Human iPSC-derived Midbrain Dopaminergic Neurons for Parkinson’s Disease Modeling and Cell Therapy

ISSCR Innovation Showcase
Thursday, June 15th, 2017
Transformative Potential of iPSC Technology

Any Human Donor

Blood Sample

Reprogramming

iPS Cell Lines

Genome Editing

Differentiation

Differentiated Human Cell Types

Enabling for Toxicology... Drug Discovery... and Regenerative Medicine
Strategic Business Moves

1. Disease Modeling

Understanding Mechanism

Drug Screening

2. Cell Therapy

Cellular Dynamics to Realign Business Units to Better Serve Therapeutic and Life Science Customers
Development of Human Midbrain Dopaminergic Neurons

- Additional differentiation tweaks and workflow improvements
- ~40 day protocol established with excellent reproducibility
- Each batch run yields nearly $4 \times 10^9$ cells
  - Equal to # of mDA neurons in 10,000 human brains!!
- Optimized cryopreservation protocol

mDA protocol based on published literature and Licensed from MSKCC (Kriks et al. *Nature* 2011)
Characterization of iCell DopaNeurons

MAP2–AF488
Nestin–AF647
FoxA2–AF647
LMX1–AF488

Map2 / Nestin / Hoechst

TH / FoxA2 / Hoechst

Characterization of iCell DopaNeurons

Thaw Viability

% Viable Cell

Lot A Lot B Lot C

Relative Expression (vs. GAPDH)

Regional Specification
Dopaminergic Identification
Neuronal Subtypes

Day 7 PT
Day 14 PT
Day 21 PT
Day 28 PT
Day 42 PT
Human SN

June 19, 2017
Numerous Applications with iCell DopaNeurons

Day 20

20 μM d-AP5

HTRF cAMP

\[
\text{Delta F\%}
\]

Log [Apomorphine] (M)

MitoTracker Red / Cyto-ID
Disease Modeling with iPSC – "Disease-in-a-Dish"

**INNATE**
- Healthy Donor
- Donor with Genetic Disease
- Reprogramming to iPSC
- iPSC Differentiation

**ENGINEERED**
- Healthy Donor
- Donor with Genetic Disease
- Genome Engineering
- Controls

**INDUCED**
- Healthy Donor
- Healthy Conditions
- Disease-inducing Conditions

Phenotypic and Functional Analysis
Starting material is an apparently healthy normal (male) iPSC line
- Genome engineering of iPSC to introduce A53T mutation into *SNCA* gene
- Differentiation into human mDA neurons just as for iCell DopaNeurons
- Multiple batches / lots have been made and tested
- Phenotypic and functional analysis: qPCR, ICC, Seahorse, Ca²⁺ assay, MEA
- Still TBD: cell death accelerated? A53T release less DA? α-syn aggregation?
Differential Gene Expression in SNCA A53T DopaNeurons

qPCR analysis reveals decreased TH & DDC and increased COMT expression, suggesting that biosynthetic levels of dopamine in the SNCA A53T neurons should be decreased.
SCNA A53T DopaNeurons Have Strong α-Syn Staining

Immunofluorescence staining shows detectable levels of alpha-synuclein in the control cells and more in the mutant line (data not quantified); several Abs screened and this clone is the best
SCNA A53T Have Higher Mito Respiratory Capacity

Bioenergetic analysis of WT vs. A53T cells indicates that the response to FCCP and the max respiratory capacity is greater in the mutant line; data is normalized based on cell number.
Both iCell and MyCell DopaNeurons display high activity and synchronous bursting on MEA; A53T cells have fewer bursts per minute, but the intensity of those bursts are ~5X greater.
SNCA A53T Display Spontaneous Ca$^{2+}$ Oscillations Earlier

MyCell SNCA A53T DopaNeurons display spontaneous Ca$^{2+}$ oscillations earlier in culture (e.g. Day 7) with an amplitude and bursting pattern that is significantly different than WT control.
Modeling Parkinson’s Disease with iPSC – Summary

- Familial PD mutation SNCA A53T was engineered in iPSC to make isogenic lines and then differentiated to human midbrain dopaminergic neurons
- Distinct differences in multiple phenotypic assays was observed and repeated
What’s Next? More DopaNeurons from More Donors

• In connection with Indiana University, CDI and MJFF are working to reprogram 85 iPSC from PPMI volunteers (with and without Parkinson’s disease)
• Expanding access to research tools is something iPSC and PD field need now
• iPSC technology provide unique advantages for improving understanding of PD
CDI’s Cell Therapy Program for Parkinson’s Disease

Chris McMahon, Ph.D.
Director
The Time is Right for iPSC-derived Cell Therapies
Cell Therapy Paradigms

- **Autologous**
  - Blood or skin cells
  - Reprogramming

- **Allogeneic** or HLA-matched
  - iPS cells
  - Manufacturing
  - Terminally Differentiated Cells
Generation of a Therapeutic iPSC Bank

**Project Status**

- **Completed**
  - Phase I – N = 5
    - 3 GMP Master Cell Banks completed, all 5 donors reprogrammed
    - 35% coverage of US population

- **In Progress**
  - Phase II – N = 12, N = 200
    - 12 donors = >50% population coverage
    - Top 200 = >95% population coverage

- **Planning**
  - Phase III – Global Banks; N ~ TBD
    - Additional genotypes in each geography (Japan, Europe, China)
    - In planning and evaluation stage

**Donor Database**

- 12M people in database, all high-resolution genotyped
- 200,000 HLA homozygotes
- Includes top 5 HLA genotypes for Japan, Europe, China, etc.

**Donor Database**

- Top 500 Homozygous HLA Targets
  - Top 183 haplotypes
    - Cover 95%
  - Top 110 haplotypes
    - Cover 90%
  - Top 12 haplotypes
    - Cover 50%

**cGMP iPSC lines from common HLA haplotypes available to CDI partners**

Potential Donors

- HLA Homozygous
  - (~1% of general population)

Potential Recipients

- HLA Matching

- US Population Coverage

- Top 500 Homozygous HLA Targets
# Cell Therapy Division of CDI: Pipeline

<table>
<thead>
<tr>
<th>Indication</th>
<th>Therapeutic</th>
<th>Discovery</th>
<th>Pre-clinical</th>
<th>IND</th>
<th>Phase I</th>
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<td><strong>OCULAR</strong></td>
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<td>Age-related Macular Degeneration</td>
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<td>Retinitis Pigmentosa and Cone Dystrophies</td>
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<td><strong>CARDIAC</strong></td>
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<td>Heart Failure (ischemic cardiomyopathy)</td>
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<tr>
<td><strong>NEURODEGENERATIVE</strong></td>
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<tr>
<td>Parkinson's Disease</td>
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<td><strong>ONCOLOGY</strong></td>
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<tr>
<td>CAR-T/NK</td>
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</table>
Parkinson’s Disease

Prevalence  >200/100,000 : 1M in USA
(2nd most common neurodegenerative disease)

Symptoms  • Loss of motor control
• Tremors and rigidity
• Dementia, depression, etc.

Mechanism  Loss of A9 midbrain dopamine neurons
(α-synuclein accumulation, Lewy body inclusions)

Cause  Idiopathic (95%); genetic mutation (5%)

Treatments  • L-DOPA
• Deep brain stimulation

Cell Therapy  Fetal tissue grafts
Midbrain Dopaminergic (mDA) Neuron Engraftment Trials

Meta-analysis of ventral mesencephalic transplant trials
From Barker et al. (2013) Lancet Neurol 12:84

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Estimate (95% CI)</th>
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<tr>
<td>Halifax</td>
<td>-43.20 (-65.02 to 21.38)</td>
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<tr>
<td>Lund</td>
<td>-37.79 (-49.95 to -25.64)</td>
</tr>
<tr>
<td>Colorado/Columbia</td>
<td>-19.09 (-29.58 to -8.60)</td>
</tr>
<tr>
<td>Paris</td>
<td>-11.47 (-21.83 to -1.12)</td>
</tr>
<tr>
<td>Tampa</td>
<td>2.96 (-9.92 to 15.85)</td>
</tr>
</tbody>
</table>

Sham groups
- Tampa: 13.77 (-4.27 to 31.81)

Fetal Cell transplant trial

Consensus
- Dissection
- Cell prep
- Patient selection
- Surgery
- Endpoints
- Follow-up

Stem Cell Therapy Trials
- ESC-mDA
- iPSC-mDA
- NSC

UPDRS score (% change)

1990 2000 2010 2020
Pre-clinical Evidence for mDA Neurons in PD

- Allogeneic DA Neurons
- Cryopreserved in DMSO

**Modes of Action**

- Engraftment
- Innervation
- Integration into host circuitry
- Dopamine release

Starter > traced neuron model for synaptic connections to graft (Grealish et al., 2015)

Dopamine release from synapse before and after sensitization
Transitioning the Process from Life Sciences to Cell Therapy

**Need:** Candidate for PD Therapeutic

**Goal:** Modify existing mDA differentiation process for clinical use

1. Transition away from research grade reagents
2. Develop a new purification approach
3. Optimize robust process for scale out: Multiple HLA-matched or autologous iPSC lines
4. Identify best stage of differentiation for therapeutic candidate
## Transition Away From Research Grade Reagents

<table>
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<tr>
<th>Material</th>
<th>iCell DopaNeurons</th>
<th>Transitioned to</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPSC Line</td>
<td>CDI internal</td>
<td>✓ cGMP HLA homozygous</td>
</tr>
<tr>
<td>Surface ECMs</td>
<td>Animal origin</td>
<td>✓ Recombinant Human</td>
</tr>
<tr>
<td>Growth Factors</td>
<td>Xeno protein or expression system</td>
<td>✓ Recombinant human, Human or non-animal expression</td>
</tr>
<tr>
<td>Media and Buffers</td>
<td>R&amp;D grade</td>
<td>✓ Cell therapy grade</td>
</tr>
<tr>
<td>Other materials</td>
<td>Unacceptable endotoxin</td>
<td>✓ Endotoxin-free</td>
</tr>
</tbody>
</table>
New Purification Scheme Yields Similar Neuron Purity

No Purification  

iCell Purification  

NEW Purification  

MAP2  

Nestin  

Day 14 Post-thaw  

Day 26 Post-thaw  

$cGMP$-compliant purification removes non-target cells
Establishing Process Robustness Across iPSC Lines

- **Large matrix experiments** to optimize timing and concentration of all patterning factors
- **Additional process standardization** required to make process more robust and efficient
- End result = **Defined Process for PD Therapeutic Cells**
Scale-out of Therapy Process Across HLA iPSC Lines

iPSC Line 1
HLA Rank: #3

iPSC Line 2
HLA Rank: #1

iPSC Line 3
HLA Rank: #1

Progenitor
FoxA2 / Lmx1

Cells at Cryo

MAP2

FoxA2

Nestin

TH
Scale-out of Therapy Process Across PD Patient iPSCs

4 PD Patients

cGMP-compatible iPSC-derived mDA Neurons

cGMP-compatible iPSCs

- Feasibility for Autologous PD cell therapy
- Collaboration with Rush University (Kordower, Wakeman, Hall, and Goetz)

| Donor | iPSC Clone | Progenitor FOXA2+/LMX1+ | Final mDA Purity (FOXA2+) | Final Neuron Purity (Tuj1+ / nestin-)
<table>
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<td>1</td>
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<td>++++</td>
<td>96%</td>
<td>99%</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>+++</td>
<td>62%</td>
<td>95%</td>
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</table>

• 7 of 9 iPSC lines exhibit excellent mDA neuron purity at end of process (successful lines from all three donors)
• Demonstrates robustness of process across iPSC lines and feasibility of autologous and HLA donor therapy approaches
Current PD Therapy Differentiation Process

- PATTERNING
- SPECIFICATION
- NEUROGENESIS
- DIFFERENTIATION

**cGMP**
**iPSC**
**WCBs**

In vivo *range-finding experiments*

- 200-300 vials
  ~10⁷ cells each

Purified mDA neurons

Immature mDA neurons 1

Immature mDA neurons 2

Purification

A | B | C
---|---|---
PATTERNING | SPECIFICATION | NEUROGENESIS | DIFFERENTIATION | Purification
Neural progenitors | Dopaminergic progenitors | Dopaminergic neuroblasts | Dopaminergic neurons | Purified mDA neurons
mDA progenitors

June 19, 2017
PD Therapy Cells Show Consistency by Gene Expression

Late Stage Gene Expression

Fold Gene Expression Relative to GPDH

mDA Markers

Other Markers

Batch 1
Batch 2
Batch 3
Batch 4
iCell DopaNeurons
Substantia Nigra
PD Therapy Cells are Similar to iCell DopaNeurons by ICC

iCell GABANeurons | iCell DopaNeurons | PD Therapy Cells

FoxA2 Lmx1

TH FoxA2
PD Therapy Cells Show Equivalent Functionality \textit{in vitro}

**Dopamine Release – ELISA (DIV 14)**

![Dopamine Release Graph](image1)

**Electrophysiology – MEA (DIV 14)**

![Electrophysiology Graph](image2)

June 19, 2017
Quality Control – Developing a Cell Type to a Specification

<table>
<thead>
<tr>
<th>Specification</th>
<th>Assay</th>
<th>In-Process</th>
<th>Final Product</th>
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</thead>
<tbody>
<tr>
<td>Viability/ Cell Count</td>
<td>Trypan Blue exclusion (ViCell)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Progenitor purity</td>
<td>flow cytometry</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Neuron purity</td>
<td>flow cytometry</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Midbrain purity</td>
<td>flow cytometry</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DA Neuron purity</td>
<td>flow cytometry</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Residual iPSC</td>
<td>qPCR</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Other Residual(s)</td>
<td>Analytical</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Genetic Identity</td>
<td>STR ID</td>
<td></td>
<td>X</td>
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<tr>
<td>Sterility</td>
<td>USP &lt;71&gt; Sterility test</td>
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<tr>
<td>Endotoxin</td>
<td>LAL method</td>
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<tr>
<td>Mycoplasma</td>
<td>PTC method</td>
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<tr>
<td>Proliferative Cells</td>
<td>EdU flow cytometry</td>
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<tr>
<td>Off-target Cell Types</td>
<td>TBD</td>
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<tr>
<td>Karyotype</td>
<td>SNP assay</td>
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<tr>
<td>Potency</td>
<td>TBD</td>
<td>X</td>
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</tr>
</tbody>
</table>
Examples of QC Assay Development (Converting ICC → Flow)

Progenitor
FoxA2/Lmx1 ICC

Isotype
FoxA2/Lmx1
PASS

FAIL

Proliferative Cell Assay (EdU)

Progenitor
iPSC
Day 24

No EdU
DA 3dpt
iPSC

EdU
Cryo

EdU
FoxA2

FSC
Pre-Clinical Animal Studies – Completed

- Short-term engraftment in rats
  - Cryopreserved vs. Fresh cells
  - Differentiation process variations

- Efficacy in rats
  - Differentiation process variations
  - Purification strategies

- Short-term engraftment in primates
  - iCell DopaNeurons
  - PD Therapy Cells

Jeffrey Kordower
Director, Research Center for Brain Repair and Neuroscience Section Head
Rush University Medical Center

Dustin Wakeman
Senior Research Scientist, RxGen, and Adjunct Assistant Professor
Yale School of Medicine
iCell DopaNeurons Survive Transplantation into Rats

**Substantia Nigral Grafts**

- DopaNeurons transplanted to rat substantia nigra (SN) **survive and extend neurites along the nigrostriatal tract** at 6 weeks post-engraftment and mDA neuron phenotype (FoxA2⁺ / TH⁺) is maintained
- DopaNeurons transplanted to the striatum survive and innervate well **without proliferation** (2 weeks)

**Striatal Grafts**

- Striatal Grafts
  - DopaNeurons transplanted to rat substantia nigra (SN) survive and extend neurites along the nigrostriatal tract at 6 weeks post-engraftment and mDA neuron phenotype (FoxA2⁺ / TH⁺) is maintained
  - DopaNeurons transplanted to the striatum survive and innervate well without proliferation (2 weeks)

**Wakeman et al. Stem Cell Reports 2017**

**Summary Table**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Mean (Survival %)</th>
<th>Standard Error (%)</th>
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</thead>
<tbody>
<tr>
<td>iCell DopaNeurons</td>
<td>23.96</td>
<td>8.00</td>
</tr>
<tr>
<td>iCell DopaNeurons Early Cryo</td>
<td>18.18</td>
<td>2.89</td>
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</table>
6-OHDA Rodent Model of Parkinson’s Disease

- Unilateral injection of 6-OHDA to medial forebrain bundle
- Toxin damages DA neuron terminals
- Retrograde degeneration

Unilateral loss of midbrain DA neurons

Hemi-parkinsonian behavioral deficits
- Amphetamine-induced rotations
- Apomorphine-induced rotations
- Cylinder test

mDA neuron engraftment

Test for behavioral deficit improvement
Testing Functional Efficacy of iPSC-mDA Neurons in Rats

- **PD Animal Model:** Spague-Dawley rats lesioned with 6-OHDA and tested for PD-like symptoms; then engrafted with iCell DopaNeurons (n=10) or vehicle control (n=4)
- Amphetamine- and Apomorphine-induced rotational asymmetry testing demonstrates **significant behavioral improvements at 5 and 6 months post-engraftment** in animals with successful grafts

Wakeman et al. Stem Cell Reports 2017
• iCell DopaNeurons transplanted into the striatum of MPTP-treated cynomolgus macaques survived and displayed fiber outgrowth into the host striatum after 3 months
• Engrafted cells have mature neuron morphology (large, arborized soma) and express tyrosine hydroxylase (TH)
Graft Survival of PD Therapy Cells in Primates

- PD Therapy mDA Neurons (cGMP-compliant process) transplanted into the striatum of MPTP-treated African Green Monkeys survived and displayed fiber outgrowth into the host striatum after 3 months.
- Engrafted cells have mature mDA neuron morphology (large, arborized soma) and express tyrosine hydroxylase (TH).
## Status of CDI / FUJIFILM Cell Therapy Program for PD

<table>
<thead>
<tr>
<th>Category</th>
<th>Current Status</th>
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</thead>
<tbody>
<tr>
<td>HLA iPSC Bank</td>
<td>Stage 1 <strong>Complete</strong> (n=5); Stage 2 <em>In Progress</em></td>
</tr>
<tr>
<td>Transition to Clinical Grade Reagents</td>
<td><strong>Complete</strong></td>
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<tr>
<td>Purification Approach to Ensure Safety</td>
<td><strong>Complete</strong></td>
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<tr>
<td>Robust Process Across iPSC Lines</td>
<td><strong>Complete</strong></td>
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<tr>
<td>Process Scale Up</td>
<td><strong>Complete</strong> for Phase I &amp; Phase II trial needs</td>
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<tr>
<td>Pre-Clinical Studies</td>
<td>Multiple promising pilot studies with respect to safety and efficacy; more <em>In Progress</em></td>
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<tr>
<td>Clinical Studies</td>
<td><em>Planning stages</em></td>
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</table>
Thank You!