Neural Progenitor Cells: Better Biological Models of Neurodegenerative Disease

Brian Shapiro, Ph.D.
Technical Writer, ATCC
ATCC - Credible leads to Incredible

- ATCC has provided credible biomaterials for over 90 years
- We continue to cultivate collaboration
  - Among scientists across disciplines
  - Essential for accelerating innovative research
  - Leading to incredible, high-impact results.
- Our Cultivating Collaboration pledge: We bring scientists together to discuss
  - Breakthroughs in the state of science
  - Multidisciplinary approaches to key areas of research
  - Breaking the silos that impede research
- Our partnership with you, the scientific community, allows us all to reach the incredible
Agenda

Neural Progenitor Cells (NPCs) and Media

- Background information
- Differentiation potential of ATCC NPCs
- Toxicological studies
- Summary
Challenges associated with current models of the nervous system

- Primary cells from animals (mouse and rat neurons)
  - Not predictive
  - Donor variation

- Continuous cell lines (originally isolated from tumors)
  - Not normal
  - Not predictive

- Induced pluripotent stem cells (iPSCs; commercial or self-made)
  - Time and labor intensive
  - Often not validated for neural development
What is neurobiologists need in a better biological model

Advanced, biologically relevant models

- A true disease model
- Validated neural functioning
- Predictive for screening applications
Neural progenitor cells - Neuronal differentiation

NPCs:  
- Shorten research time  
- Human model  
- Predictive screening

Embryoid body

6-8 weeks

Astrocyte

3-4 weeks

Neuron

2-3 weeks

Oligodendrocyte

6-7 weeks
NPCs: An advanced model of the nervous system

A better biological model:
- Human models with no donor variation
- Live imaging is possible
- Cells exhibit full differentiation spectrum
- Complete system of cells and media is available

More meaningful results:
- More biologically relevant results/more predictive system
- Parkinson’s NPCs better replicate the disease state *in vitro*
- Markers allow for easy endpoint readout
- Can differentiate to neuronal and glial cells
- Easy to use and saves time
<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS-3003™</td>
<td>NPC Growth Kit – <em>add to DMEM/F12</em></td>
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<tr>
<td>ACS-3004™</td>
<td>NPC Dopaminergic Differentiation Kit – <em>add to DMEM/F12</em></td>
</tr>
<tr>
<td>ACS-5001™</td>
<td>NPCs derived from ATCC-DYS0530 Parkinson’s Disease (ACS-1013) <strong>New!</strong></td>
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<tr>
<td>ACS-5003™</td>
<td>NPCs derived from ATCC-BXS0117 (ACS-1031)</td>
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<td>ACS-5004™</td>
<td>NPCs derived from ATCC-BYS0112 (ACS-1026)</td>
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<td>ACS-5005™</td>
<td>Neural Progenitor Cells derived from XCL-1 DCX-GFP <em>for late neuron differentiation</em></td>
</tr>
<tr>
<td>ACS-5006™</td>
<td>Neural Progenitor Cells derived from XCL-1 GFAP-Nanoluc®-Halotag® <em>for astrocyte differentiation</em></td>
</tr>
<tr>
<td>ACS-5007™</td>
<td>Neural Progenitor Cells derived from XCL-1 MAP2-Nanoluc®-Halotag® <em>for early neuron differentiation</em></td>
</tr>
<tr>
<td>ACS-2103F™</td>
<td>Screening Fee – For Profit</td>
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</tbody>
</table>

ATCC® ACS-1026 – iPSC derived from bone marrow CD34+ cell from Caucasian male
ATCC® ACS-1031 – iPSC derived from bone marrow CD34+ cell from Asian female

Reporter lines from iPSC derived from cord blood CD34+ from a Caucasian male (XL-1 iPSCs from NIH)
QC testing of ATCC® NPCs

- Post-thaw cell viability: >80%
- Post-thaw viable cell number: >1x10^6 cells/vial
- Longevity: >15 PDLs or 5 passages
- NPC marker expression: Nestin^+, Pax-6^+, and Tra-1-60^-
- Differentiation potential:
  - Tuj1^+ early neurons
  - TH^+ dopaminergic neurons
- Identity: STR profile matching parental iPSC line
- Sterility, mycoplasma, and viral panel testing: None detected
Agenda

Neural Progenitor Cells (NPCs) and Media

- Background information
- **Differentiation potential of ATCC NPCs**
- Toxicological studies
- Summary
ATCC normal NPCs express NPC markers but not iPSC markers

- NPC Marker
  - Nestin
  - Pax-6

- iPSC Marker
  - Tra-I-60
ATCC Parkinson’s NPCs express NPC markers but not iPSC markers.
Dopaminergic neuron differentiation of NPCs

TuJ1  TH/DAPI
Dopaminergic neuron differentiation of Parkinson’s disease NPCs

TuJ 1

TH

TH + DAPI
Astrocyte and oligodendrocyte differentiation

Astrocyte differentiation

GFAP

Oligodendrocyte differentiation

O4

ACS-5003

ACS-5001
Dopaminergic neuron differentiation of NPC reporter lines

A. c. D. F.

MAP2- NanoLuc®-HaloTag®
(ACS-5007)

DCX-GFP
(ACS-5005)

GFAP-NanoLuc®-HaloTag®
(ACS-5006)
Expression of the luciferase reporter during dopaminergic neuron or astrocyte differentiation

Luciferase secretion during dopaminergic neuron differentiation of NanoLuc®-HaloTag® NPCs

![Image of dopaminergic neuron](image)

Luciferase secretion during astrocyte differentiation of GFAP-NanoLuc®-HaloTag® NPCs

![Image of astrocyte](image)
Expression of the GFP or HaloTag® reporter during dopaminergic neuron or astrocyte differentiation

DCX-GFP (Live imaging)

GFAP-Nanoluc®-Halotag® (ICC)

MAP2-Nanoluc®-Halotag® (ICC)

+ Dopaminergic differentiation medium
Expression of genes associated with the differentiation of NPCs

TaqMan® primers were used to identify the presence of other types of neurons during dopaminergic neuron differentiation using ATCC® ACS-3004™ media

- Dopaminergic neurons: TH, NURR1, VMAT2, AADC
- Glutamatergic neurons: GLS2, vGLUT1, vGLUT2
- GABAergic neurons: GABA (GABRB3)
- Motor neurons: EN1, LIM3, and Hb9
- Cholinergic neurons: ChAT
Early and dopaminergic neuron gene expression

Upregulation of early and dopaminergic neuron genes in ACS-5001, ACS-5003, and ACS-5007 NPCs during dopaminergic neuron differentiation

NPC-derived dopaminergic neurons
Expression of early neuron gene MAP2

Dopaminergic Differentiation

Fold Induction of MAP2 mRNA

ACS-5003
ACS-5007

ACS-5001

0 1wk 2wk 3wk

MAP2

Dopaminergic Differentiation

Fold Induction of MAP2 mRNA

0 1wk 2wk 3wk

ACS-5001

**
Expression of dopaminergic neuron gene TuJ1

**TuJ1**

<table>
<thead>
<tr>
<th>Fold Induction of TuJ1 mRNA</th>
<th>Dopaminergic Differentiation</th>
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<tbody>
<tr>
<td>ACS-5003</td>
<td>0</td>
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<tr>
<td>ACS-5007</td>
<td>1wk</td>
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<tr>
<td></td>
<td>2wk</td>
</tr>
<tr>
<td></td>
<td>3wk</td>
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**TuJ1**

<table>
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<tr>
<th>Fold Induction of TuJ1 mRNA</th>
<th>Dopaminergic Differentiation</th>
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</thead>
<tbody>
<tr>
<td>ACS-5001</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1wk</td>
</tr>
<tr>
<td></td>
<td>2wk</td>
</tr>
<tr>
<td></td>
<td>3wk</td>
</tr>
</tbody>
</table>
Expression of dopaminergic neuron gene TH

Dopaminergic Differentiation

Fold Induction of TH mRNA

TH

ACS-5003
ACS-5007

0 1wk 2wk 3wk

Fold Induction of TH mRNA

TH

ACS-5001

0 1wk 2wk 3wk
Expression of dopaminergic neuron gene NURR1

Dopaminergic Differentiation

Fold Induction of NURR1 mRNA

0 1wk 2wk 3wk

ACS-5003
ACS-5007

NURR1

Fold Induction of NURR1 mRNA

0 1wk 2wk 3wk

ACS-5001

NURR1

***

**
Expression of VMAT2

Fold Induction of VMAT2 mRNA

Dopaminergic Differentiation

VMAT2

Fold Induction of VMAT2 mRNA

Dopaminergic Differentiation

ACS-5003
ACS-5007

ACS-5001

***
**
***
***

*
Expression of DAT

Fold Induction of DAT mRNA

**Dopaminergic Differentiation**

![Graphs showing DAT induction over time and treatment with ACS-5003 and ACS-5007.](image)
Expression of AADC

AADC

Fold Induction of AADC mRNA

Dopaminergic Differentiation

ACS-5003
ACS-5007

0 1wk 2wk 3wk

Fold Induction of AADC mRNA

Dopaminergic Differentiation

ACS-5001

0 1wk 2wk 3wk
Gene expression other neuronal subtypes

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Gutamatergic</th>
<th>GABAergic</th>
<th>Motor</th>
<th>Cholinergic</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>GLS2</td>
<td>vGLUT1</td>
<td>vGLUT2</td>
<td>GABRB3</td>
</tr>
<tr>
<td>ACS-5001</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>ACS-5003</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>ACS-5007</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

- = no significant increase in expression after 3 weeks
+ = increased expression within 3 weeks, fold over control
Protein expression

Confirmation of protein expression in ACS-5007 NPCs during dopaminergic differentiation by immunocytochemistry

NPC-derived neurons
Confirmation of dopaminergic neuronal-specific protein expression during differentiation by immunocytochemistry
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Neurotoxicity studies – undifferentiated NPCs

ACS-5003 NPCs

Viability (% of Vehicle Control)

DMSO Control  Paclitaxol  Cisplatin  Piperine  Vincristine  Hydroxyurea  Amiodarone  Chlorodexine

1 µM  10 µM  100 µM

*  **  ***
Neurotoxicity studies – undifferentiated NPCs

ACS-5001 NPCs
Dose-response curves for cell viability of ACS-5003 ACS-5007 NPCs treated with paclitaxel for two days

![Graph showing dose-response curves for ACS-5003 and ACS-5007 NPCs treated with paclitaxel. The x-axis represents the log concentration of paclitaxel in µM, ranging from -3 to 5. The y-axis represents viability as a percentage of vehicle control, ranging from 0 to 100. The graph shows a decrease in viability with increasing concentration of paclitaxel.]
Neurotoxicity studies – NPCs-derived neurons

ACS-5007 NPCs-derived neurons

Viability (% of Vehicle Control)

- Paclitaxel
- Cisplatin
- Vincristine
- Hydroxyurea
- Amiodarone
- Chlorhexidine
- Pipeline
- DMSO control

Comparisons:
- 1 µM
- 10 µM
- 100 µM
High content imaging analysis of neurotoxicity in normal NPC-derived neurons

![Image of cellular imaging showing effects of different treatments on neuron morphology.](image1.png)

![Graph showing total neurite length per neuron for different treatments.](image2.png)
## Overall neurotoxicity studies

<table>
<thead>
<tr>
<th>Toxin</th>
<th>ACS-5001 NPCs</th>
<th>ACS-5003 NPCs</th>
<th>NPC-derived neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>Toxic</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Toxic</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Resistant</td>
<td>Weakly toxic</td>
<td>Resistant</td>
</tr>
<tr>
<td>Piperine</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Toxic</td>
<td>Toxic</td>
<td>Weakly toxic</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>Resistant</td>
<td>Weakly Toxic</td>
<td>Resistant</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Toxic</td>
<td>Toxic</td>
<td>Resistant</td>
</tr>
</tbody>
</table>
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NPCs - Summary

- Cells and media with easy to use protocols
  - Expansion and Differentiation Medium

- Human model with no donor variation
  - Ability to expand and bank

- Differentiation across a wide spectrum of neural and glial lineages
  - Neurons
  - Astrocytes
  - Oligodendrocytes

- Live imaging of differentiation
  - GFP expression upon neural differentiation
NPCs - Summary

- Our studies demonstrated that ATCC normal and PD NPCs have the potential to be differentiated into:
  - Dopaminergic neurons
  - GABAergic neurons
  - Glutamatergic neurons
  - Motor neurons
  - Cholinergic neurons
    after treatment of NPCs with ATCC dopaminergic differentiation media

- ATCC NPCs are suitable for drug screening applications
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Cultivating collaboration to elevate biological models

Let’s continue to cultivate collaboration:

- Help us elevate our Better Biological Models
- Advanced biological models enable greater
  - Specificity
  - Functionality
- Join our community of early adopters
- Our partnership with you, the scientific community, allows us all to reach the incredible

2020.atcc.org/elevating-biological-models

for more information about becoming an early adopter of NPCs