Neuroprotective and Regenerative Stem Cell Therapies in a Pig Stroke Model

Franklin D. West
Regenerative Bioscience Center
University of Georgia
Financial Disclosure

• No financials to disclose
Second leading cause of death globally

Number one cause of long term disability in US

Every 40 seconds someone has a stroke

Every 4 minutes someone dies of stroke
Stroke Death Rates, 2015 - 2017
Adults, Ages 35+, by County

Rates are spatially smoothed to enhance the stability of rates in counties with small populations.

Data Source:
National Vital Statistics System
National Center for Health Statistics
Over 700 treatments have gone to clinical trial for stroke and only two have been FDA approved:

- Tissue Plasminogen Activator (tPA)
- Thrombectomy

- Limited window of treatment
- No regenerative potential
- <25% of patients can receive tPA and/or thrombectomy

Why Do All the Drugs Fail?

An assessment of failed treatments by the Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) group has resulted in the identifying 2 major needs of the research community:

1) a regenerative cell therapy that will not only protect cells from ischemic injury but replace the lost and damaged tissues

2) a translational animal model more similar to humans for testing treatments

Induced Pluripotent Stem Cell-Derived Neural Stem Cells (iNSC) to Treat Neural Injury
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Neural stem cell therapy for stroke: A multimechanistic approach to restoring neurological function

Emily W. Baker | Holly A. Kinder | Franklin D. West

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iPSC-NSCs Express SOX1 and Nestin
Differentiated iPSC-NSCs Express βIII-Tubulin and MAP2 Neuron Markers
Differentiated iPSC-NSCs Express the GFAP Astrocyte Marker
Differentiated iPSC-NSCs Express the O4 Oligodendrocyte Marker
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1. Size

2. Lissencephalic Vs Gyrencephalic

3. White Matter

Development and characterization of a Yucatan miniature biomedical pig permanent middle cerebral artery occlusion stroke model

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Simon R Platt,1,2,*, Shannon P Holmes,1,3, Elizabeth W Howerth,1,6, Kylee Jo J Duberstein,1,5, C. Robert Dove,1,5, Holly A Kinder,1,6, Emily L Wyatt,1,6, Amie Y Linville,1,6, Vivian W Lau,1,6, Steven L Stice,1,5, William D Hill,1,5, David C Hess,1 and Franklin D West,1,6

Gait analysis in a pre- and post-ischemic stroke biomedical pig model

Kylee Jo Duberstein,1,6, Simon R. Platt,1,6, Shannon P. Holmes,1,5, C. Robert Dove,1,5, Elizabeth W. Howerth,1,6, Marc Kent,1, Steven L. Stice,1,5, William D. Hill,1,5, David C. Hess,1, Franklin D. West,1,6

T2-FLAIR

DWI

ADC
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Overarching Hypothesis

Transplanted iNSCs will produce regenerative trophic factors and undergo differentiation leading to cellular, tissue and functional recovery in a pig model of ischemic stroke.

Emily Baker
Sample Collection:

1. Magnetic Resonance Imaging (MRI)
2. Immunohistochemistry
DWI and ADC Maps Confirm Ischemic Stroke
iNSC Treatment Leads to Slowed Tissue Atrophy

Tissue Volume Change (% of Contralateral Hemisphere)

-20  -10  0  10  20

Non-Treated  iNSC Treated

24hr  1wk  4wk  12wk

*  *  #

*indicates significance from contralateral hemisphere
# indicates significance between treatment groups
iNSC Treatment Leads to Improved White Matter Integrity

*indicates significance from 24 hours, within treatment
iNSC Treatment Leads to Recovery in NAA

![Graph showing change in NAA over time]

- * indicates significance between treatment groups
- # indicates significance from 24 hours, within treatment
iNSC Treatment Leads to Recovery in Choline

- Normal
- Non-Treated
- iNSC Treated

*indicates significance between treatment groups
iNSC Treatment Leads to Recovery in Creatine

Change in Cr

Non-Treated
iNSC Treated

* indicates significance between treatment groups

Normal

24hr 1wk 4wk 12wk

Non-Treated
iNSC Treated

Cho 2.08
NAA 1.18

Cho 3.64
NAA 2.49

Cr 0.26
Cr 1.92

ppm

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iNSC Treatment Improves Cerebral Blood Perfusion

**Mean Transit Time**

- **Non-Treated**
- **iNSC Treated**

<table>
<thead>
<tr>
<th>Time</th>
<th>Non-Treated</th>
<th>iNSC Treated</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>24hr</td>
<td></td>
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<td>4wk</td>
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<tr>
<td>12wk</td>
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</table>

*indicates significance between treatment groups

**Time to Peak**

- **Non-Treated**
- **iNSC Treated**

<table>
<thead>
<tr>
<th>Time</th>
<th>Non-Treated</th>
<th>iNSC Treated</th>
<th>Normal</th>
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<tbody>
<tr>
<td>24hr</td>
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<tr>
<td>12wk</td>
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*indicates significance between treatment groups
iNSC Treatment Reduces Neuron Loss at the Lesion Border

A Normal
B Non-Treated
C iNSC Treated

NeuN

D

<table>
<thead>
<tr>
<th>Condition</th>
<th>NeuN+ cells/mm²</th>
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<tbody>
<tr>
<td>Normal</td>
<td>350</td>
</tr>
<tr>
<td>Non-Treated</td>
<td>250</td>
</tr>
<tr>
<td>iNSC Treated</td>
<td>300</td>
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</table>

* Indicates significant difference compared to Non-Treated.
iNSC Treatment Reduces Microglia Activation at the Lesion Border

E Normal  F Non-Treated  G iNSC Treated

H

% Iba1+ Area

0 5 10 15 20 25

Normal Non-Treated iNSC Treated

# #

# #

Iba1

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iNSC Treatment Promotes Endogenous Neuroblast Proliferation and Migration to the Lesion Border

![Images of DCX+ cells/mm² for Normal, Non-Treated, and iNSC Treated conditions]

- **A**: Normal
- **B**: Non-Treated
- **C**: iNSC Treated

![Bar graph showing DCX+ cells/mm²]

- Normal: 0
- Non-Treated: 5
- iNSC Treated: 15

* indicates statistical significance.
Transplanted iNSCs Differentiate into Neurons
Transplanted iNSCs Differentiate into Neurons
Transplanted iNSCs Differentiate into Oligodendrocytes
Transplanted iNSCs Differentiate Primarily into Neurons

% Colocalization with HNA

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<th></th>
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<th>Olig2</th>
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<tbody>
<tr>
<td>0</td>
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Conclusions

iNSC treatment promotes brain tissue recovery through:

– Improved white matter integrity
– Improved neurometabolism
– Enhanced cerebral perfusion
– Neuroprotective
– Promoted neurogenesis
– Cell replacement
– Immunomodulatory

Human iNPC therapy leads to improvement in functional neurologic outcomes in a pig ischemic stroke model
**Funding Sources:**
NIH-NINDS, DoD, Bill and Melinda Gates Foundation, USDA, ArunA Biomedical, REM, BIRC

**West Laboratory:**
Emily Baker, PhD  
Vivian Lau, DVM, MS  
Harrison Grace, PhD  
Holly Kinder, PhD  
Erin Kaiser  
Kelly Scheulin  
Elizabeth Waters  
Soo Shin  
Sydney Sneed

**Collaborators:**
Simon Platt, DVM  
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