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Center for Biologics Evaluation and Research

ADVANCING REGULATORY SCIENCE

Development of an assay to predict the capacity of mesenchymal stromal cells to suppress immune system activity

FDA scientists developed an assay that identifies cellular features of mesenchymal stromal cells (MSCs) associated with their immunosuppressive capacity following stimulation by interferon-gamma (IFN-γ). The assay is an important step toward predicting the efficacy of MSCs in treating inflammatory diseases, such as Crohn's disease and multiple sclerosis.

"Morphological features of IFN-γ-stimulated **Mesenchymal Stromal Cells** predict overall immunosuppressive capacity"

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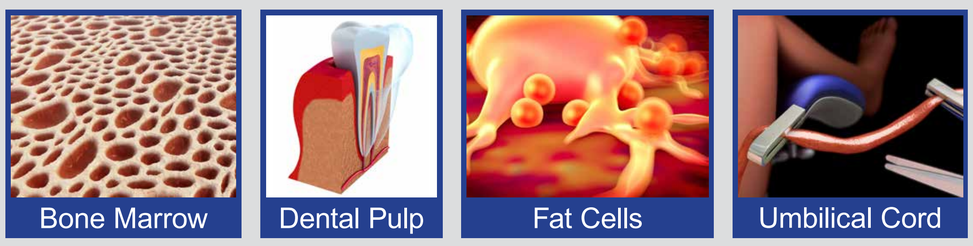
Matthew W. Klinker[a,1], Ross A. Marklein[a,1], Jessica L. Lo Surdo[a], Cheng-Hong Wei[a], and Steven R. Bauer[a,2]

[a]Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, USA [1]M.W.K. and R.A.M contributed equally to this work.
[2]To whom correspondence may be addressed Email: steven.bauer@fda.hhs.gov

Mesenchymal Stromal Cells



- Derived from various tissues: bone marrow, fat, dental pulp, and umbilical cord.
- Can give rise to cells associated with bone, fat, and cartilage.
- Interferon-γ enhances MSC ability to suppress T Cells

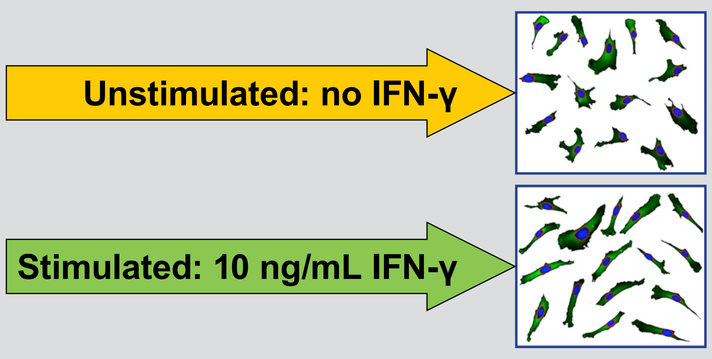
Why FDA scientists developed this assay

- Clinical trials are investigating MSCs as potential therapies for many diseases including inflammatory diseases such as Crohn's disease and multiple sclerosis.
- Sponsors need new tools to reliably predict immunosuppressive capacity of specific batches of MSCs.
- Well-defined predictive markers to identify MSC immunosuppressive capacity would advance MSC-based therapeutics.

How FDA scientists laid the groundwork for this new assay

- Integrated several measurements of T Cell activation at various MSC densities, thus enabling quantification of MSC capacity to suppress T cell activity
  - T Cell surface proteins related to immune activation (e.g., CD25)
  - T Cell-produced cytokines (e.g. TNF-α, IFN-γ)
- Identified morphological features of MSCs that predicted immunosuppressive capacity, as well as the magnitude of IFN-γ-mediated immunosuppression enhancement
- Quantitatively assessed overall immunosuppressive capacity of MSCs, which enabled identification and quantification of differences in immunosuppression that are related to donor and cellular expansion.

Distinct MSC morphological changes following stimulation by IFN-γ are correlated to their ability to suppress T Cell activity



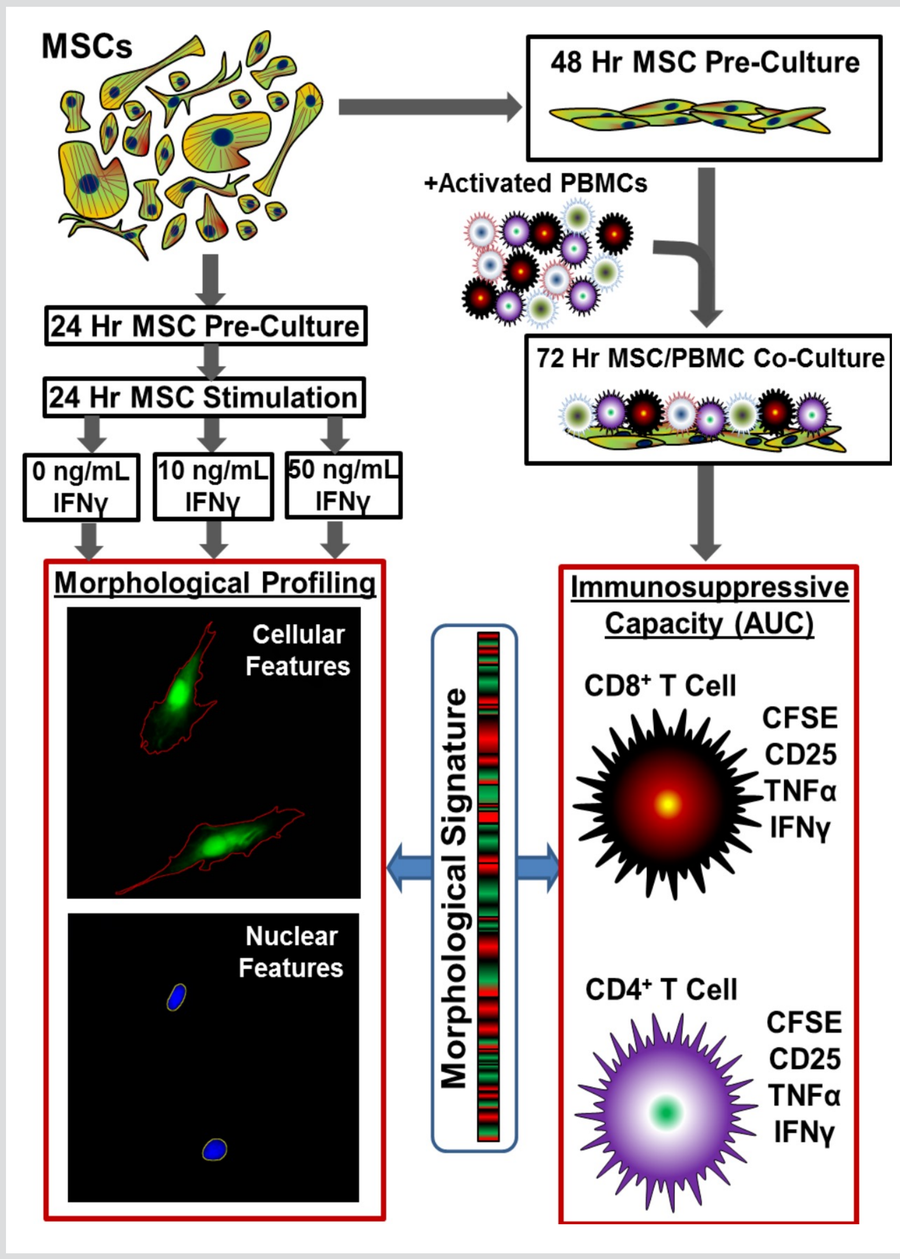
How the assay correlates T Cell activity with enhanced MSC immunosuppressive activity

- Run MSC morphological profiling and immunosuppression assays concurrently
- Correlate resulting morphology and immunosuppressive activity data

Morphological Profiling — **Immunosuppression Assay**

Step 1: MSC Stimulation
Pre-culture MSCs from known source and then stimulate with IFN-γ

Step 2: MSC Stimulation
Record cellular and nuclear morphological changes in treated MSCs



Step 1: Co-culture MSCs & Peripheral Blood Mononuclear Cell (PBMC)
Pre-culture MSCs from a known source; then co-culture with PBMCs with T Cell "activating beads."

Step 2: Assess T Cell activation and compare with MSC morphological profiles
Track the extent of T Cell proliferation; identify secretion by T Cells of pro-inflammatory cytokines (IFN-γ and TNF-α) and surface expression of activation marker CD25

This work supports further development of an assay that can 1) identify MSC preparations with a desired immunosuppressive activity; 2) facilitate discovery of optimal conditions for preconditioning MSCs to enhance their immunosuppressive function.

Such an assay could also potentially identify stimulatory MSC preconditioning regimens that are optimal for a specific patient or disease and thus support development of personalized therapies for treatment of some inflammatory diseases.