CMC Strategy Forum North America Winter 2023 and WCBP 2023

Parallel Session 2 - Cell and Gene Therapy – New Frontier and Our Best Hope to Cure

Regenerative Medicine for the 21st Century

January 24, 2023

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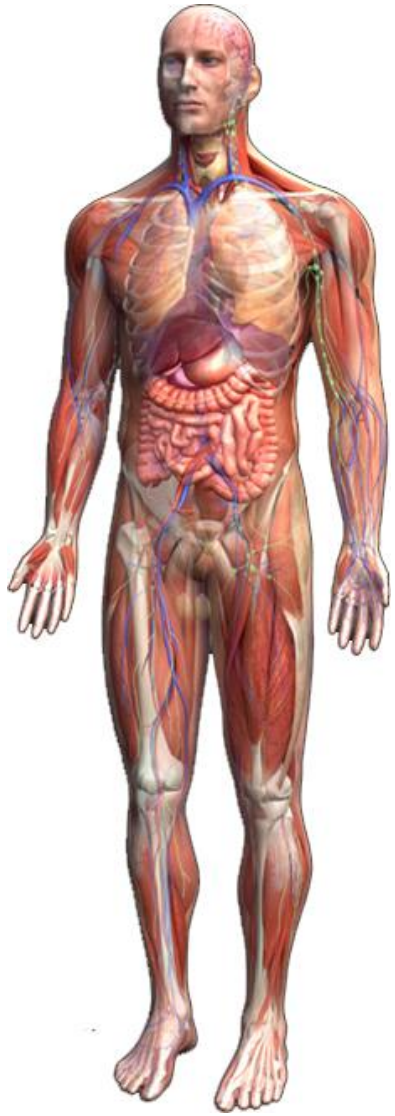
Wake Forest Institute for Regenerative Medicine

Wake Forest School of Medicine

Institute for Regenerative Medicine

Therapeutic Promise of Regenerative Medicine



Dental repair

Heart repair

Lung repair

Spinal cord repair

Graft Vs. Host disease

Stroke

Skin replacement

Diabetes

Crohn's disease

Artificial organs

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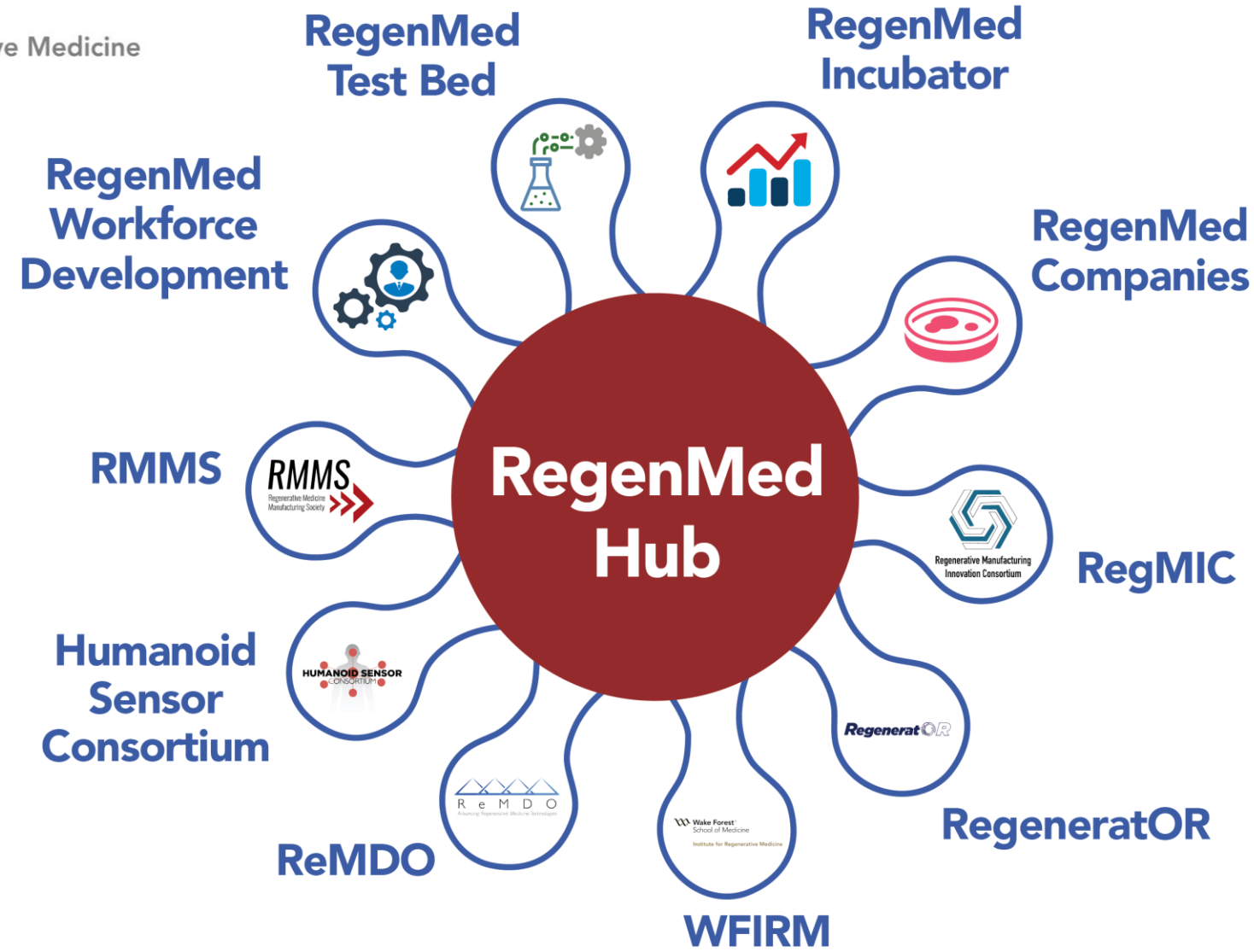


http://remdo.org/the-hub/

https://school.wakehealth.edu/research/institutes-and-centers/wake-forest-institute-for-regenerative-medicine

Goal: Cell-Based Product Biologics License

Demonstrate through analytical and clinical testing:

Identity

Purity

Potency

Safety

Sterility

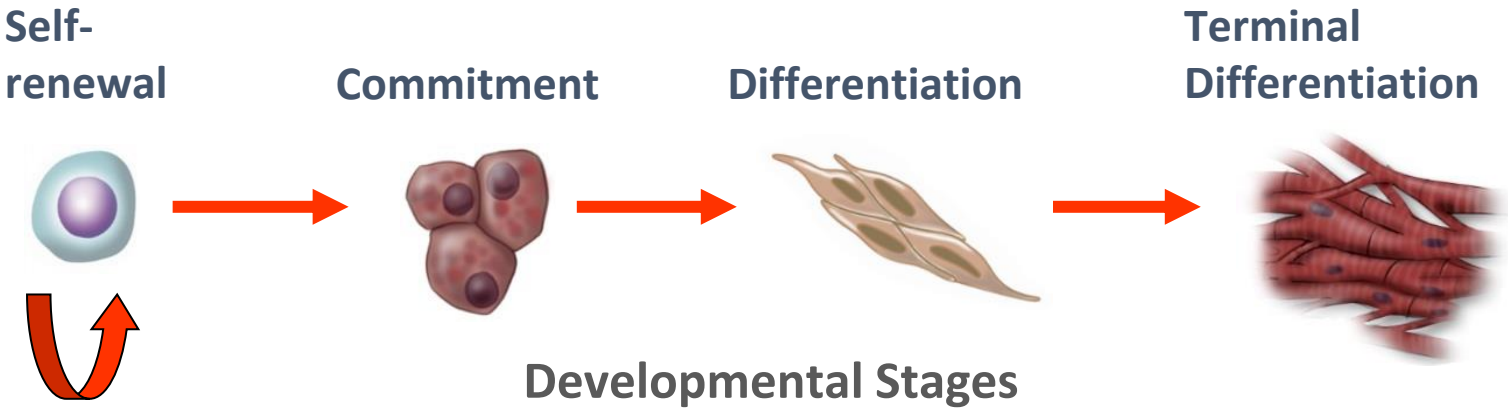
Stability

Efficacy

Code of Federal Regulations for Food and Drugs

(21 CFR 600 - BIOLOGICS)

How Can We Help Fulfill the Tremendous Promise?



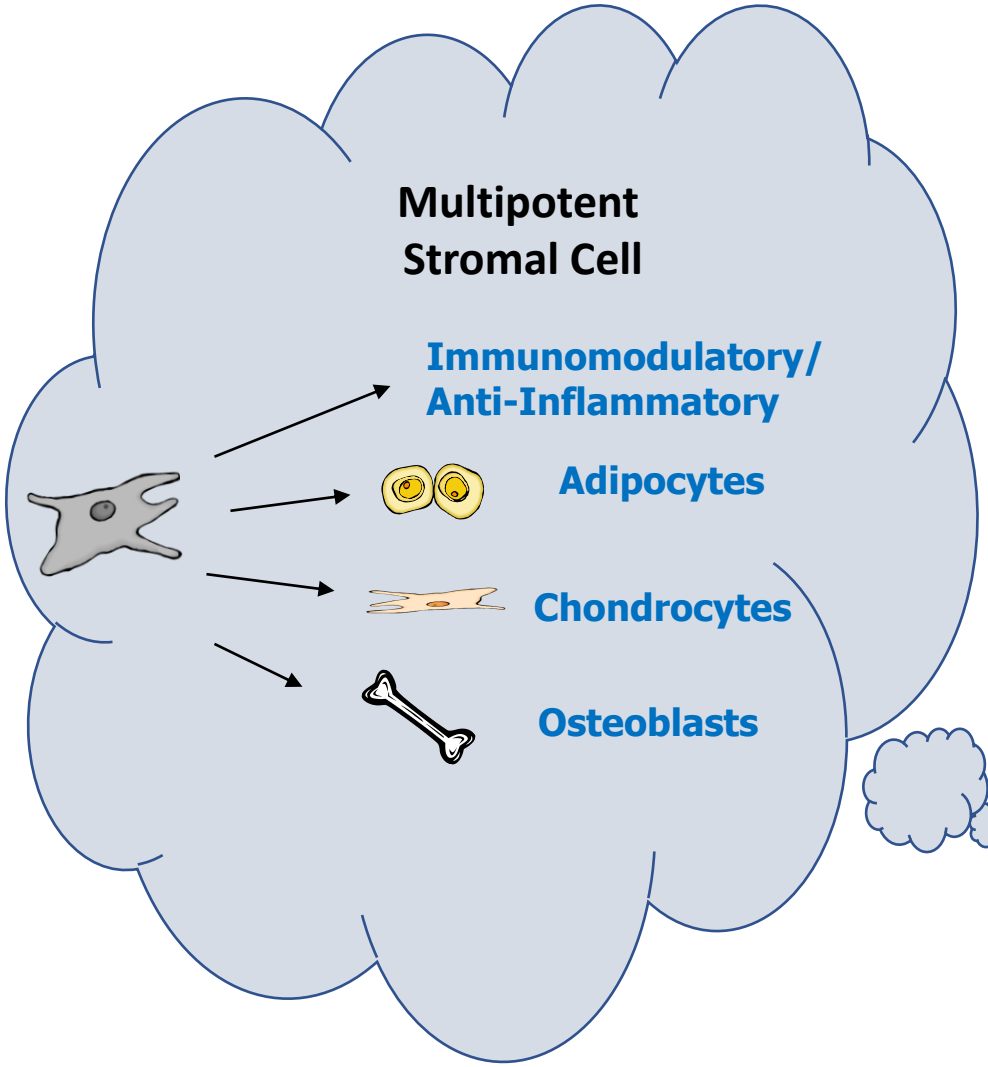
Can we develop ways to identify Quality Attributes that predict safety and effectiveness?

(Purity, Identity, Potency)

Challenges for Regenerative Medicine

- Cell-based products
  - Cell heterogeneity
    - Donor, manufacturing environment
- Meaningful characterization schemes
  - Potency, Identity, Purity
    - Ideally potency will predict effectiveness
- Understanding phased product development
  - Safety gets you in the door (IND allowed to proceed)
  - Effectiveness gets you over the goal line (BLA approved, License issued)
  - High Quality Product is required
- FDA does not dictate scientific approach but oversees regulatory requirements based on protecting patient rights, safety, and assuring quality and effectiveness

MSCs: Are We Measuring the Right Things?



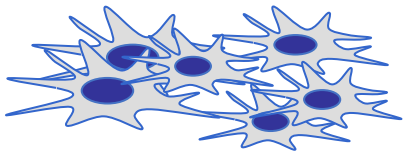
?

Manufactured MSCs

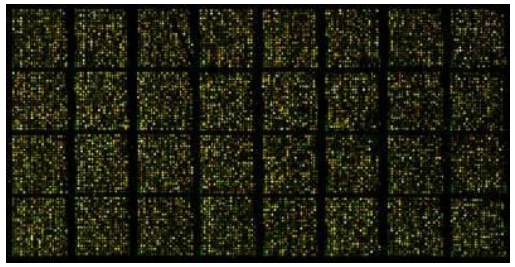
Adherent in culture
CD73+, CD90+, CD105+
Negative for blood cell markers

CBER/FDA MSC Consortium: Identification and correlation of MSC attributes with *in vivo* and *in vitro* assays of safety and efficacy

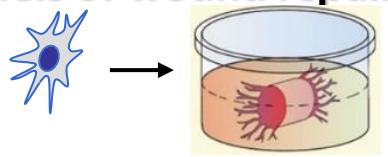
MSC Characterization



Puri Lab: genomics

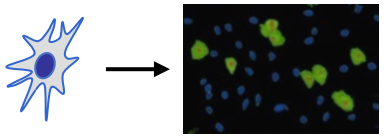


McCright Lab: *in vivo, in vitro* models of wound repair

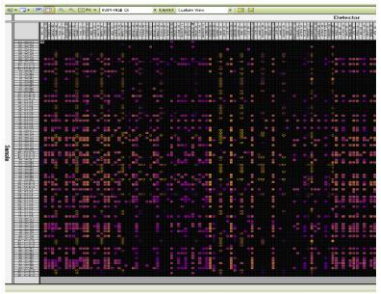


PRODUCT CHARACTERISTICS ⟷ **CORRELATE CANDIDATE ATTRIBUTES WITH ASSAY OUTCOMES**

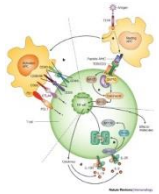
Bauer Lab: *in vitro* quantitative differentiation



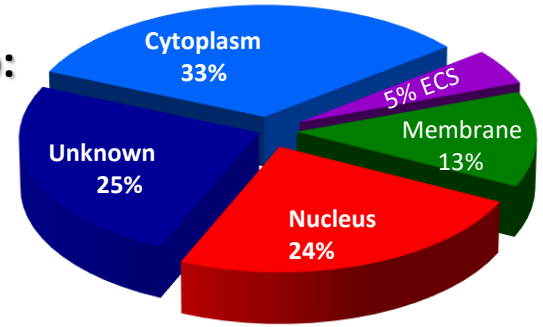
Moos Lab: gene expression, qRT-PCR, single cell PCR, NGS



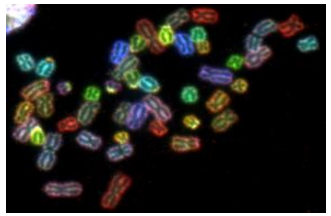
Wei/Bauer Labs: *in vitro, in vivo* immunosuppression

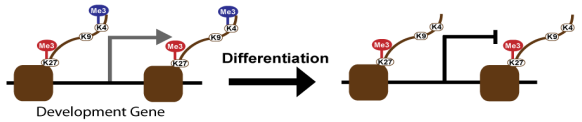


Alterman Lab: proteomics



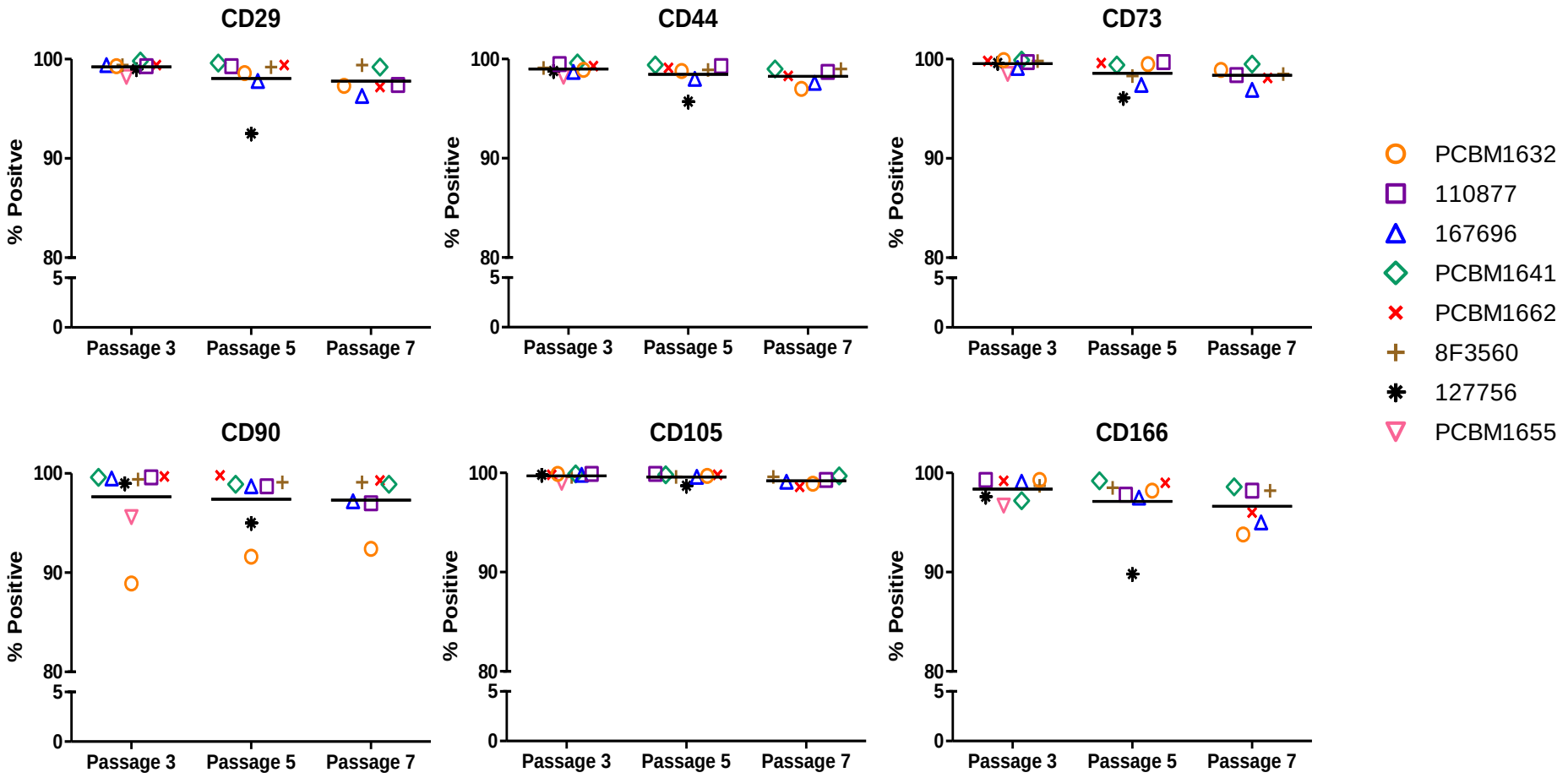
Hursh lab: epigenetics, karyotypes





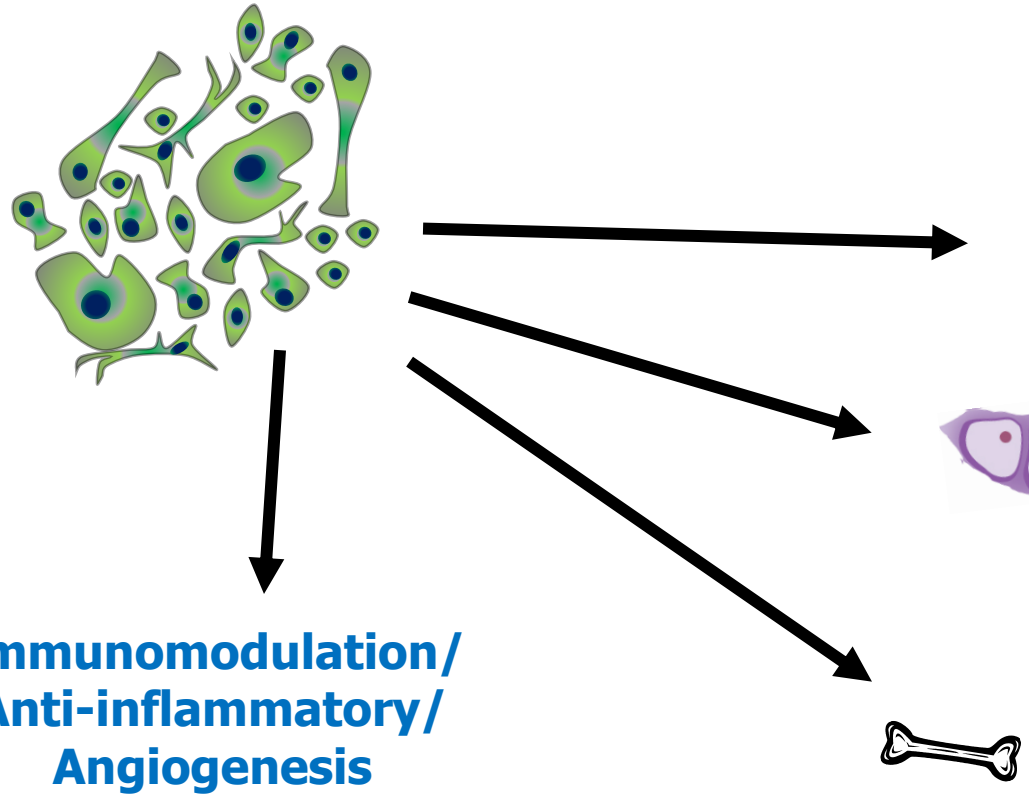
Sung Lab: *3D in vitro* tissue/organ microfluidic models

Consensus MSC Surface Markers Do Not Differ Between Cell lines or With Time in Culture

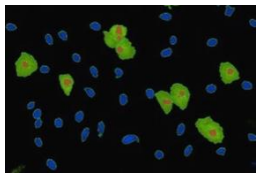


Lo Surdo JL et al., Cytotherapy, 2013.

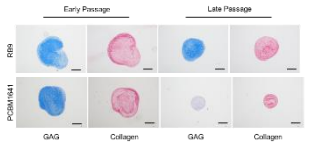
Quantitative MSC Differentiation Assays



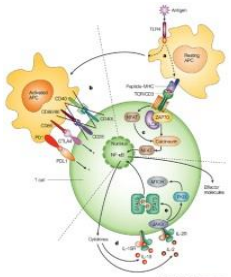
Adipogenesis



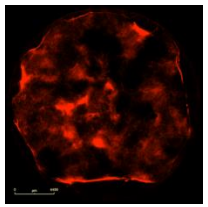
Chondrogenesis Sung Lab



Immunomodulation/ Anti-inflammatory/ Angiogenesis



Osteogenesis



Quantitative Measures to Assess MSC Characteristics

- Proliferation
- Cell Size
- Colony forming units (CFU-F)
- Adipogenic Activity

- **Osteogenic Activity**
  - Marklein, et al. 2016. Stem Cells, 34:935–947
- Chondrogenic Activity
  - Lam J, et al . 2018. Stem Cells Translational Medicine, 7: 664-675
- **Immunosuppressive Activity**
  - Klinker , and Marklein et al. 2017. PNAS. 114: 2598-2607
  - Marklein, et al 2018. Cytotherapy: available online 11/28/2018

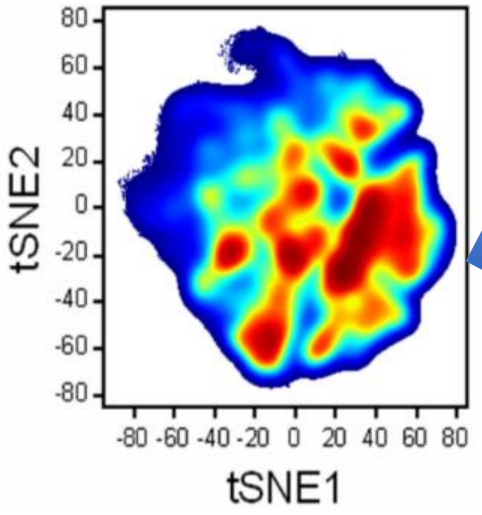
- Lo Surdo, JL, and Bauer, SR. 2012. Tissue Engineering: Part C 18: 877-889.

- Lo Surdo, et al. 2013. Cytotherapy 15: 1527-40

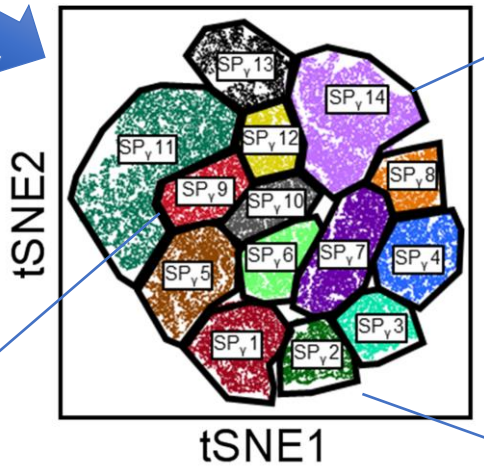
Detect differences among MSCs from different donors, cultured for different lengths of time, and manufactured under different conditions!

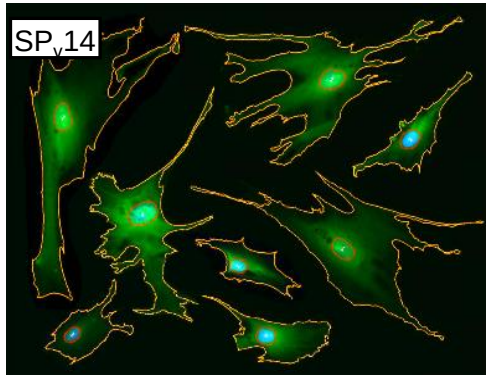
Machine Learning Identifies Immunosuppressive MSC Morphological Sub-populations

Morphological Data

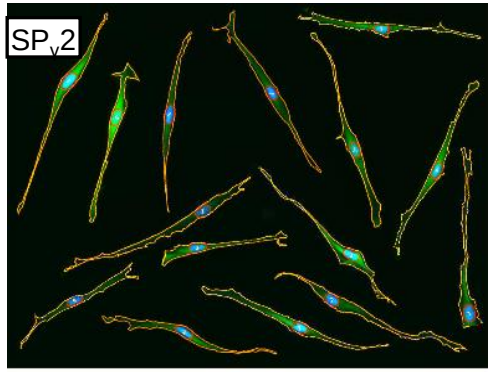


Manual "gating"

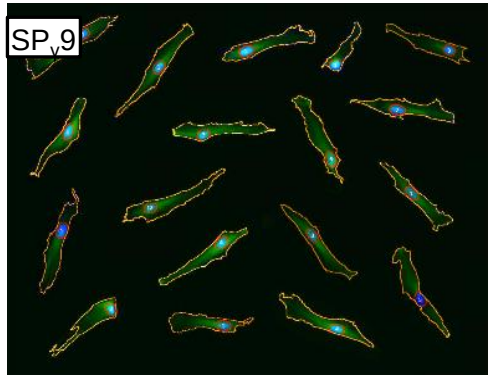




Low correlation with immunosuppression



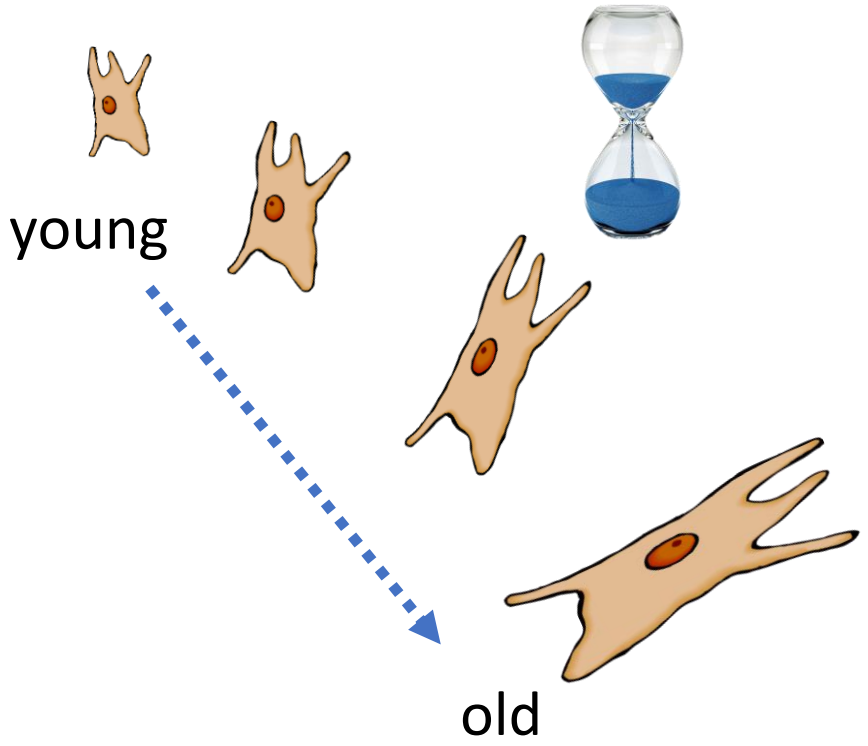
High correlation with immunosuppression



Marklein et al. Cytotherapy 2018

MSCs Can Change Over Time

- **MSC biological properties that can diminish with tissue culture age:**
  - **“Stemness” (Frequency of CFU-F)**
  - **Proliferation**
  - **Frequency of adipogenic precursors**
  - **Osteogenic activity**
  - **Chondrogenic activity**
  - ***In vitro* immunosuppressive capacity**
- **MSC properties that increase with tissue culture age:**
  - **Cell size**
- **MSC qualities that do not change:**
  - **Expression of CD73, CD105, CD90 (also CD44, CD29, CD166)**
  - **These are the markers that are most often used to define MSCs**



“As they age, MSCs get big and lazy”

Outcomes

- Consensus markers do not predict functional biological heterogeneity of MSCs
- Morphological characteristics predict relevant biological properties of MSCs
  - Functionally relevant morphological profiling
    - Osteogenesis
    - Immunosuppression

Potential Applications (1)

- Cell Source/Donor
  - Screen samples for desired biological activity
- Manufacturing
  - Evaluate impact of manufacturing process
    - Tissue culture conditions and duration
- Identify Quality Attributes
  - Activity/Potency
    - Quantitative Bioassays
    - Molecular markers correlated with bioassay outcomes
- Guide cell enrichment techniques

Potential Applications (2)

- **Standards Development**
  - **Quantitative bioassays**
    - **Osteogenesis**
    - **Adipogenesis**
    - **Immunosuppressive Capacity**
    - **Others?**
      - **Chondrogenesis**
      - **Angiogenesis/Wound repair**

Strategies to Identify Predictive CQAs

- Sophisticated, powerful
- Slow
- Finicky
- Labor intensive
- Often expensive
- Comprehensive
- Time consuming

- Robust
- Rapid compared to product expiry
- GMP friendly
  - Easy to validate
  - Operator independent
- Economical
- Focused

Potency ideally based on MOA, predictive of in vitro or in vivo result, related to clinical outcomes

Acknowledgements

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