


Developing Cell Therapeutics for Lung Diseases


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WAISMAN BIOMANUFACTURING

PACT Production Assistance for Cellular Therapies
National Heart Lung and Blood Program

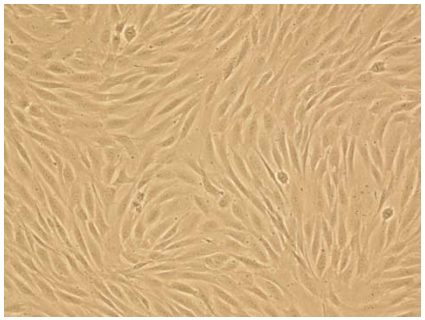
THE UNIVERSITY of WISCONSIN MADISON

Production Assistance for Cellular Therapies

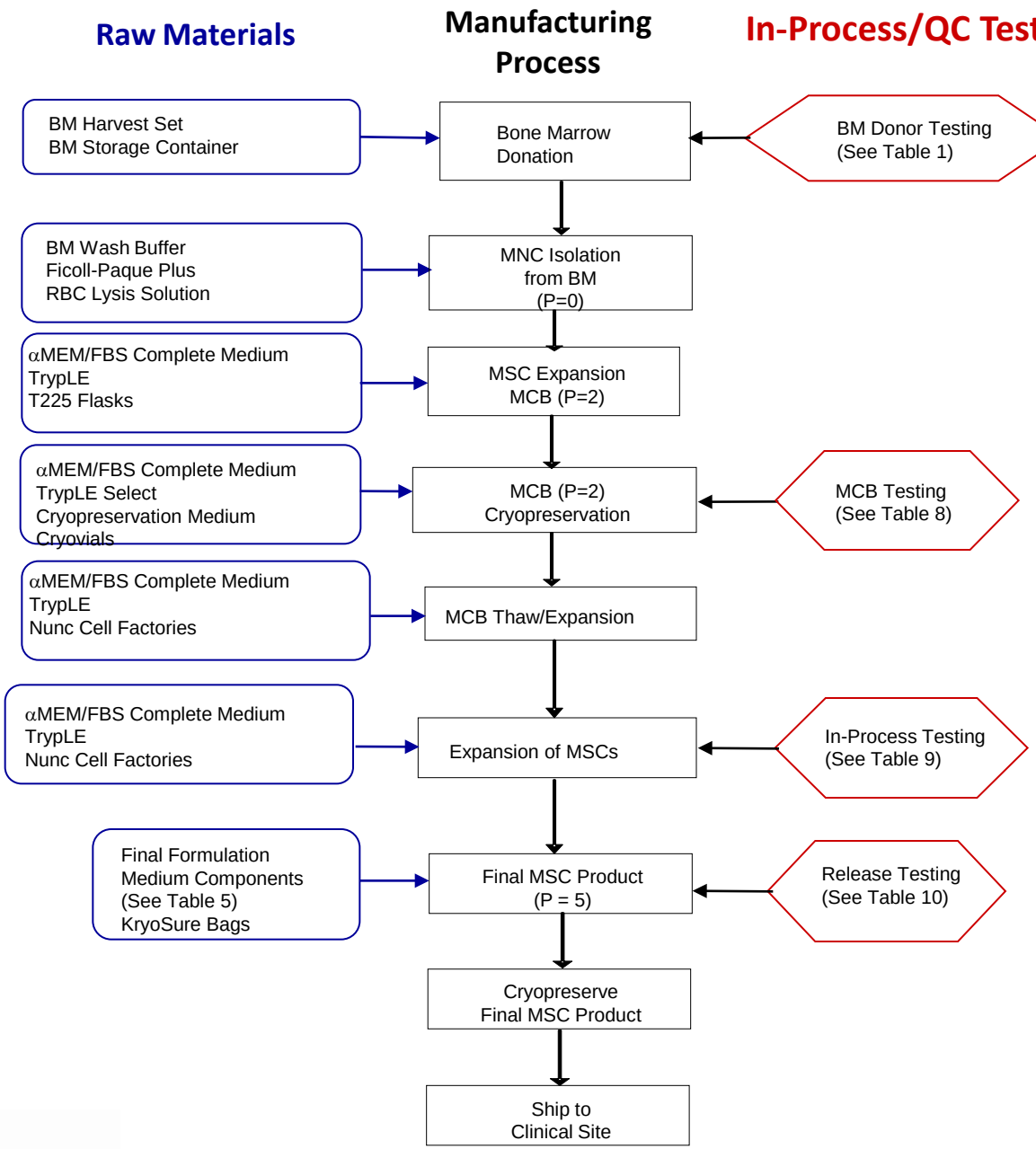
- UW PACT team services
  - Technology transfer, process/assay development
  - Cell production for pre-clinical testing and clinical trials
  - Support for pre-clinical animal studies
  - Regulatory Affairs support through IND filing

- Mesenchymal Stromal Cell projects
  - Abba Zubair (Mayo) – Bronchiolitis obliterans in lung transplant
  - Amish Raval – Preclinical animal studies for Acute Myocardial Infarction
  - Susan Thibeault – MSCs in biocompatible gel for vocal fold injuries
- Pluripotent Stem Cell projects
  - Human Embryonic Stem Cells – Master Cell Banks
  - Induced Pluripotent Stem Cells (non-PACT)

Challenges in Cell Therapy Development



- cGMP compliance issues -
  - Donor eligibility requirements – 21 CFR 1271
  - Development of manufacturing batch records
  - Development of Quality Control test methods
  - Validation of aseptic processing for full manufacturing process
- Manufacturing process challenges –
  - Variability in starting material – donor variability
  - Variability in raw materials (e.g., FBS)
  - Scalability of manufacturing process
- Quality Control challenges -
  - Appropriate potency assay may be challenging to identify
  - **Cell therapeutics are extremely complex - the product is the process**



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Summary of QC Testing for MSC MCB and Final Product

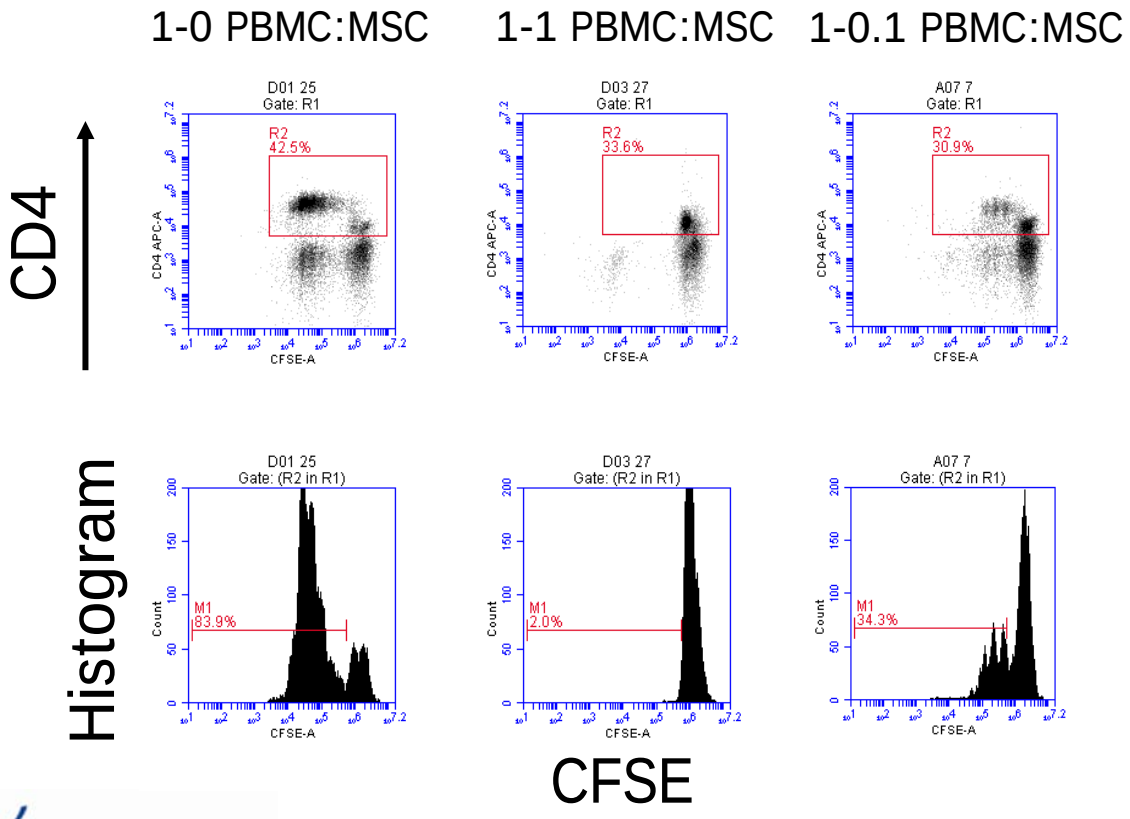
Test	Test Method	Specification	MCB	FP
Cell Count	Viable cell count	2-6 x 10^6/mL > 70% Viable	X	X
Identity	HLA or STR Testing	Report Profile	X	X
Sterility	Direct transfer method, 21 CFR 610.12	No contamination detected	X	X
Mycoplasma	PTC method – direct culture and Vero culture with Hoechst stain	No contamination detected	X	X
Karyotype	G-band analysis 20 metaphase spreads	Normal	X	X
Flow Cytometry	Positive: CD105, CD73, CD90 Negative: CD34, CD45, CD14, CD19, HLA-DR	Positive > 95% Negative < 5%	X	X
Adventitious Agents	*In Vitro* Adventitious Agent Testing – MRC-5, Vero, HeLa cells	No contamination detected	X	
Endotoxin	Kinetic Turbidometric LAL assay	< 2.5 EU/mL		X
Residual FBS	BSA ELISA assay	Report Value		X
Potency	**T-Cell Proliferation Assay**	TBD		X
Potency	**Cytokine/paracrine factor expression assay**	TBD		X

Establishing a Potency Assay

- Development timeline
  - Establish early in program based on hypothesized mode of action
  - Correlate assay data with *in vivo* performance
  - Goal of establishing assay and validating by Phase 3 clinical trials
- Reference Standard
  - Create reference standard prior to initiating assay development
  - Use to establish assay characteristics- accuracy, precision…
  - Monitor stability of standard
- Process development support
  - Impact of donor variability on product quality
  - Screening of raw materials (e.g., FBS)
  - Evaluation of process changes and scale-up on product quality

PACT Immunopotency Assay
Inhibition of CD4+ T cell Proliferation

- CFSE-label PBMCs to track proliferation
- MSCs added at titrated ratios
- anti-CD3 and anti-CD28 used in 4 day T cell stimulation
- Flow cytometry using anti-CD4 APC



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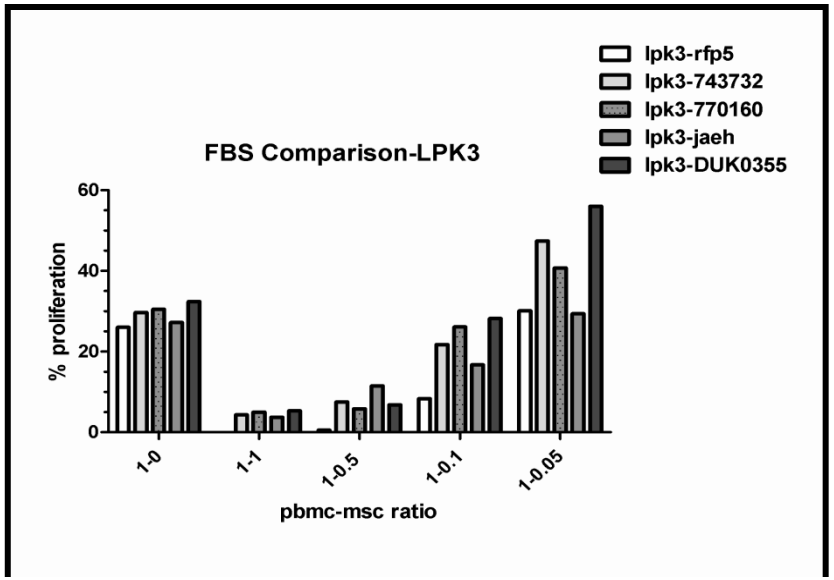
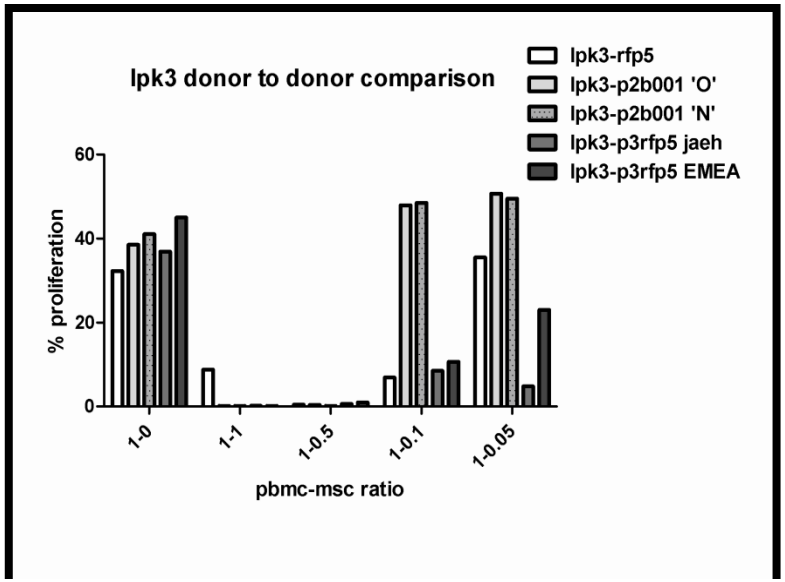
Application of the Potency Assay





Impact of process parameters on MSC performance -

- FBS vs. serum-free media formulations
- Effect of donor variability
- Selection of FBS lots for MSC production
- Impact of media components - growth factors
- Impact of passage number
- Impact of crysopreservation and shipping/thaw
- Comparison of MSCs produced at PACT facilities

Moving Through Clinical Trials - Future Challenges

- Continued development of potency assays
  - Look at additional markers for MSC potency
  - Develop indication-specific assays
  - Establish relationship with *in vivo* performance
- Scale-up of MSC manufacturing process
  - Cell Factories vs. HYPER Stacks vs. novel bioreactors
  - Cell harvest methods
  - Media - identify alternative formulations to enhance cell production and process reproducibility
- Validation studies
  - Process validation studies
  - QC method validation – complete by Phase 3
  - Product shipping and stability studies

Preclinical Animal Study Challenges

- Selection of appropriate animal model – discuss with the FDA before initiating studies
- Cell product should be comparable to cells to be used in human clinical trials – same cell bank, manufacturing process…
- Cell formulation and storage conditions should be comparable to those proposed for human trials
  - Establish formulation and packaging for cell product
  - Perform shipping studies with cell processing at clinical site
  - Perform stability studies under proposed clinical use conditions (thaw, hold time)
- Cell administration should mimic human dosing –
  - IV infusion over extended period
  - Cell settling during administration

Waisman Biomanufacturing and PACT Teams

Waisman Biomanufacturing

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