# 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 $CD34^+$ 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

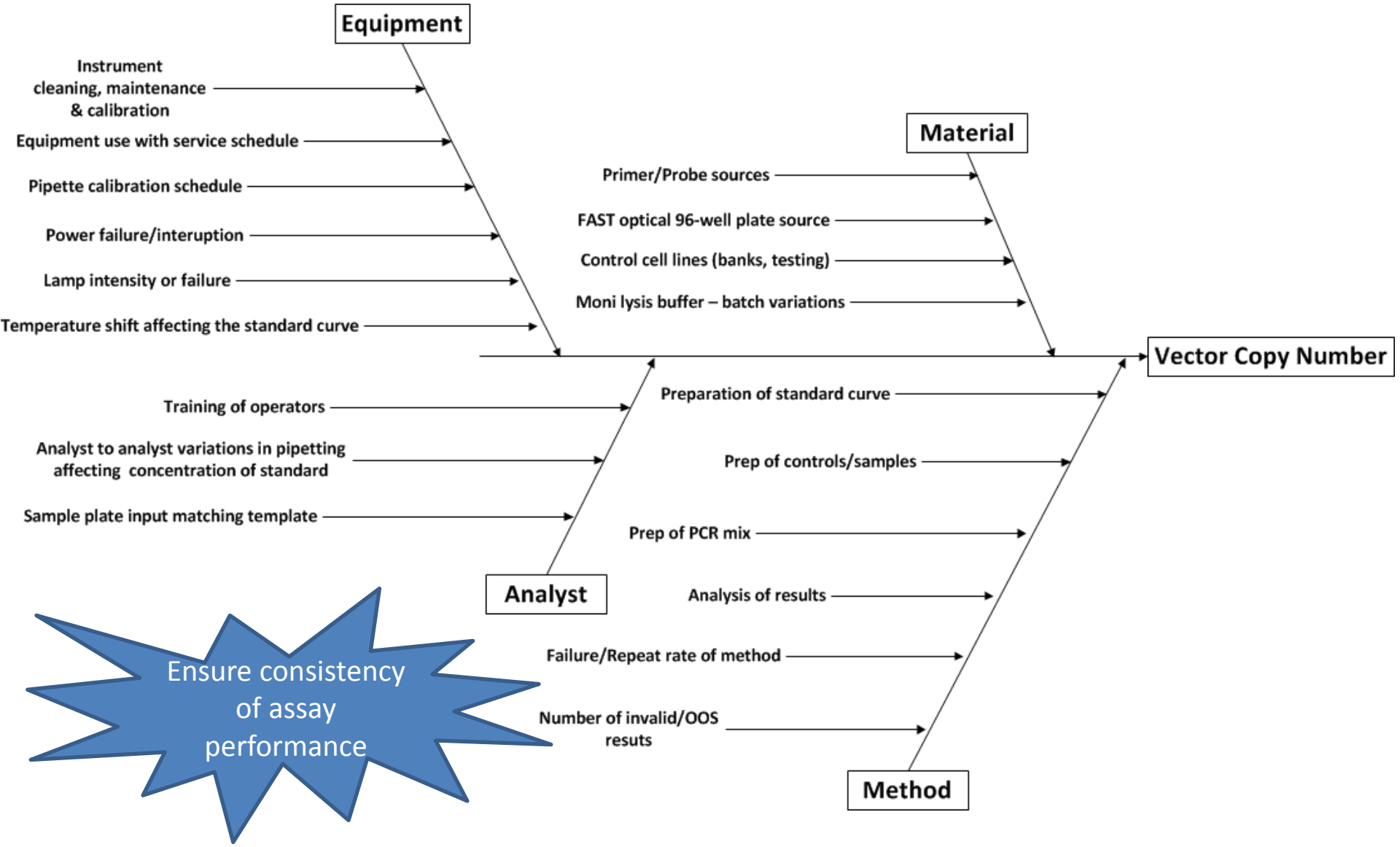
| Cell Product CQAs | |
|---|---|
| Identity | Percent CD34+ |
| | Vector copy number |
| Potency / Purity | CD34+ Stem Cell Potential |
| | Enzyme Activity |
| | Cell Viability (%) |
| | Transduction efficiency |
| | Vector copy number (VCN) |
| Safety | Endotoxin |
| | Mycoplasma |
| | Microbiological 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 |

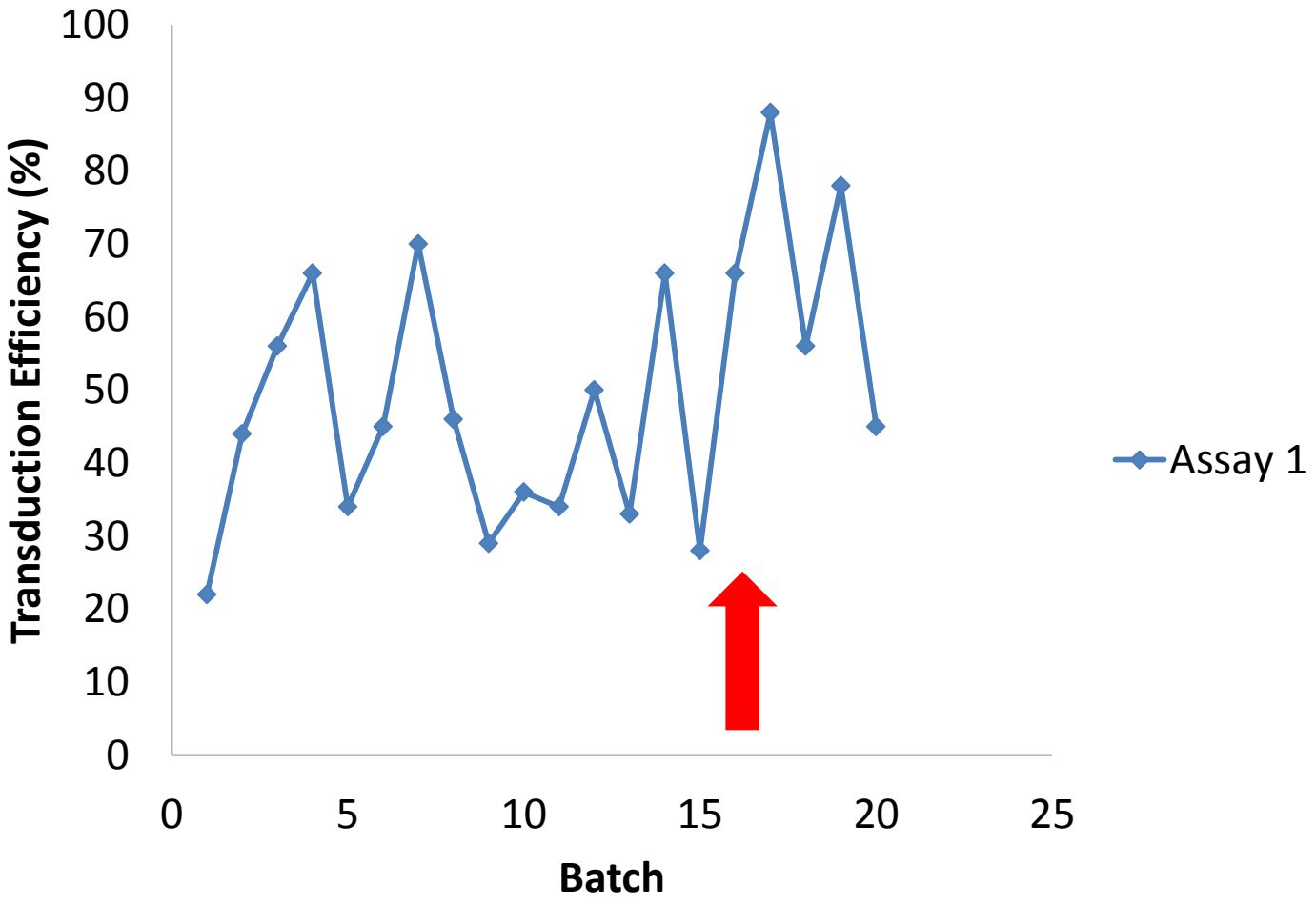
# Step 2: Identify Analytical Methods

| 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



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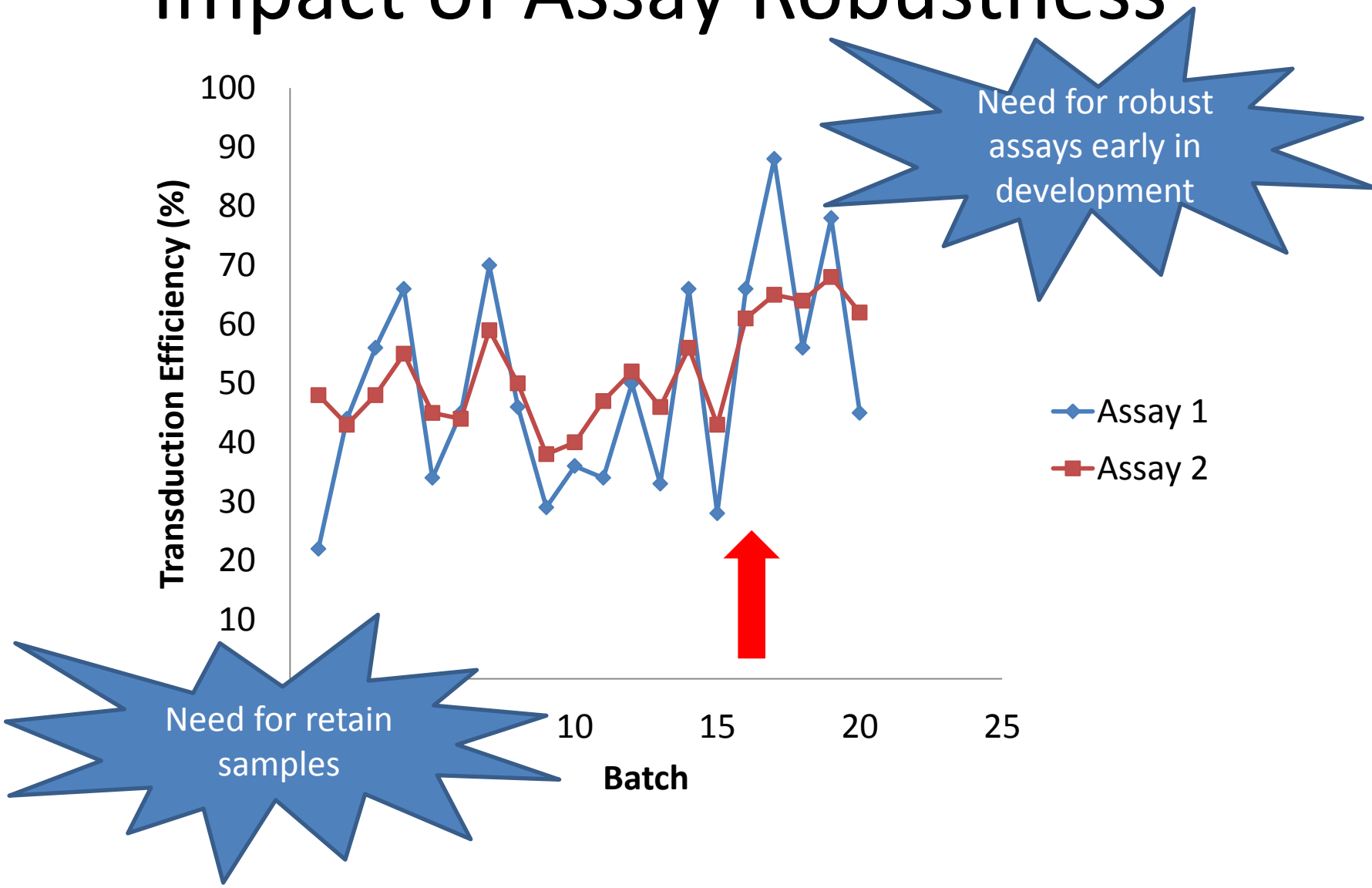


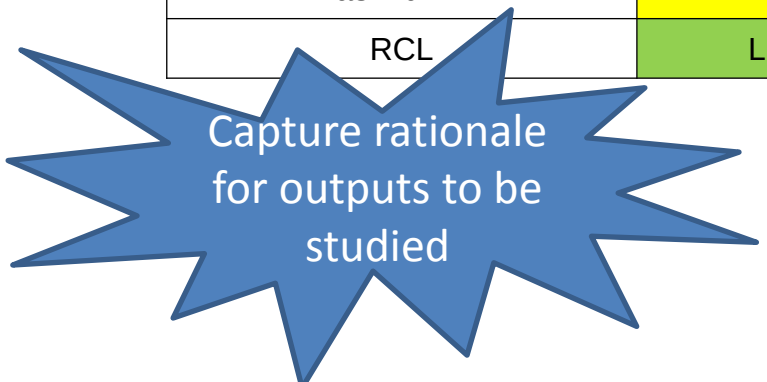
# Proposed Changes

| Manufacturing Process Component | | Process v 1.0 | Proposed Process v 2.0 | Rationale for Change |
|---|---|---|---|---|
| Vector Process Case 1 | Cell expansion | Adherent | Suspension | • Enable treatment of larger population of patients including some older patients<br>• Improve supply chain robustness |
| Cell Process Case 2 | Cell manipulation | Manual production | Implementation of automation | |
| | Final product formulation | Fresh product with 4 hour shelf life | Cryopreserved product. | |

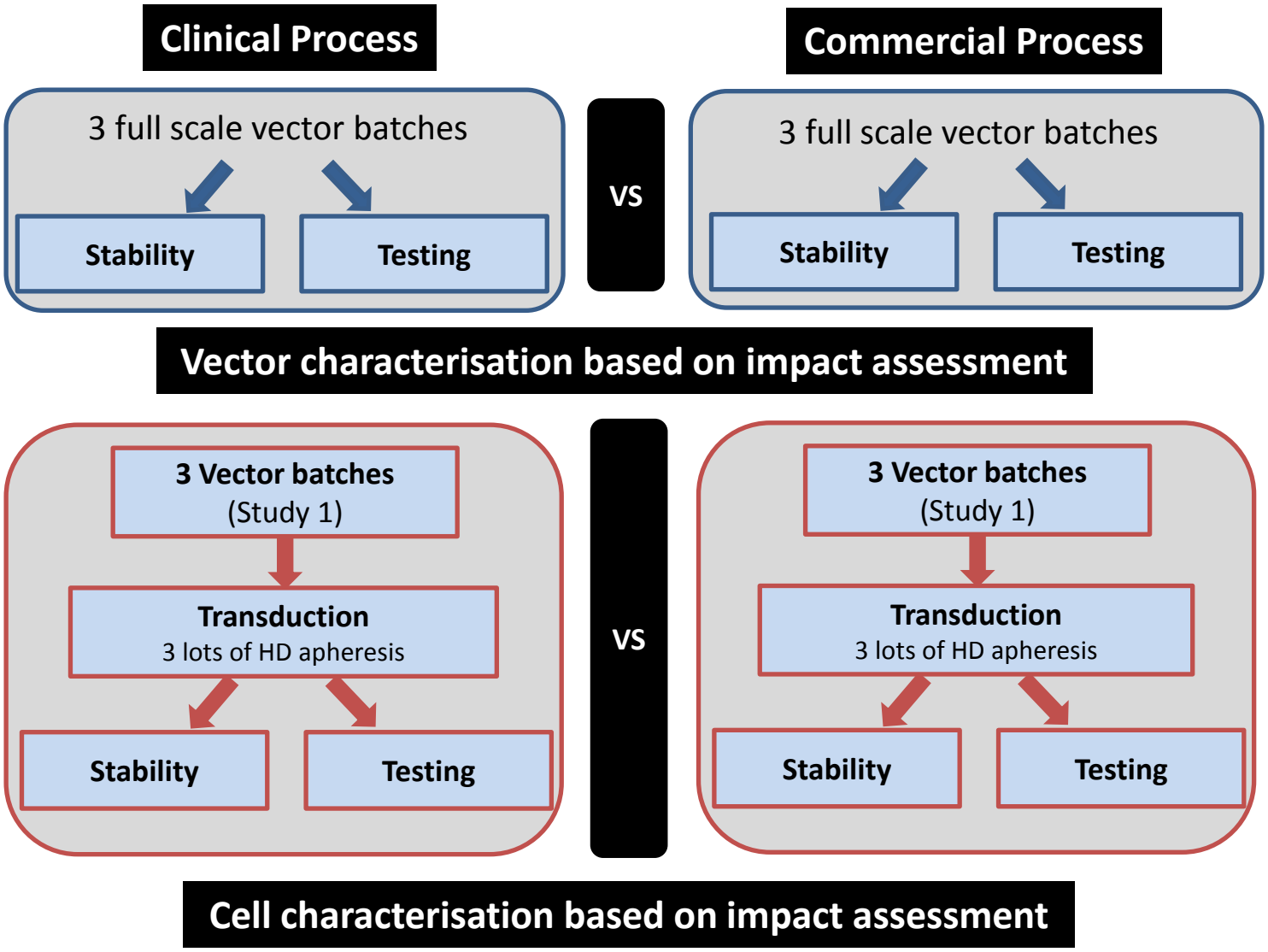
# Assess Impact of Vector Process Change

| 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 |



# Comparability Study Design



# 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 |

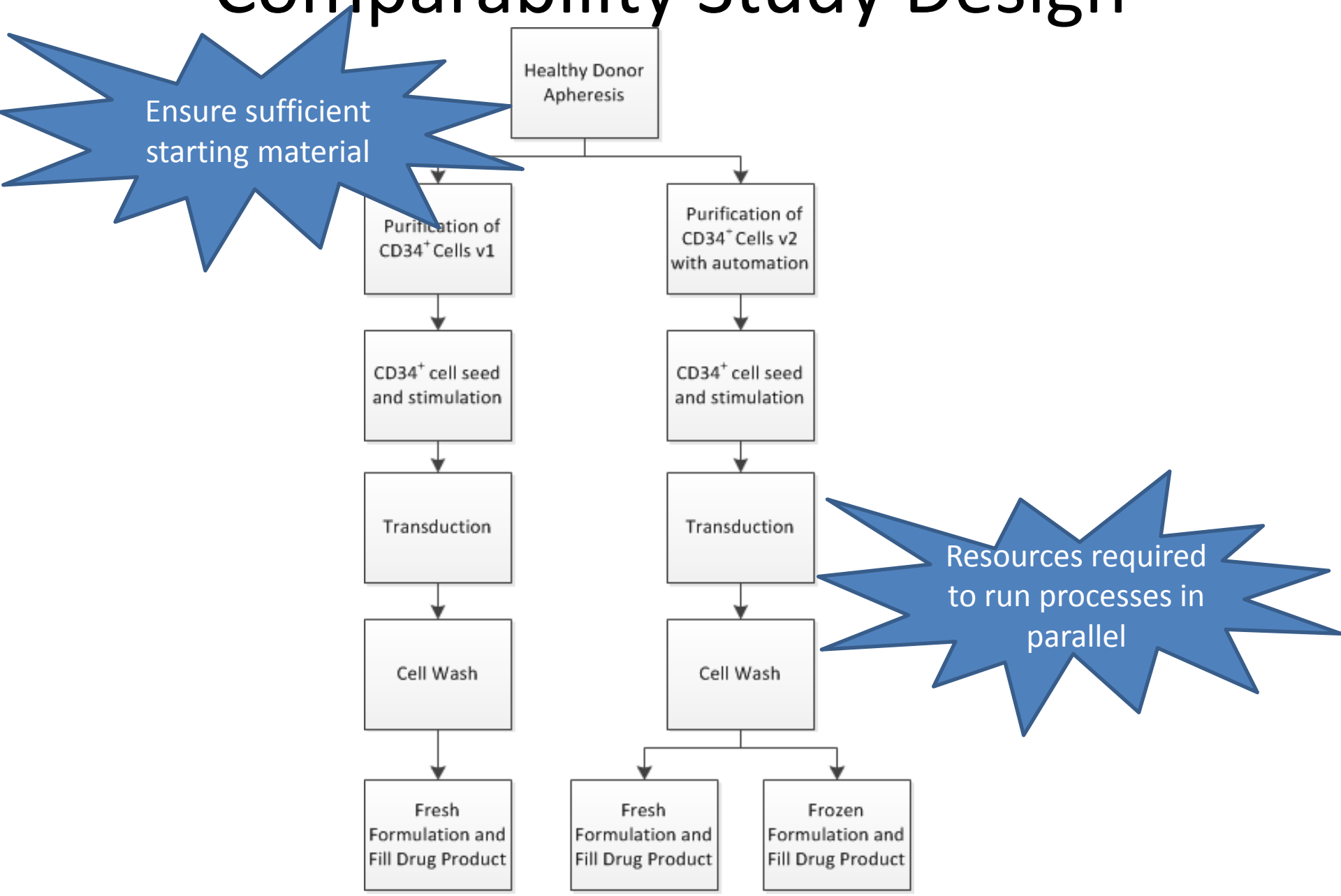
# Proposed Changes

| Manufacturing Process Component | | Process v 1.0 | Proposed Process v 2.0 | Rationale for Change |
|---|---|---|---|---|
| Vector Process Case 1 | Cell expansion | Adherent | Suspension | • Enable treatment of larger population of patients including some older patients<br>• Improve supply chain robustness |
| 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 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



# 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