)	M	H
Transduction efficiency	H	L
Endotoxin	L	L
Mycoplasma	L	L
Microbiological control	L	L
RCL	L	L
Adventitious virus	L	L
HCP	L	L
Plasmid DNA	L	L
Host Cell DNA	L	L
Residual cytokines	L	L

# Comparability Study Design

Ensure sufficient starting material



Resources required to run processes in parallel



# Discussion Points

1. The need for *in vivo* comparability studies
  - a) *In vitro* comparability studies, including product characterization, are a sufficient measure of potential product quality changes, as such, no further nonclinical testing is required prior to initiation of the new clinical trial
  - OR**
  - b) The *in vitro* potency tests should be supplemented with a further *in vivo* study to allow assessment of stem cell potential.
2. Need for additional analytical characterization methods.
3. Will cell product comparability always be required to support vector process changes?
4. How should *in vitro* comparability studies be designed when considering manufacturing site changes (e.g. sites in Europe and US)?
  - a) Split apheresis between two sites (logistical risks)
  - b) How to set acceptance criteria despite inherent variability of starting material?  
Use (sometimes limited) clinical and development data?
5. Need for analytical method control (reference standards)
6. Need for analytical method comparability (bridging studies) when assays are changed