Outcomes in Mesenchymal Stem Cell Manufacturing

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Background

• HCTL established in 1992 to support BMT programs of Mayo Clinic and Wolfson Children’s Hospital

• HCTL began receiving support from the Mayo Clinic Center for Regenerative Medicine in 2012 for developing investigational cellular therapies to support INDs
Background

• Products developed thus far:
  • Allogeneic bone marrow-derived mesenchymal stem cells (MSC)
  • Autologous bone marrow aspirate concentrate
  • Autologous adipose stromal vascular fraction cells
Background

• Products in development:
  • Allogeneic adipose-derived mesenchymal stem cells
  • Autologous adipose-derived mesenchymal stem cells
  • Mesenchymal stem cell-derived extracellular vesicles
Outcome measures of IND products

- Guidance regarding acceptable outcomes for drug or cell manufacturing originates from regulatory agencies (FDA) and accreditation agencies (FACT)
  - FACT Common Standards D4.7.1: Criteria for cellular therapy product safety, product efficacy and/or clinical outcome, shall be determined and shall be reviewed at regular time intervals
- Each product may have a different set of outcome measures based on the unique characteristics of each product
- Focus on manufacturing outcomes of allogeneic BM-derived MSC
Critical Quality Attributes

• FDA defines CQA as “a physical, chemical, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.”¹

• FDA expects manufacturers to “identify critical parameters in the manufacturing process and critical product attributes to ensure the desired clinical effect of the final product.”²

¹ US FDA. Guidance for Industry: Q8(R2) Pharmaceutical Development.
² US FDA. Guidance for FDA Reviewers and Sponsors: Content and Review of Chemistry, Manufacturing and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)
Critical Quality Attributes

- Quality outcome parameters important for cellular manufacturing of clinical grade therapeutic products:
  - Safety
  - Identity
  - Purity
  - Potency
Critical Quality Attributes

• CQAs are primarily concerned with the safety of the product, and secondly, with thorough characterization of the product

• Critical to establish a comprehensive understanding of the components of the product to be delivered, as well as how the characteristics of the product relate to the intended therapeutic application
Safety

• Sterility
  • Bacterial and fungal cultures
  • Mycoplasma testing
  • Adventitious viral agent assays

• Karyotype
  • Absence of chromosomal abnormalities due to *ex vivo* culture

• Tumorigenicity
  • Absence of tumorigenic transformation due to *ex vivo* culture

• Also, preclinical animal studies establish safety
Identity

• Surface markers
  • Flow cytometric analysis of defined surface marker profiles most common identity assay

• HLA testing
  • Distinguish individual products from others manufactured in the same facility
  • Detection of cross-contamination of cell products
Identity

• Other possible identity assays:
  • mRNA profiling
    • cellular identity based on gene expression
  • Other genetic profiling
    • miRNA profiling, epigenetic profiling

• Genetic profiling assays can help detect phenotypic (i.e. identity) alterations that may occur as a result of manufacturing processes
Purity

• Endotoxin
• Residual reagents
  • e.g. if using FBS, residual BSA assay must be performed
• Must fully characterize what is in the product and how pure it is
  • e.g. if there are multiple cell types present, must provide some indication of what cell types and in what ratios/percentages
Potency

• Quantitative measure of the desired biological activity with regard to the intended clinical indication
• Correlate as closely as possible with the presumed mechanism of action
• Can be the most challenging assays to develop and may or may not be a direct measure of *in vivo* efficacy
Potency

- Cytokine secretion
  - e.g. anti-inflammatory cytokines, angiogenic cytokines, neuroprotective cytokines, etc.

- Immunomodulation assays
  - MSC play a role in dampening and/or modulating immune responses
  - Useful assay for indications such as organ rejection (BOS/CLAD), chronic inflammation (Crohn’s disease), autoimmune disorders (Type 1 diabetes), GVHD
Potency assays are designed with clinical outcomes in mind

- Treatment of Chronic Lung Allograft Dysfunction/Bronchiolitis Obliterans Syndrome with MSC
  - Clinical outcome measures: Forced expiratory volume (FEV), O₂ saturation (SaO₂), fraction of inspired oxygen (FiO₂), dyspnea index
  - MSC immunomodulatory functions hypothesized to improve clinical outcomes by moderating organ rejection processes
  - Potency assayed designed to test MSC product’s ability to suppress T cell activation and proliferation
Potency assays are designed with clinical outcomes in mind

- Treatment of recent Intracerebral Hemorrhage using MSC
  - Clinical outcome measures: neurological function tests, CT/MRI
  - MSC anti-inflammatory and neuroprotective functions hypothesized to improve clinical outcomes by reducing tissue damage and encouraging neuronal recovery
  - Potency assayed designed to measure ability of MSC product to secrete anti-inflammatory cytokines and factors shown to play a role in neuro-recovery in preclinical *in vitro* studies
Other important outcome measures

• Cell number
  • Need to generate large numbers of MSC while maintaining acceptable product attributes

• Cell viability
  • Manufacturing processes must be engineered to maximize viability
  • Related to product purity and potency
  • *FACT Common Standards D4.14: The QM Plan shall include SOPs for validation and/or verification of critical procedures to achieve expected end-points, including viability of the cells and cellular therapy product characteristics.*
Initial assay development strategy for measuring outcomes

• Identify multiple candidate assay targets (e.g. for potency or identity).
• Generate end product from many donors.
• Identify trends in the data. Determine which assays are the most reliable and reproducible batch-to-batch across.

  • FACT Common Standards D4.7.2: Both individual cellular therapy product data and aggregate data for each type of cellular therapy product shall be evaluated.
Process improvement in cellular manufacturing

- Comparability testing is key when investigating or implementing any potential process changes or improvements

- Must monitor how process changes affect CQA parameters
  - FACT Common Standards D4.14.3: Changes to a process shall include evaluation of risk to confirm that they do not create an adverse impact anywhere in the operation and shall be validated or verified as appropriate
Process improvement examples

• Safety
  • Improving risk of contamination
    • Transitioning from open to closed, or functionally closed, system protocol
    • Animal product elimination
      • Reduction in Mycoplasma and adventitious agent risks
      • Also associated with allergic reactions
Process improvement examples

• Purity
  • Improving risk of endotoxin contamination
    • Transitioning from open to closed, or functionally closed, system protocol
  • Improving residual reagent contamination
    • Animal product elimination
• Purification methods
  • If working with heterogenous cell populations, cell selection approaches could be considered
Process improvement examples

• Potency
  • Improving cellular potency
    • Cellular activation or stimulation
    • Directed differentiation
  • Potency assay improvement
    • Good practice to conduct frequent literature searches to identity different expressed genes or secreted proteins that can be assayed that may be a more valuable parameter to measure for a specific therapeutic indication
Process improvement examples

• Cell yields
  • Scalability of processing method a huge consideration for any manufacturing program
  • Introducing automated cell expansion methods may be necessary
  • Any improvements in manufacturing method will require comparability testing to ensure acceptable outcome measures are maintained
Process quality engineering approach to development and improvement

• Certain process inputs in cellular manufacturing contribute to significant variability and unpredictability
  • Donor-to-donor variability from the source material
  • Lot-to-lot variability of components such as serum or platelet lysate
Donor variability based on source material
Process quality engineering approach to development and improvement

• Design factorial-type screening experiments to test different combinations of process input parameters to determine which combinations result in positive or negative effects on process outcomes

Process quality engineering approach to development and improvement

• Identify process inputs that could affect manufacturing outcomes
  • Donor demographics
  • Mononuclear cell isolation methods
  • Cell seeding density
  • Medium type
  • Supplement concentration
  • Supplement vendors
  • Medium change schedule
  • Passage confluence
Process quality engineering approach to development and improvement

• Individual input parameters affect process outputs
  • Higher seeding densities resulted in lower proportions of MSC expressing STRO-1

• Multiple factors interact to produce different outcomes when paired together
  • Seeding cells into larger volumes of medium resulted in more STRO-1+ cells if serum concentration was low, but fewer STRO-1+ cells if serum concentration was high
Process quality engineering approach to development and improvement

- Need to repeat these factorial experiments using a large number of donors to determine the most reliable methods that will produce the best outcomes for the majority of donor samples
- Large amount of statistical analysis required
- Could demand large amount of resources
  - Time, development funds, staff, etc.
- Absolutely necessary before settling into a process on track for commercialization, or even Phase III trial
Process quality engineering approach to development and improvement

• For early stage process development, process parameters can be decided based on smaller pilot studies, established industry standards, or literature

• Efforts can be focused mainly on determining effective processes for attaining desired identity and potency outcomes
Quality by Design overview for process development and outcome monitoring

Thank you!