Towards Improved Characterization of MSCs

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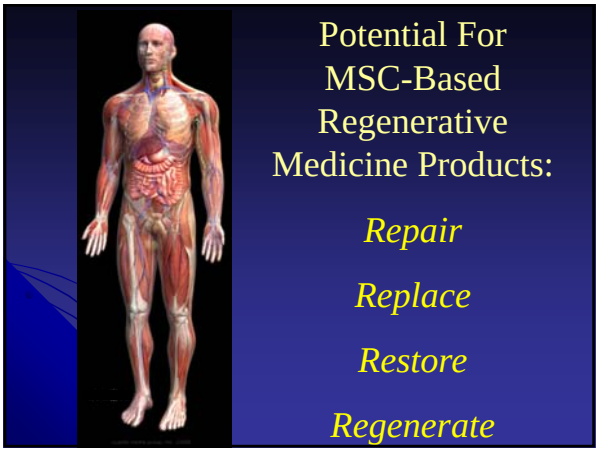


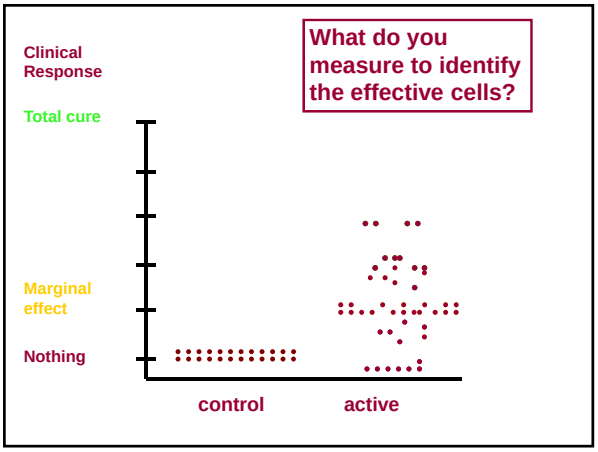
Potential For MSC-Based Regenerative Medicine Products:

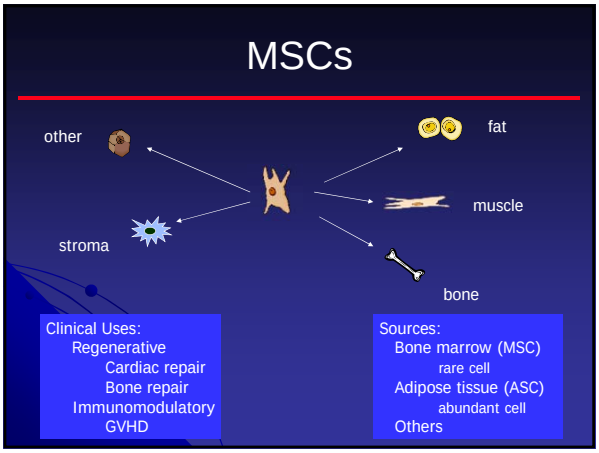
Repair

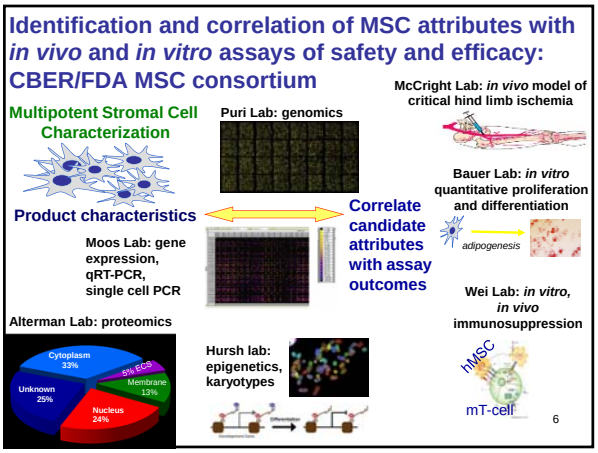
Replace

Restore

Regenerate







MSC Manufacturing

Goal: 8 donors
- Expand to ~100 T175 flasks
- Change media every third day
- Expand to ~ 80% confluence
- Harvest at p3, p5, p7
- Cryopreserve, snap freeze, or initiate analytical procedures

Yield ~ 2-3 x 10^8 cells per passage

Jess Lo Surdo
Eva Rudikoff

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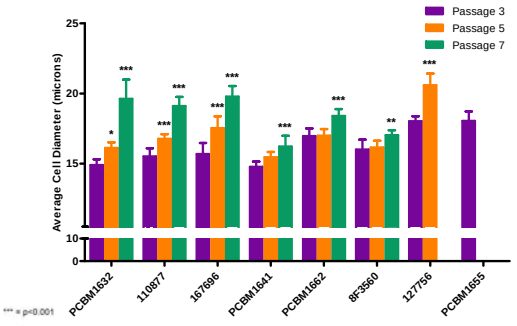
Cell Size Increases with Tissue Culture Passage: Donor Differences



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MSC Production

- Publicly available sources
- Single method, single lab, uniform media
  - One lot of FCS
- Sufficient MSCs for multiple characterization approaches

Cell Manufacturing Information

Designation*	Sex*	Age*	P3	P4	P5	P7	Cryopreserved	Comments
PCBM 1641	F	23	124	169	116	93	502 x 10e6	P3, P5, P7
PCBM1632	M	24	178		171	48	397 x 10e6	P3, P5, P7
PCBM 1662	F	31	244		156	199	599 x 10e6	P3, P5, P7
110877	M	22	194		251	106	551 x 10e6	P3, P5, P7
8F3560	F	24	195		169	183	547 x 10e6	P3, P5, P7
167696	F	22	208		121	166	495 x 10e6	P3, P5, P7
127756	M	43	87		49		136 x 10e6	P3, P5
PCBM 1655	F	47	35				35 x 10e6	P3

** Information supplied by cell line source*

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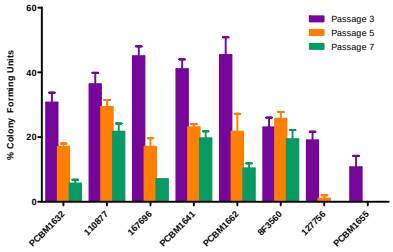
Source: ALLCELLS, LONZA

CFU-F Activity Decreases with Tissue Culture Passage: Donor Differences



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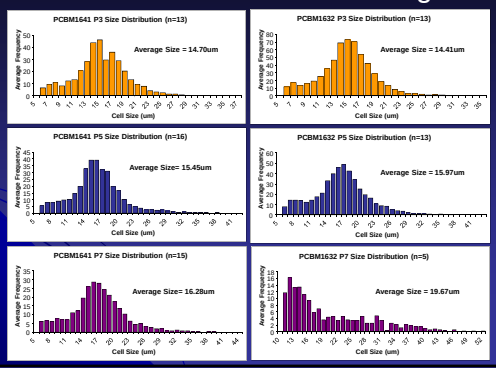
Cell Size Distribution Patterns Changes with Tissue Culture Passage



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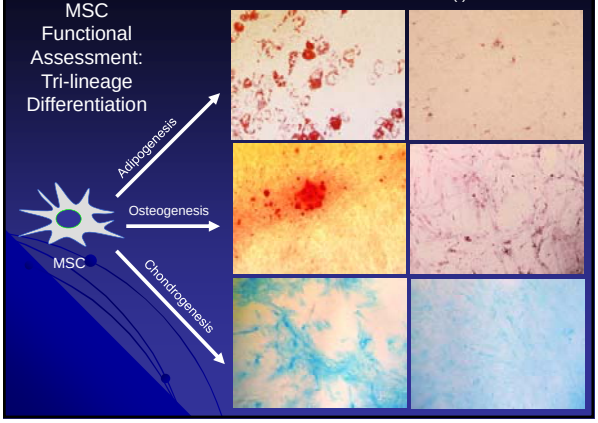
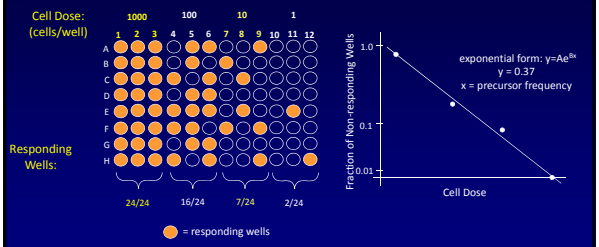
MSC Functional Assessment: Tri-lineage Differentiation

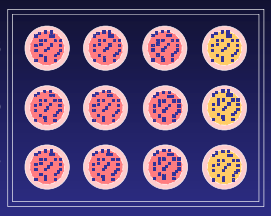


Limiting Dilution Assay



Lo Surdo, JL, and Bauer, SR. Quantitative Approaches to Detect Donor and Passage Differences in Adipogenic Potential and Clonogenicity in Human Bone Marrow-Derived Mesenchymal Stem Cells. 2012. Tissue Engineering: Part C 18: 877-889.

Automated Microscopy For Adipogenesis



= Adipogenic stimulation

= Undifferentiated control

- To exclude edge effects, outer 15% of well was not sampled
- 25 images per well
- Nile Red positive cells associated with nuclei counted and normalized to total nuclei count per image
- n=3/Donor/passage
- Imaging ~4% of specified area at 10X

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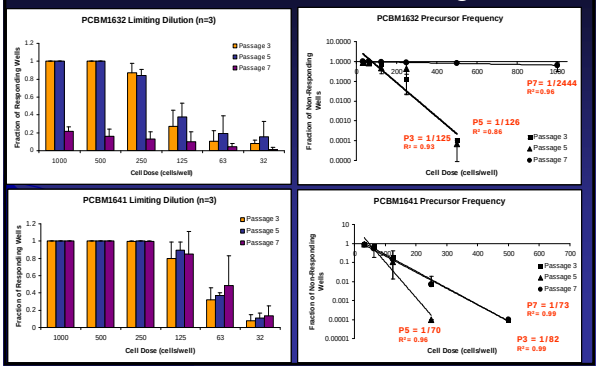
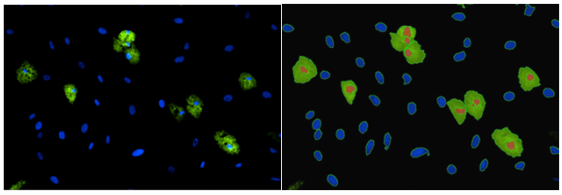
Adipogenic Precursors Can Change with Tissue Culture Passage



Automated Microscopy for Adipogenesis

Image

Processed for Counting



DAPI (DNA)

Nile Red (intracellular fat)

Nuclei/ Fat /Nuclei with Fat

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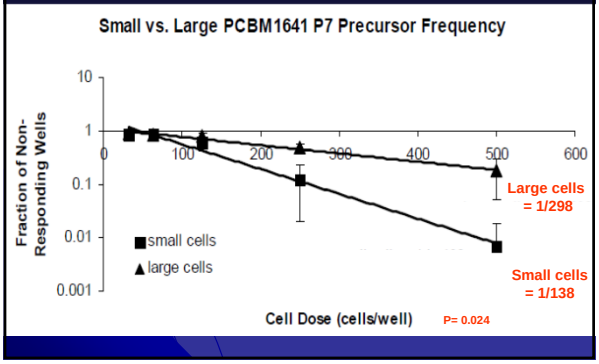
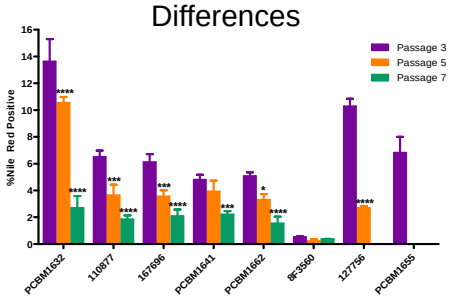
Adipogenic Precursors are Associated with Smaller MSCs



Adipogenic Activity Decreases with Tissue Culture Passage: Donor Differences



*P<0.05
**P<0.01
***P<0.001
****P<0.0001
Relative to P3

Lo Surdo JL et al., *Cytotherapy, 2013. In Press.*

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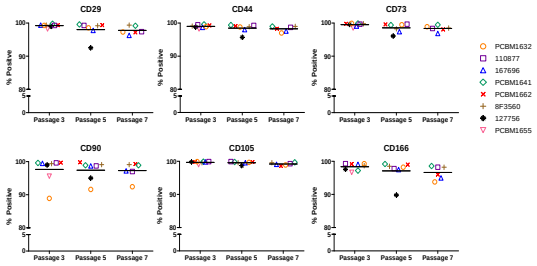
Consensus MSC Markers do not Correlate with Functional Heterogeneity, Donor or Tissue Culture Age Differences



International Society for Cell Therapy: Cytotherapy (2006) Vol. 8, No. 4, 315-317. MSCs are characterized as being adherent to plastic, capable of tri-lineage differentiation, ≥95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression (≤ 2%positive) of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II.

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MSC Immunosuppresion

- Important attribute for allogeneic use
- Measured by cell-based assays
- Use of human cells T-cells contributes to variability of assay read out
- Can mouse T-cell functions be suppressed by human MSCs?
  - Use T-cell receptor transgenic mice
    - All T-cells express same antigen receptor
    - Known antigen
    - No genetic variability

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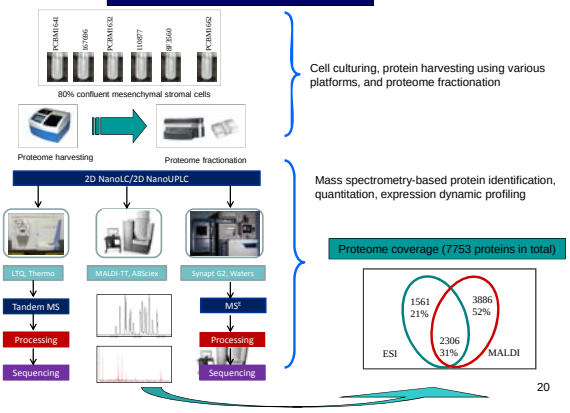
1. Proteomic characterization scheme



Cell culturing, protein harvesting using various platforms, and proteome fractionation

Mass spectrometry-based protein identification, quantitation, expression dynamic profiling

Proteome coverage (7753 proteins in total)

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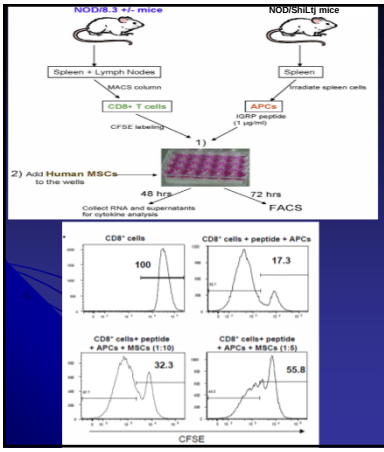

Immuno-suppression Assay

- MSC concentration-dependent
- MSC specific
  - HepG2 hepatocarcinoma
  - HT-180 epithelial line
- Ongoing
  - Apply to all donors, all passages

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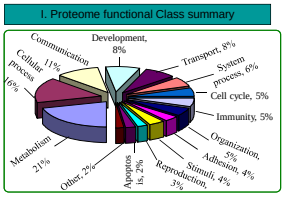
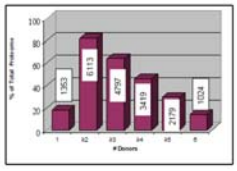
2. Overview of proteome summary and comparison between donors

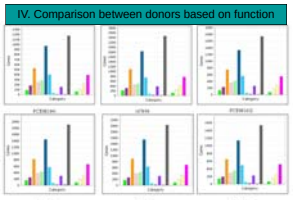
I. Proteome functional Class summary



II. Shared proteins between donors



IV. Comparison between donors based on function



III. Proteomic variability between donors

Donor ID	% Variability
PCBM1641	28.4
167696	8.7
PCBM1632	23.3
110877	22.2
8F3650	17.1
PCBM1662	25.7

Average of 21%

% variability: ratio of proteome size of a donor as compared to proteome size constructed using proteins expressed in at least 3 donors

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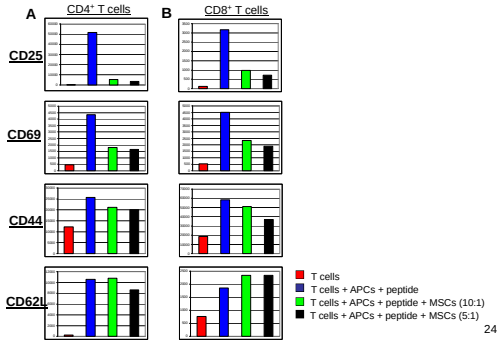
Modulation of surface activation markers on murine T cells by human MSCs, as determined by mean fluorescence intensity (MFI)



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Significance for Cell Therapy

- Quantitative functional assays; a crucial part of a systematic approach to identify and qualify predictive product characteristics for cell therapy products
  - Consensus MSC Markers do not Correlate with Functional Heterogeneity: Donor or Tissue Culture Age Differences
  - Robust enumeration of functional sub populations illustrates importance of extensive single cell analyses
- Assess
  - Differences between MSC donors
  - Impact of tissue culture conditions and duration
  - Correlation with other characteristics of MSCs
  - Enrichment techniques
- Application to understanding mechanisms controlling stem-cell differentiation and function
  - Heterogeneity
  - Improved understanding of "stemness"
  - Mechanisms of function

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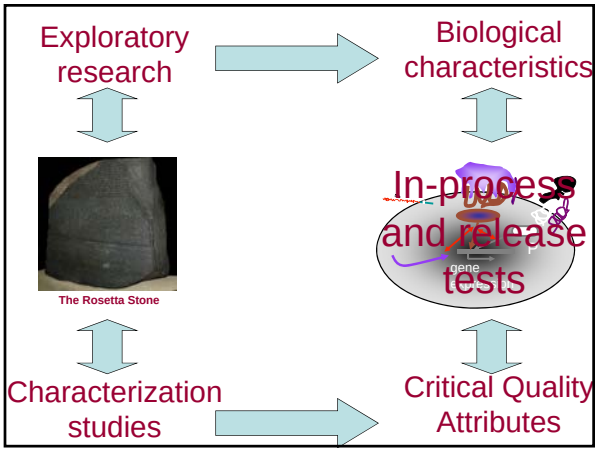

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  - **Malcolm Moos**
  - **Raj Puri**
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