INDUCED PLURIPOTENT AND MESENCHYMAL STEM CELLS – CELLS WITH A LOT OF POTENTIAL

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Senior Scientist
ASCB Vendor Showcase
Dec. 15, 2013
Outline

- Human induced pluripotent stem cells (iPSCs)
- ATCC iPSC quality standards
- Characterization of Parkinson’s iPSC lines
- Human reference iPSC lines
- Human mesenchymal stem cells
What are iPSC?

Yamanaka Factors

Oct3/4\textsuperscript{1,2}
Sox2\textsuperscript{1,2}
Klf4\textsuperscript{1}
Myc\textsuperscript{1}
Nanog\textsuperscript{2}
Lin28\textsuperscript{2}

Yamanaka Factors reprogramming factors

iPS reprogramming factors

Self-renewal capability

Differentiation

- Pancreatic Cells
- Hepatocytes
- Lung Cells

- Cardiomyocytes
- Skeletal Muscle
- Smooth Muscle

- Neurons
- Glial
- Epithelial

ENDODERM

MESODERM

ECTODERM

www.sabiosciences.com
The promise of iPSCs

## Overview of ATCC iPSC lines collection

### iPSC lines derived from apparent normal donors

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Designation</th>
<th>Reprogramming System</th>
<th>Tissue and Cells Origin</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS-1019™</td>
<td>ATCC-DYS0100</td>
<td>OSKM / Sendai Virus</td>
<td>Foreskin, Fibroblast</td>
<td>Normal</td>
</tr>
<tr>
<td>ACS-1020™</td>
<td>ATCC-HYS0103</td>
<td>OSKM / Sendai Virus</td>
<td>Liver, Fibroblast</td>
<td>Normal</td>
</tr>
<tr>
<td>ACS-1021™</td>
<td>ATCC-CYS0105</td>
<td>OSKM / Sendai Virus</td>
<td>Heart, Fibroblast</td>
<td>Normal</td>
</tr>
<tr>
<td>ACS-1007™</td>
<td>ATCC-HYR0103</td>
<td>OSKM / Retrovirus</td>
<td>Liver, Fibroblast</td>
<td>Normal</td>
</tr>
<tr>
<td>ACS-1011™</td>
<td>ATCC-DYR0100</td>
<td>OSKM / Retrovirus</td>
<td>Foreskin, Fibroblast</td>
<td>Normal</td>
</tr>
</tbody>
</table>

### iPSC lines derived from donors with diseases

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Designation</th>
<th>Reprogramming System</th>
<th>Tissue and Cells Origin</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS-1012™</td>
<td>ATCC-DYR0530</td>
<td>OSKM / Retrovirus</td>
<td>Skin, Fibroblast</td>
<td>Parkinson’s Disease, Asthma, Depression</td>
</tr>
<tr>
<td>ACS-1013™</td>
<td>ATCC-DYS0530</td>
<td>OSKM / Sendai virus</td>
<td>Skin, Fibroblast</td>
<td>Parkinson’s Disease, Asthma, Depression</td>
</tr>
<tr>
<td>ACS-1014™</td>
<td>ATCC-DYP0530</td>
<td>OSKM / Episomal</td>
<td>Skin, Fibroblast</td>
<td>Parkinson’s Disease, Asthma, Depression</td>
</tr>
<tr>
<td>ACS-1003™</td>
<td>ATCC-DYP0730</td>
<td>OSKM / Episomal</td>
<td>Foreskin, Fibroblast</td>
<td>Down syndrome</td>
</tr>
<tr>
<td>ACS-1004™</td>
<td>ATCC-DYP0250</td>
<td>OSKM / Episomal</td>
<td>Skin, Fibroblast</td>
<td>Cystic fibrosis: Homozygous CFTRΔ508</td>
</tr>
</tbody>
</table>
Outline

- Human induced pluripotent stem cells (iPSCs)
- ATCC iPSC quality standards
- Characterization of Parkinson’s iPSC lines
- Human reference iPSC lines
- Human mesenchymal stem cells
# iPSC quality control

<table>
<thead>
<tr>
<th>Description</th>
<th>QC Methods</th>
<th>QC Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Thaw Viable Cell Recovery</td>
<td>hiPSC culture</td>
<td>≥ 30 colonies in 5 days</td>
</tr>
<tr>
<td>Expression of Stem Cell Markers</td>
<td>Immunocytochemistry</td>
<td>Tra1-60, Tra1-81, SSEA-4, Nanog</td>
</tr>
<tr>
<td>Surface Antigen Expression of Stem Cell Markers</td>
<td>Flow Cytometry</td>
<td>Pluripotency (SSEA4, Tra-1-60) &gt;85%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Differentiation (SSEA1) &lt;15%</td>
</tr>
<tr>
<td>Karyotype</td>
<td>G banding</td>
<td>Normal karyotype, 46,XY or 46,XX</td>
</tr>
<tr>
<td>Germ Layer Differentiation *</td>
<td>qRT-PCR analysis of EBs</td>
<td>Gene expression relative to pluripotent cells (endoderm, mesoderm and ectoderm layers)</td>
</tr>
<tr>
<td>PluriTest (Transcriptome analysis)</td>
<td>Illumina Human HT-12v4</td>
<td>Pluripotency and Novelty Scores</td>
</tr>
<tr>
<td></td>
<td>Expression Beadchip</td>
<td></td>
</tr>
<tr>
<td>Sterility (Bacterial and Fungal Testing)</td>
<td>Growth on agar</td>
<td>No bacterial growth after 21 days</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Direct culture and</td>
<td>None detected</td>
</tr>
<tr>
<td></td>
<td>Hoechst DNA staining</td>
<td></td>
</tr>
<tr>
<td>Identity</td>
<td>STR</td>
<td>Consistent with donor sample</td>
</tr>
<tr>
<td>Viral Panel Testing</td>
<td>PCR</td>
<td>None detected for CMV, EBV, HBV, HIV1 and HPV</td>
</tr>
</tbody>
</table>

**EB differentiation is being replaced by transcriptome-based pluripotency analysis**
ATCC iPSCs are monitored for pluripotency

Immunocytochemistry: Tra 1-60, Tra 1-81, SSEA-4 and Nanog
ATCC iPSCs are monitored for pluripotency

Flow Cytometry: SSEA-4, Tra 1-60 and SSEA-1
ATCC iPSCs are tested for their capacity to differentiate into the three germ layers

HFF iPS cell line (ATCC® ACS-1011™)
Transcriptome-based pluripotency: PluriTest

- Assesses pluripotency and differentiation based on a comparison of gene expression profiles from a large database of known pluripotent cell samples
  - Pluripotent stem cells (223 hESCs, 41 hiPSCs)
  - Differentiated cell types, developing and adult tissues (204 somatic cells)

- Pluripotency is based on empirically determined thresholds
  - Pluripotency Score: indication of a sample containing a pluripotent signature
  - Novelty Score: based on existing data from other well-characterized PSC lines

ATCC iPSC lines
Somatic cells used for reprogramming
EBs collected at 2, 3 and 4 weeks
BG01V hESC
Easy to use, all-in-one culture system

<table>
<thead>
<tr>
<th></th>
<th>Feeder-Dependent</th>
<th>Feeder-Free</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Media</strong></td>
<td>Pluripotent Stem Cell SFM XF (Serum Free, Xeno Free)</td>
<td>Pluripotent Stem Cell SFM XF/FF (Serum Free, Xeno Free)</td>
<td>DMEM:F12 ES Qualified FBS</td>
</tr>
<tr>
<td><strong>Substrate</strong></td>
<td>MEF/HFF Mitomycin C treated; $\gamma$-irradiated</td>
<td>Cell Basement Membrane Gel</td>
<td>MEF/HFF Mitomycin C treated; $\gamma$-irradiated</td>
</tr>
<tr>
<td><strong>Passaging</strong></td>
<td>Dissociation Reagent</td>
<td>Dissociation Reagent</td>
<td>Dissociation Reagent</td>
</tr>
<tr>
<td><strong>Cryopreservation</strong></td>
<td>Stem Cell Freezing Media</td>
<td>Stem Cell Freezing Media</td>
<td>Stem Cell Freezing Media</td>
</tr>
<tr>
<td><strong>Supporting Reagent</strong></td>
<td>ROCK inhibitor</td>
<td>ROCK inhibitor</td>
<td>ROCK inhibitor</td>
</tr>
<tr>
<td><strong>Growth Factor</strong></td>
<td>N/A</td>
<td>N/A</td>
<td>bFGF</td>
</tr>
</tbody>
</table>

No adaptation necessary, all reagents are formulated to work together!

Visit [www.atcc.org](http://www.atcc.org) for a complete list of feeder cells
ATCC iPSC cultured in different media system retain consistent differentiation capabilities

HFF iPS cell line (ATCC® ACS-1011™)
Outline

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- Human mesenchymal stem cells
ATCC Parkinson’s iPSC lines

- Patient-specific iPSCs provide an opportunity to model human disease in culture – ‘Disease-in-a-dish’
- **Parkinson’s Disease** – Second most common neurodegenerative disorder
- **Donor information**: 63 years old Caucasian male diagnosed with Parkinson’s disease, asthma, and depression
- Exome sequencing identified multiple missense mutations in Leucine-Rich Repeat Kinase 2 (LRRK2) gene: R50H, I1723V, M2397T

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<td>ATCC-DYR0530</td>
<td>Retrovirus</td>
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<td>ACS-1013™</td>
<td>ATCC-DYS0530</td>
<td>Sendai virus</td>
</tr>
<tr>
<td>ACS-1014™</td>
<td>ATCC-DYP0530</td>
<td>Episomal</td>
</tr>
</tbody>
</table>

Fonzo A.D. et al., Eur J Hum Genet. 2006; 14: 322-331
Reprogramming methods do not affect differentiation potential

Normal

Parkinson’s/Retroviral

Parkinson’s/Sendai virus

Parkinson’s/Episomal
Outline

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Reference iPSC collection

• Derivation criteria

<table>
<thead>
<tr>
<th>Somatic cell of origin</th>
<th>CD34+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease state</td>
<td>Clinically normal</td>
</tr>
<tr>
<td>Reprogramming method</td>
<td>Sendai virus (Foot-print free)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>Age</td>
<td>Adult, 25-60</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>African American</td>
</tr>
<tr>
<td></td>
<td>Hispanic</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
</tr>
<tr>
<td>Somatic cell of origin</td>
<td>CD34+ cells</td>
</tr>
</tbody>
</table>

• In-depth characterization
  – Differentiation potential
  – Stability over long-term in *vitro* culture
  – Whole exome sequencing
Outline

Human induced pluripotent stem cells (iPSCs)

ATCC iPSC quality standards

Characterization of Parkinson’s iPSC lines

Human reference iPSC lines

Human mesenchymal stem cells
Mesenchymal stem cells

Mesenchymal stem cells, or MSCs, are multipotent stromal cells that can differentiate into a variety of cell types, including: osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells).

The cells could potentially be used to treat diseases by providing immunomodulation, anti-inflammatory actions, and cell replacement as well as delivering therapeutic agents.

Easy to use MSC Culture System from ATCC

• Mesenchymal Stem Cells derived from various tissue sources

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Name</th>
<th>Source</th>
<th>Age</th>
<th>Passage #</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCS-500-010</td>
<td>Umbilical Cord-Derived MSCs; Normal, Human</td>
<td>Umbilical cord matrix</td>
<td>Neonatal</td>
<td>2</td>
</tr>
<tr>
<td>PCS-500-011</td>
<td>Adipose-Derived MSCs; Normal, Human</td>
<td>Lipo-Aspirate</td>
<td>Adult</td>
<td>2</td>
</tr>
<tr>
<td>PCS-500-012</td>
<td>Bone Marrow-Derived MSCs; Normal, Human</td>
<td>Bone Marrow</td>
<td>Adult</td>
<td>2</td>
</tr>
</tbody>
</table>

• Optimized Mesenchymal Stem Cell Culture Medium to ensure:
  – Functional expression of MSC markers
  – Growth and proliferation
  – Multi-lineage differentiation capability

• Multi-lineage Differentiation Kit
  – Adipocyte Differentiation Toolkit
  – Chondrocyte Differentiation Toolkit
  – Osteocyte Differentiation Toolkit

Visit [www.atcc.org](http://www.atcc.org) for more information
Characterization of BM-MSCs

Post-thaw recovery and growth curve of BM-MSCs
Characterization of BM-MSCs

Flow analysis of a panel of MSC-specific CD markers recommended by ISCT (International Society for Cellular Therapy) in BM-MSCs after 3 or 6 passages of *in vitro* culture.

BM-MSCs exhibit consistent immunophenotype over extended culture
Characterization of BM-MSCs

Immunocytochemistry analysis of MSC markers expression

CD44

CD90

CD45

CD105
Characterization of BM-MSCs

Adipogenic, osteogenic, and chondrocyte differentiation of BM-MSCs

Adipocytes

Adipocytes stained with Oil Red O

Osteoblasts stained with Alizarin Red

Chondrocytes stained with Alcian Blue