Extracellular matrix free microcarrier cultures of human pluripotent stem cells induced by inhibition of ROCK-Myosin II signaling

Steve Oh, Allen Chen, Chen Xiaoli, Lim Yuming, Shaul Reuveny

Stem Cell Group,
Bioprocessing Technology Institute,

International Society of Cell Therapy 2013 Meeting
22 to 25 April 2013
Press Releases

ACT Treats First Patient with Better Vision in Clinical Trial for Stargardt's Macular Dystrophy

Patient injected with 100,000 human embryonic stem cell (hESC) derived Retinal Pigment Epithelial Cells

MARLBOROUGH, Mass. — April 15, 2013 — Advanced Cell Technology, Inc. (ACT: OTCBB: ACTG), a leader in the field of regenerative medicine, today announced treatment of the first patient in patient cohort 2a, consisting of patients with better vision, in its U.S. clinical trial for Stargardt's Macular Dystrophy (SMD), a form of juvenile macular degeneration. The surgery was performed on Thursday, April 11 at Willis Eye Institute in Philadelphia, by Joseph Maguire, M.D., a co-investigator on the trial. The protocol is overseen by Principle Investigator, Carl D. Regillo, M.D., Chief of the Willis Eye Institute Retina Service, and professor of ophthalmology at Thomas Jefferson University. The patient was injected with 100,000 human embryonic stem cell-derived retinal pigment epithelial (RPE) cells. As the company announced on Jan. 22, patients a visual acuity of 20/100 are

Press Contact

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844-450-3616

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Microcarrier Technology Platform

Expansion of anchorage dependent stem cells on commercial microcarriers

Stem Cell Research, 2009
Stem Cell & Development, 2010, 11
Tissue Eng. 2010, 11

Differentiation of stem cells

Stem Cell Research, 2011
Current Protocols, 2012
Tissue Eng. 2012
Bioresearch Open Access, 2013
Regenerative Med. 2013

Updated:
06/05/2013
ROCK inhibitor supports expansion of pluripotent cells on cellulose cylindrical microcarriers without extracellular matrix coatings
Long term culture over 15 passages

HES-3

Matrigel

- mAb84
- Oct4
- Tra-1-60

ROCK inhibitor

- mAb84
- Oct4
- Tra-1-60

Cell concentration ($\times 10^6$ cells/ml)

% Positive cells

0 20 40 60 80 100

3 6 9 12 15
**Long term culture over 12 passages**

**H7**
- Matrigel
- ROCK inhibitor

**Matrigel**
- mAb84
- Oct4

**ROCK inhibitor**
- mAb84
- Oct4

Cell concentration ($\times 10^6$ cells/ml)

% Positive cells

0 20 40 60 80 100

0 3 6 9 12

0 20 40 60 80 100

0.5 1.0 1.5 2.0
Long term culture over 15 passages

**hiPS (IMR90)**

- Matrigel
- ROCK inhibitor

**Cell concentration (×10^6 cells/ml)**

<table>
<thead>
<tr>
<th>Passage</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
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<tr>
<td>Matrigel</td>
<td></td>
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<td>ROCK inhibitor</td>
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**% Positive cells**

- Matrigel
- ROCK inhibitor

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<td>100</td>
<td>80</td>
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<tr>
<td>ROCK inhibitor</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
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- ROCK inhibitor
- mAb84

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<td>mAb84</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
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</table>
Morphology of pluripotent stem cells treated with ROCK inhibitor on microcarriers

Replated cells stained
With Oct4 and DAPI
Blebbistatin also supports microcarrier culture of pluripotent stem cells without ECM coating.
MYPT1 and MLC are dephosphorylated by ROCK inhibitor and Blebbistatin.
Non-coated, ROCK inhibitor or Blebbistatin treated microcarrier cultures causes dephosphorylation of MYPT1 and MLC2 in the ROCK pathway.
Non-coated, ROCK inhibitor treated microcarrier cultures causes dephosphorylation of MYPT1 and MLC2 in the ROCK pathway.
Suspension bioreactor cultures of pluripotent stem cells on microcarriers treated with ROCK inhibitor

HES-3

- ROCK inhibitor
- Matrigel

H7

Cell concentration (x10^6 cells)

Time (Day)

Cell concentration (x10^6 cells)

Time (day)
Suspension bioreactor cultures of pluripotent stem cells on microcarriers Treated with ROCK inhibitor – FACS and morphology

B Matrigel coated microcarrier culture

ROCK inhibitor treated microcarrier culture

C

D
Tri-lineage differentiation ability of expanded pluripotent stem cells on microcarriers with ROCK inhibitor

![Graph showing fold increase in expression of various transcription factors and their roles in differentiation into pluri, endo, meso, and ecto lineages.](attachment:image.png)
Tri-lineage differentiation ability of expanded pluripotent stem cells on microcarriers with ROCK inhibitor
Karyotypic stability of expanded pluripotent stem cells

HES-3

H7

hiPSC (IMR90)
Characterization of neuroprogenitors (NPC) from microcarrier spinner flask cultures

NPCs produced in MC spinner flask cultures express PAX6 and Nestin (similar results observed with hiPSC derived NPCs)
H&E staining of neural progenitors on microcarriers

Red arrow indicates microcarrier
Neuronal differentiation of NPC and patch clamp recording of neurons

β-tubulin III DAPI  |  GFAP DAPI  |  O4 DAPI

Neurons  |  Astrocytes  |  Oligodendrocytes 100 microns

Spontaneous post synaptic currents  |  Membrane potential recordings

NPCs can differentiate to the 3 neuronal lineages
### NPC yield per hiPSC or hESC

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>2D Static Cultures</th>
<th>Suspension Microcarrier Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>hESC (HES-3)</td>
<td>$32 \pm 11$</td>
<td>$371 \pm 22$</td>
</tr>
<tr>
<td>hiPSC (IMR90)</td>
<td>$53 \pm 3$</td>
<td>$333 \pm 72$</td>
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<tr>
<td>Fold improvement</td>
<td></td>
<td><strong>6 to 11.5 times</strong></td>
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</table>

Bardy et al, Tissue Engineering 2012
5th Annual Stem Cell Society Singapore Symposium
Theme: Early Human Development & Fetal-maternal Medicine
18 – 19 November 2013, Biopolis, Singapore

Keynote:
❖ Rudolf Jaenisch, Whitehead Institute & MIT, USA

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• Jacob Hanna, Weizmann Institute, Israel
• Edith Heard, Institut Curie, France
• Takashi Hiiragi, EMBL, Germany
• Fuchou Tang, Peking University, China
• Mitinori Saitou, Kyoto University, Japan

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• De-programming/ Pluripotency
• Early Cell Fate Decisions
• Single Cell Technologies
• Germ Cell Development
• Fetal Programming/ Developmental Origins of Health & Disease
• Stem Cells of the Placenta
• Imprinting / Epigenetics

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Registration opens in May!