iPSC-derived primary cells: Expand your cell-based assays with an unlimited, biologically relevant source

Yalin Firinci, M.B.A.
Product Line Business Specialist, ATCC

Credible Leads to Incredible™
ATCC today

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA and an R&D & Services center in Gaithersburg, MD
- World wide brand name and quality recognition

- World’s premiere biological materials resource and standards development organization
  - 4,000 cell lines
  - 70,000 microbes

- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
  - Growing portfolio of products and services
  - Sales and distribution in 140 countries, 12 International distributors

- Talented team of 475+ employees; > one third with advanced degrees

- Multiple accreditations including ISO 9001 and ISO 13485
Agenda

- iPSC-derived Primary Cells Background

- iPSC-derived Primary Cells Portfolio
  - iPSC-derived Mesenchymal Stem Cells (ATCC® ACS-7010™)
  - iPSC-derived CD34+ Cells (ATCC® ACS-7020™)
  - iPSC-derived Monocytes (ATCC® ACS-7030™)

- Summary

Image courtesy of Alex Ritter, Jennifer Lippincott Schwartz, and Gillian Griffiths, National Institutes of Health
iPSC-derived Primary Cells Background Information

Image courtesy of Alex Ritter, Jennifer Lippincott Schwartz, and Gillian Griffiths, National Institutes of Health
What are iPSCs?

Yamanaka Factors
Oct3/4\textsuperscript{1,2}
Sox2\textsuperscript{1,2}
Klf4\textsuperscript{1}
Myc\textsuperscript{1}


Figure adapted from Kaebisch C, et al., 2015.
iPSC-derived Primary Cells

- ATCC iPSCs were used as feedstock for this project

- The iPSCs were terminally differentiated to the desired cell type by incubation in proprietary media formulations

- An unlimited, clonal source of cells needed for research or therapeutic purposes was developed using this method

**Scope:** ATCC iPSCs were the source for three types of differentiated cells:
- Mesenchymal Stem Cells (MSCs)
- CD34+ Progenitors
- Monocytes.
ATCC classic and advanced cell models

- ATCC is a complete solution supplier
- From basic research through discovery and development to product testing
  - Continuous cell lines
  - Primary cells
  - hTERT immortalized primary cells
  - iPSC-derived primary cells
- Portfolio features
  - Reliability
  - Fully characterized cells
  - Optimized growth protocols
### Pros and cons of different cell models

<table>
<thead>
<tr>
<th></th>
<th>Primary Cells</th>
<th>hTERT Immortalized</th>
<th>Continuous Cell Lines</th>
<th>iPSC-derived</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mimic in vivo tissue type</strong></td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Genotypic stability</strong></td>
<td>Diploid</td>
<td>Diploid/ Near Diploid</td>
<td>Aneuploid</td>
<td>Diploid</td>
</tr>
<tr>
<td><strong>Proliferative capacity</strong></td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Supply</strong></td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Inter-experimental reproducibility</strong></td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td><strong>Ease-of-use</strong></td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Predictability in toxicological studies</strong></td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Cell Type</td>
<td>ATCC® No.</td>
<td>Product Type</td>
<td>Tissue of Origin/Parental Line (ATCC® No.)</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------</td>
<td>----------------------</td>
<td>-------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Mesenchymal Stem Cells</td>
<td>PCS-500-011™</td>
<td>Primary</td>
<td>Adipose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCRC-4000™</td>
<td>hTERT immortalized</td>
<td>Adipose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACS-7010™</td>
<td>iPSC derived</td>
<td>Bone marrow BYS0112 (ACS-1026™)</td>
<td></td>
</tr>
<tr>
<td>CD34+ Progenitor Cells</td>
<td>PCS-800-012™</td>
<td>Primary</td>
<td>Bone Marrow</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACS-7020™</td>
<td>iPSC derived</td>
<td>Bone marrow – CD34+ BXS0117 (ACS-1031™)</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>PCS-800-011™</td>
<td>Primary</td>
<td>Peripheral Blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACS-7030™</td>
<td>iPSC-derived</td>
<td>Foreskin fibroblast DYS0100 (ACS-1019™)</td>
<td></td>
</tr>
</tbody>
</table>
ATCC’s iPSC-derived Cells Portfolio
iPSC-derived Mesenchymal Stem Cells (ATCC® ACS-7010™)

- High purity
  - CD29, CD44, CD73, CD90, CD105, and CD166 >95%;
  - CD14, CD19, CD31, CD34, and CD45 <5%.
  - Tra-I-60⁺ < 5%

- High post-thaw viability (>90%)

- Available in large quantity from a single source

- High osteocyte, adipocyte and chondrocyte differentiation potential

- Serum free freezing medium
Flow cytometry of surface markers of iPSC-derived MSC

Positive marker expression

- CD29 > 95%
- CD44 > 95%
- CD73 > 95%
- CD90 > 95%
- CD105 > 95%
- CD166 > 95%
Flow cytometry of surface markers of iPSC-derived MSC

Negative marker expression

- **CD14**: <2%
- **CD34**: <1%
- **CD19**: <1%
- **CD45**: <1%
- **TRA-1-60**: <1%
- **Iso**: <1%
Osteocyte differentiation potential

Undifferentiated MSC

Differentiated MSC

10X
Chondrocyte differentiation potential
Adipocyte differentiation potential

Undifferentiated MSC

Differentiated MSC
iPSC-derived MSCs immunosuppress activated PBMCs

![Bar graph showing the percent of proliferating lymphocytes with different PBMC:MSC ratios.](image)

- iPSC-MSC
- BM-MSC

CD45/EdU Double Positive

Percent of Proliferating Lymphocytes

PBMC:MSC Ratio

No MSC, 1:1, 2:1, 4:1

**** ****
iPSC-derived CD34+ progenitor cells (ATCC® ACS-7020™)

- High purity
  - CD34⁺ >90%
  - CD45⁺ < 80%
  - Tra-I-60⁺ < 5%

- High post-thaw viability (>92%)

- Suspension cells

- Available in large quantity from a single source

- High erythroid, myeloid, and megakaryocyte differentiation potential

- Serum free freezing medium
Marker analysis in iPSC-derived CD34+ cells

- **Isotype**
  - CD34: >90%
  - CD45: <80%
  - TRA 1-60: <1%

- **CD34**
  - >90%

- **CD45**
  - <80%

- **TRA 1-60**
  - <1%
Blood lineage differentiation potential

Overview of colonies in the dish

CFU-GEMM

CFU-GM

CFU-GEMM with hemoglobinized erythrocytes

CFU-E

BFU-E

1000 µm

200 µm

400 µm

100 µm

100 µm

400 µm
iPSC-derived Monocytes (ATCC® ACS-7030™)

- High purity
  - CD14+ > 90%
  - Tra-I-60+ < 5%
- High post-thaw viability (>95%)
- Suspension cells
- Available in large quantity from a single source
- High macrophage and dendritic cell differentiation potential
- High cytokine activation
- High phagocytic potential for macrophages
- Serum free freezing medium
Marker analysis of iPSC-derived Monocytes

TRA 1-60 <5%

CD 14 >90%
Macrophage differentiation potential

- Macrophages-PC
- Macrophages-GIEMSA stain
- Macrophages-Phagocytosed red bioparticles

Scale bars: 100 µm, 100 µm, 200 µm
Dendritic cell differentiation potential
Monocyte activation assays

![Graph showing iPSC derived Monocytes IL-6 stimulation](chart.png)
Summary

- ATCC developed proprietary methods for the differentiation of CD34+ cells, Monocytes, and MSCs from normal human iPSCs
- Different ATCC iPSC lines have varied efficiencies for differentiation into distinct lineages
- Differentiated iPSC-derived primary cells provide:
  - An unlimited source of cells
  - Interexperimental reproducibility
  - High biological relevance – They exhibit primary cell functionality and genetic stability
- iPSC-derived CD34+ cells can differentiate to all blood lineage cells
- iPSC-derived Monocytes can differentiate to functionally active macrophages and dendritic cells
  - Activated cells can be utilized for developing a MAT assay
- iPSC-derived MSCs can:
  - Differentiate to osteocytes, adipocytes, and chondrocytes
  - Display immunosuppressive properties
- You can order these products at [www.atcc.org/differentiatediPSCs](http://www.atcc.org/differentiatediPSCs)
Thank You

Questions?

© American Type Culture Collection. The ATCC trademark and trade name, and any other trademarks listed in this publication are trademarks owned by the American Type Culture Collection unless indicated otherwise.
Thank you for joining today!

- Register for more ATCC webinars at www.atcc.org/webinars

- Go to www.atcc.org/differentiatediPSCs

- Poster Presentation: ISSCR 2019
  Production of assay-ready iPSC-derived CD34+ cells, monocytes, and mesenchymal stem cells

  Presented by Sheela Jacob, Ph.D.
  June 28 | 7:00 PM ET
  Poster Board # F-2052

Please email additional questions to: tech@atcc.org