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SOURCES OF VARIABILITY IN PRECLINICAL AND CLINICAL RESEARCH ON STEM CELL THERAPIES FOR ALS

Exploring sources of variability related to clinical translation of regenerative engineering products – A workshop

Cedars-Sinai Regenerative Medicine Institute

Clive Svendsen, PhD

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Sources of variability in preclinical and clinical research on stem cell therapies for ALS

- Introduction
- Manufacturing human neural progenitors
- Case study : Neural progenitor-derived astrocytes for ALS
- Transitioning to iPSC-derived product

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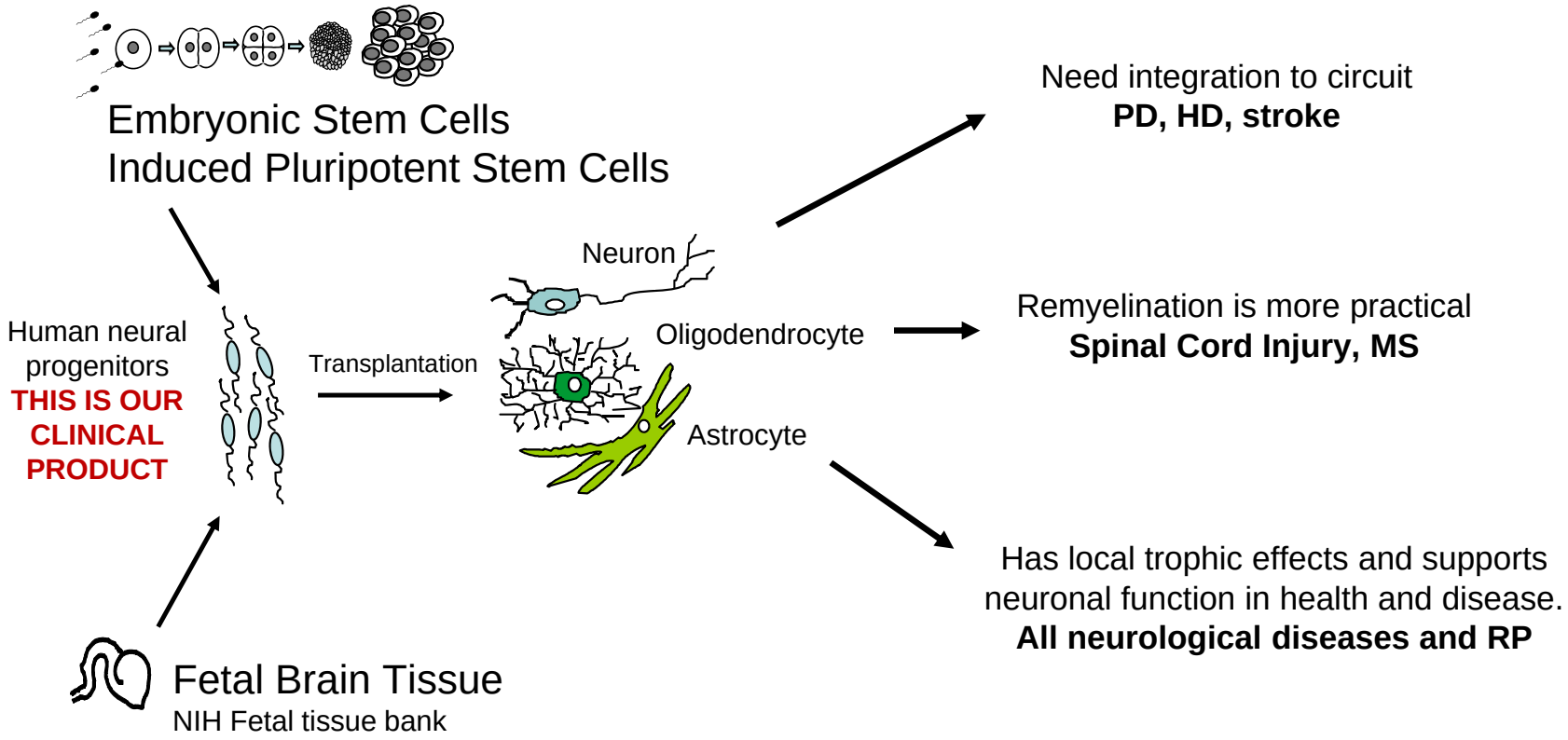
Stem cells for neurological disorders



Astrocytes are essential for normal brain function

Acta Neuropathol (2016) 131:323–345
DOI 10.1007/s00401-015-1513-1

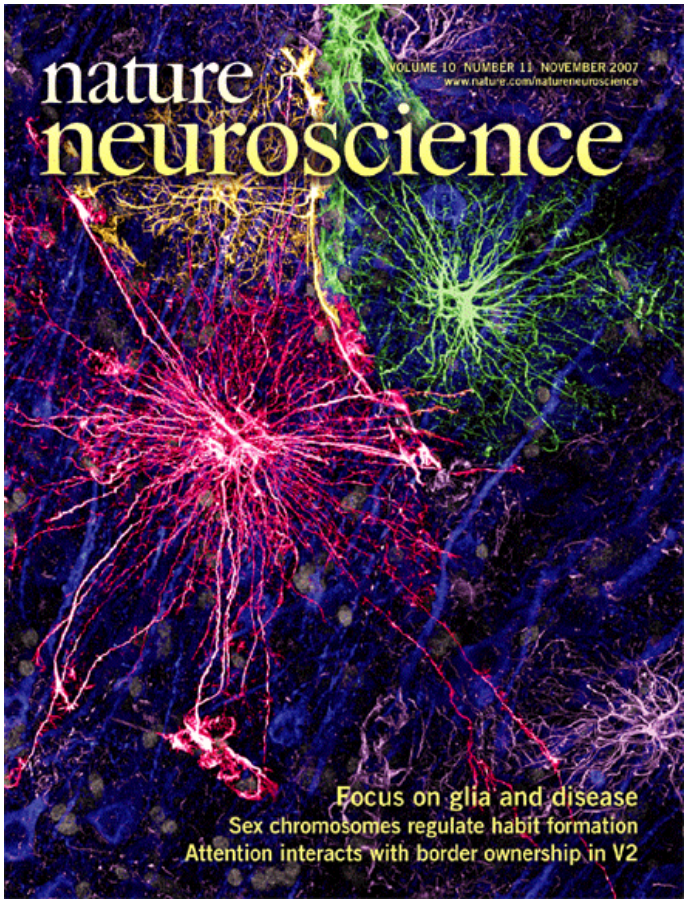
REVIEW

Astrocytes: a central element in neurological diseases

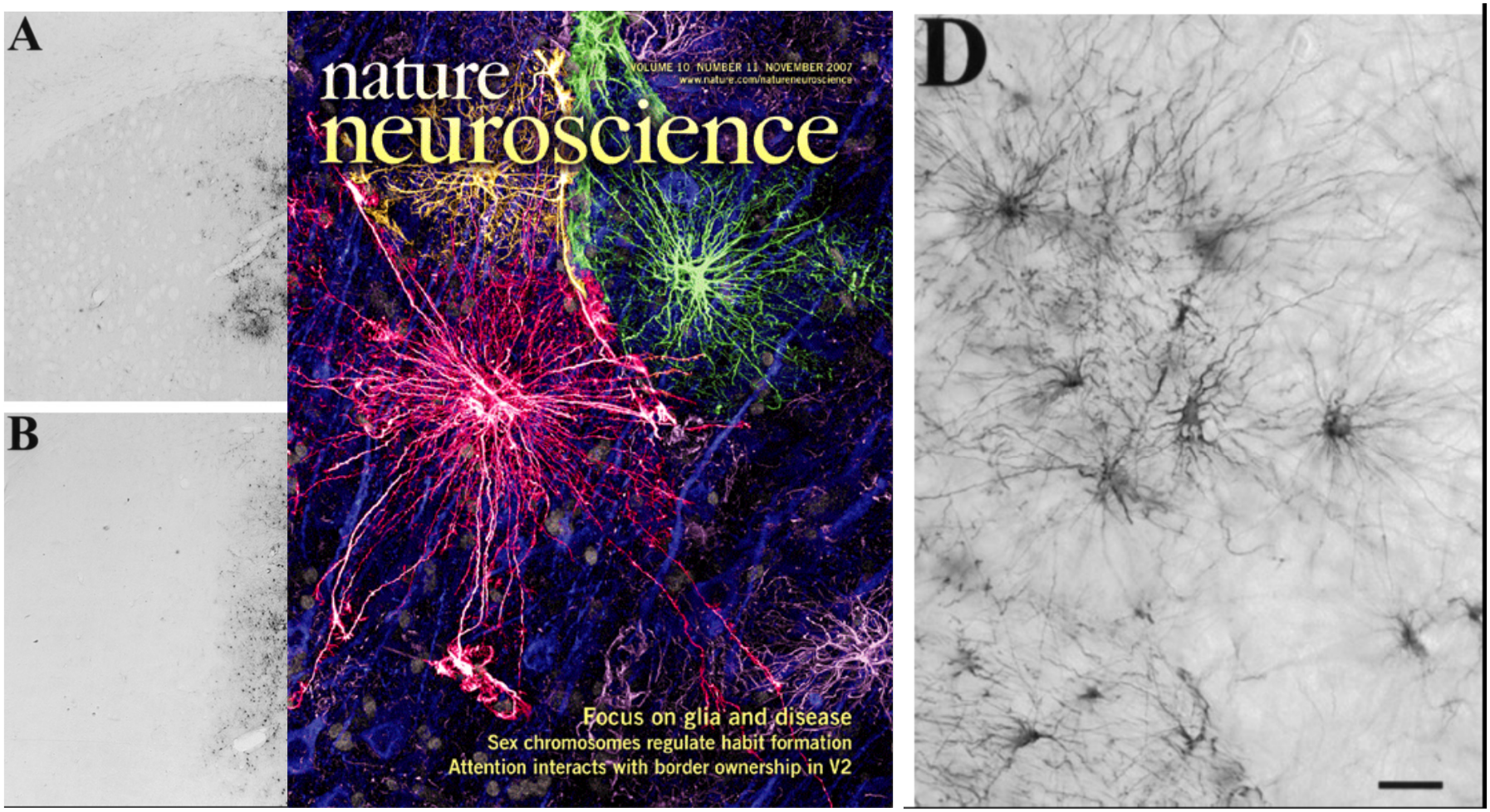
Milos Pekny[1,2,3] · Marcela Pekna[1,2,3] · Albee Messing[4] · Christian Steinhäuser[5] · Jin-Moo Lee[6] · Vladimir Parpura[7] · Elly M. Hol[8,9,10] · Michael V. Sofroniew[11] · Alexei Verkhratsky[12,13,14,15]

Received: 6 August 2015 / Revised: 28 October 2015 / Accepted: 21 November 2015 / Published online: 15 December 2015

Abstract The neurone-centred view of the past disregarded or downplayed the role of astroglia as a primary component in the pathogenesis of neurological diseases. As this concept is changing, so is also the perceived role of astrocytes in the healthy and diseased brain and spinal cord. We have started to unravel the different signalling mechanisms that trigger specific molecular, morphological and functional changes in reactive astrocytes that are critical for repairing tissue and maintaining function in CNS pathologies, such as neurotrauma, stroke, or neurodegenerative diseases. An increasing body of evidence shows that the effects of astrogliosis on the neural tissue and its functions are not uniform or stereotypic, but vary in a context-specific manner from astrogliosis being an adaptive beneficial response under some circumstances to a maladaptive and deleterious process in another context. There is a growing support for the concept of astrocytopathies in which the disruption of normal astrocyte functions, astrodegeneration or dysfunctional/maladaptive astrogliosis are the primary cause or the main factor in neurological dysfunction and

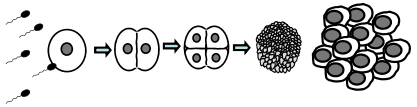


hNPC migration and maturation into astrocytes following transplantation into a rat model of Parkinson's Disease



Svendsen et al, Exp. Neurology 1997

Stem cells for neurological disorders

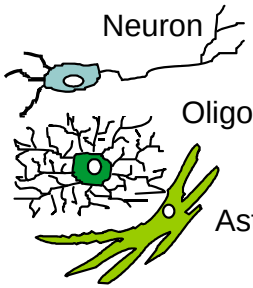


Embryonic Stem Cells
Induced Pluripotent Stem Cells

Limitless supply and make any type of neural tissue
iPSCs have no ethical issues and autologous use
Teratoma risk and stability in vitro.
Differentiation is challenging!

Human neural progenitor expansion

Transplantation



Remyelination is more practical
Spinal Cord Injury, MS

Has local trophic effects and supports neuronal function in health and disease.
All neurological diseases and RP

Fetal brain tissue
NIH Fetal tissue bank

Low risk of tumor growth
Neural progenitors make differentiated astrocytes
Limited supply, limited differentiation and ethical issues

Sources of variability in preclinical and clinical research on stem cell therapies for ALS

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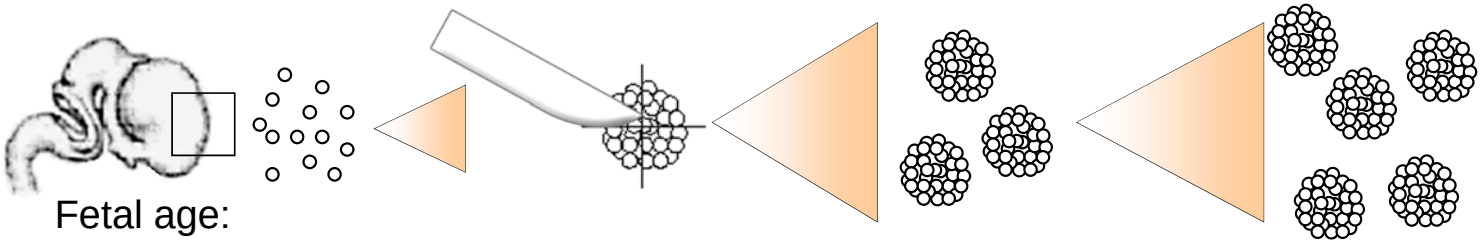
Manufacturing cortical neural progenitor cells for generating neurons and astrocytes – a unique ”chopping” method



Fetal age:
90-100 days

For manufacturing differentiated cell types from fetal tissue or iPSCs there needs to be a focus on developmental biology

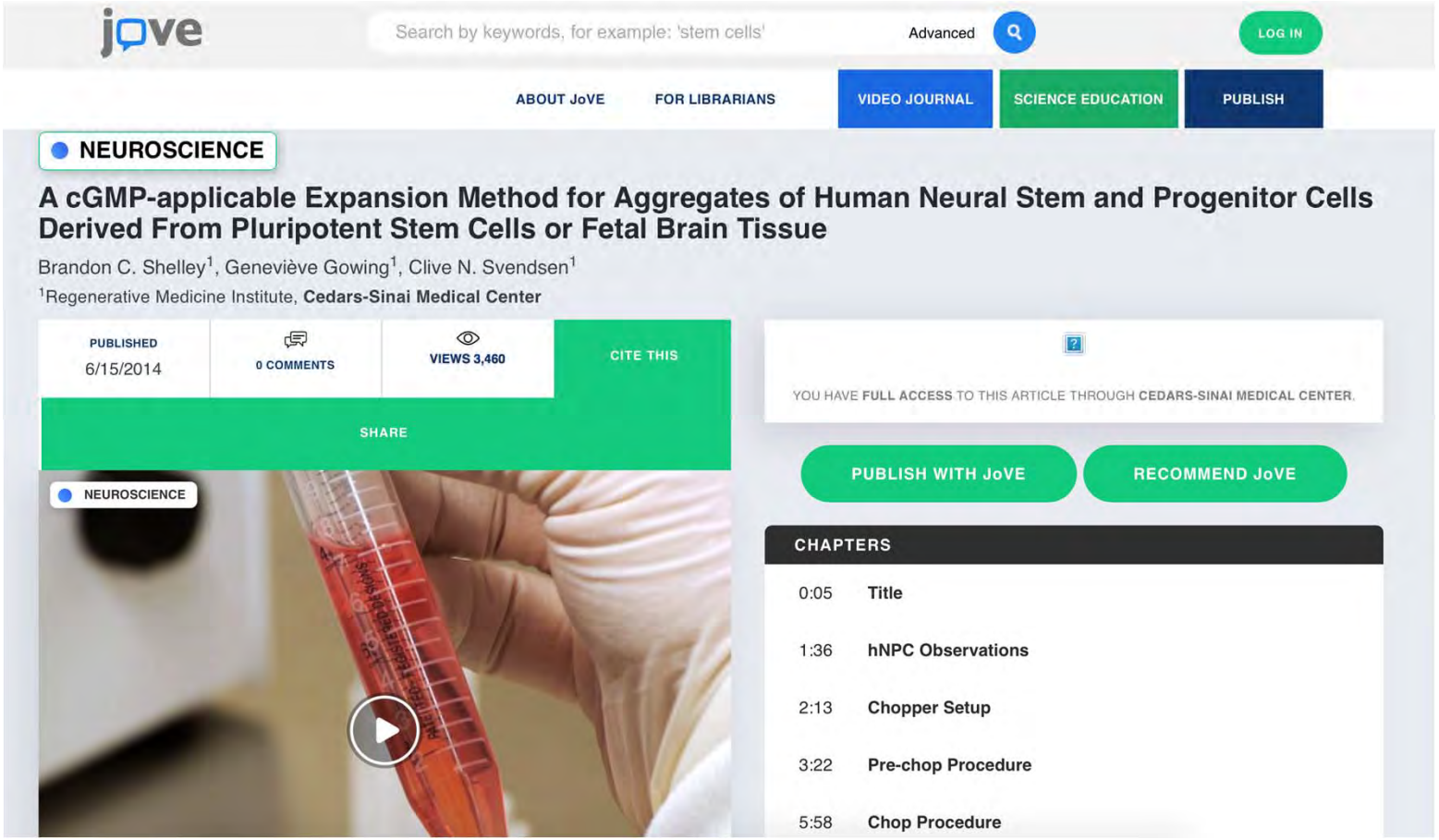
Use development rather than fight it in the manufacturing process!

Human 25 days

Developmental time for human cortex (approx 50 wks)

Svendsen et al, 1996 - 2010

Process transfer of ”chopping method” to cGMP manufacturing

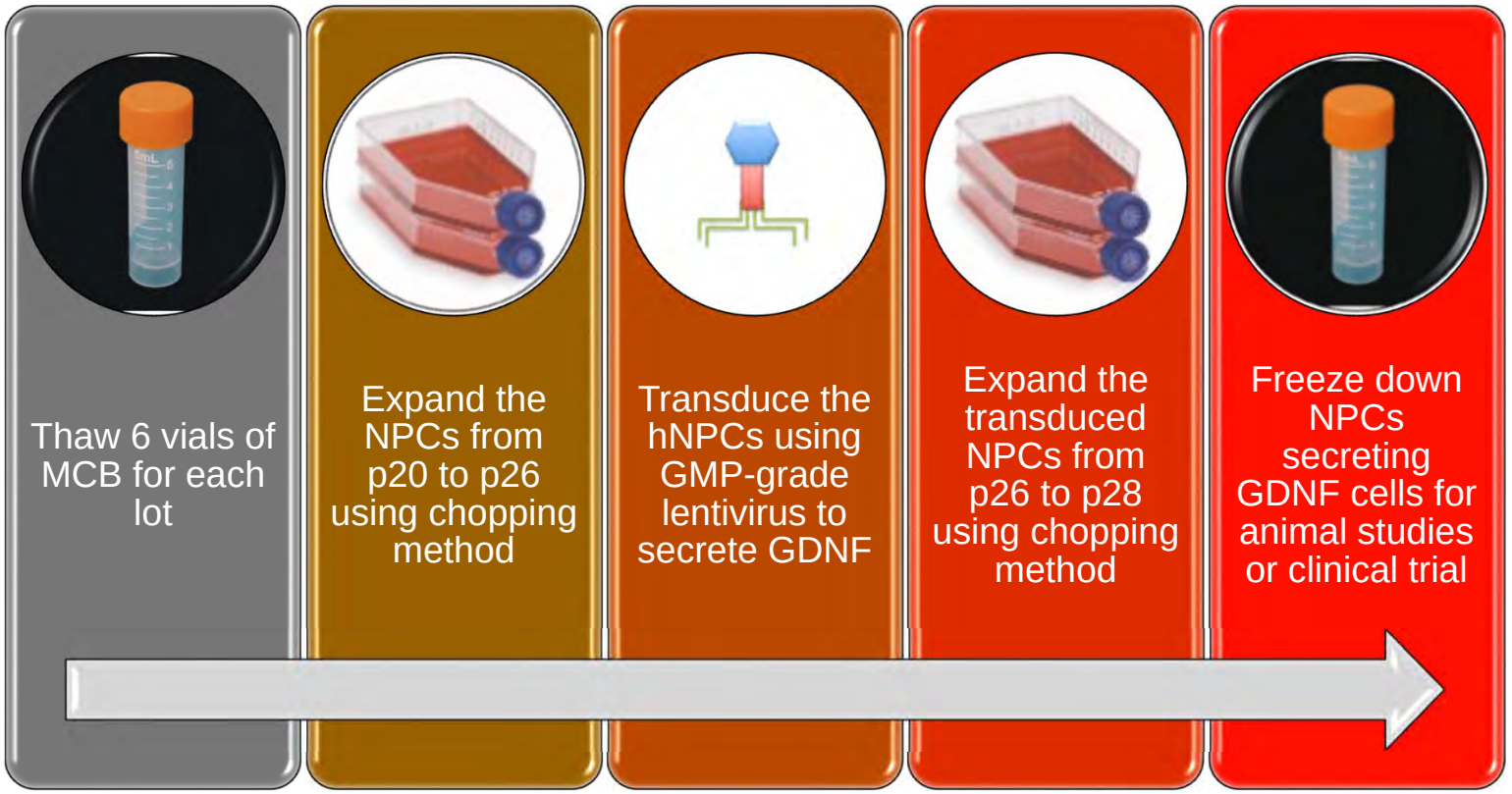


Derek Hei
Larry Couture
Joe Gold

WAISMAN BIOMANUFACTURING

City of Hope.

Global manufacturing process



Challenges with manufacturing process

- Major issues with karyotyping – trisomy 7 and translocation on chromosome 1. Fresh EGF crucial
- First GMP batch grew poorly due to wrong sourcing of EGF
- Second GMP production batch grew well. Major freeze down at scale to 1,200 vials.
- But… showed low post-thaw viability (~40%) compared to >70% historic values.
- Laboratory studies identified process-critical items at scale for successful freeze down and recovery
- A third GMP batch with serial freeze down for 4 sub-lots was successfully run to completion
- **8 vials of MCB expanded to 1,200 vials of product in 10 weeks**

Final Product

- Patient dose preparation
  - Cells thawed and counted at cGMP facility
  - Shipped to Cedars-Sinai Pharmacy day before surgery
  - Stable for up to 9 days at 4 degrees C in final Cedars-Sinai transplantation media

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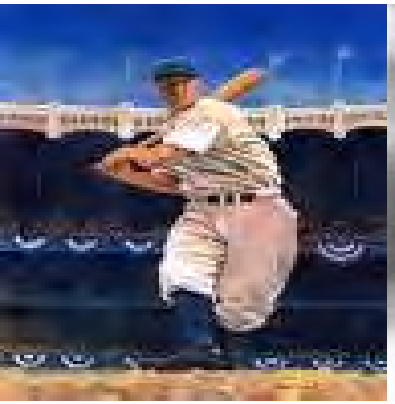
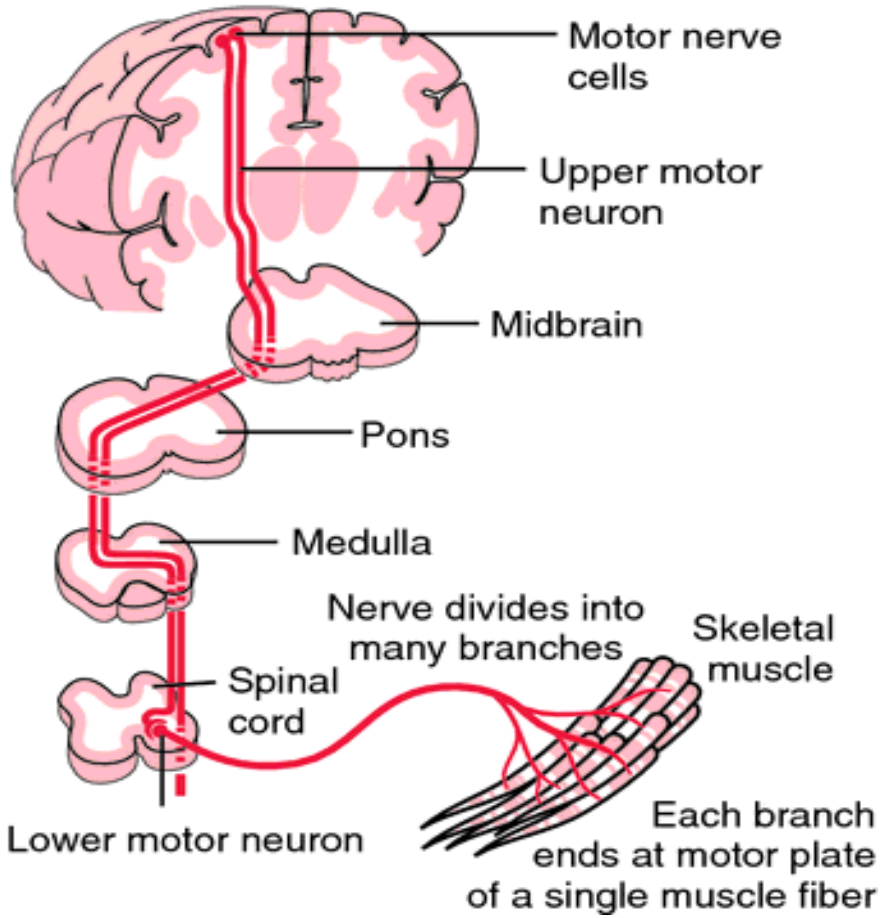


Lou Gehrig's Disease, Motor Neuron Disease or ALS

- Twitching, death of motor neurons and paralysis
- Cause unknown, no drugs, no cure
- Death normally within 3 years
- Majority of cases "sporadic" with about 10% of cases showing "familial" inheritance patterns



A New Combined Gene and Stem Cell Therapy for ALS at Cedars-Sinai

18 patient trial

ALS Association
Fighting Lou Gehrig's Disease

CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE
CIRM

CEDARS-SINAI

NATIONAL INSTITUTES OF HEALTH

- We have developed a clinical-grade line of human stem cells that are engineered to release a powerful growth factor GDNF
- These cells protect motor neurons from dying in animal models of ALS by acting as a "Trojan Horse" – releasing GDNF right where the motor neurons die…
- We have developed an innovative surgical device to deliver these cells to the spinal cord of patients with ALS
- On March 17th we filed an Investigational New Drug (IND) application to the FDA to conduct a first in man clinical study for ALS patients at Cedars-Sinai Medical Center
- Unique unilateral trial design for high efficacy

HUMAN GENE THERAPY 16:509–521 (April 2005)

studies

GDNF Delivery Using Human Neural Progenitor Cells
in a Rat Model of ALS

OPEN ACCESS Freely available online

PLoS one

SANI
KYLI

GDNF Secreting Human Neural Progenitor Cells Protect Dying Motor Neurons, but Not Their Projection to Muscl

Masatoshi Suzu

1 The Waisman
University of W
(EPFL), Lausann

Intermittent Hypoxia and Stem Cell Implants Preserve Breathing Capacity in a Rodent Model of Amyotrophic Lateral Scle

OPEN

Nicole L. Nichols[1], Gen
Masatoshi Suzuki[1], Pab
Clive N. Svendsen[2], an

[1]Department of Comparative
Biomedical Sciences, Cedars-

**Glial cell line-de
neural progenit
astrocytes, and
into the spinal**

Geneviève Gowing,
Pablo Avalos, Jesse
Clive N. Svendsen

Experimental Neurology 280 (2016) 41–49

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

ELSEVIER

Human neural progenitors differentiate into astrocytes and protect motor neurons in aging rats

Melanie M. Das [a], Pablo Avalos [a], Patrick Suezaki [a], Marlesa Godoy [a], Leslie Garcia [a], Christine D. Chang [a], Jean-Philippe Vit [b,c], Brandon Shelley [a], Genevieve Gowing [a], Clive N. Svendsen [a,c,*]

[a] The Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, United States
[b] Biobehavioral Research Core, Cedars-Sinai Medical Center, Los Angeles, CA, United States
[c] Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, United States

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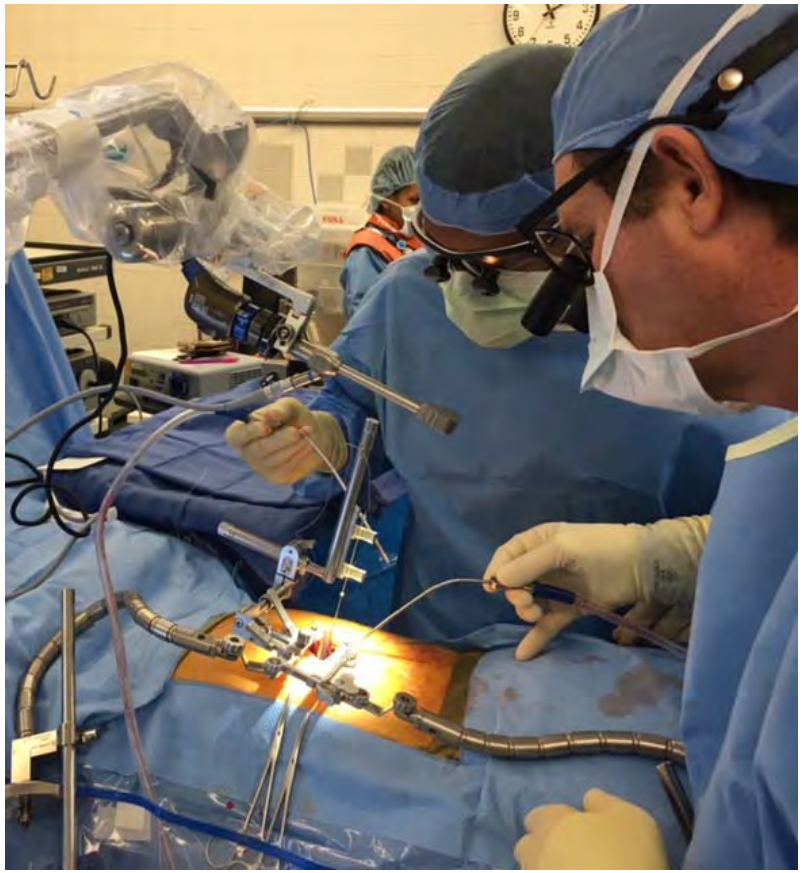
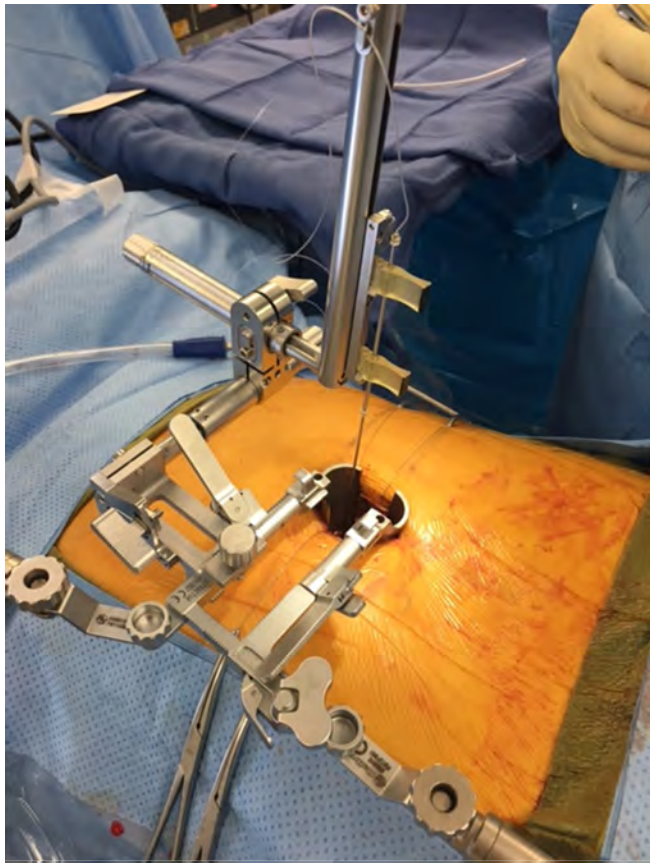
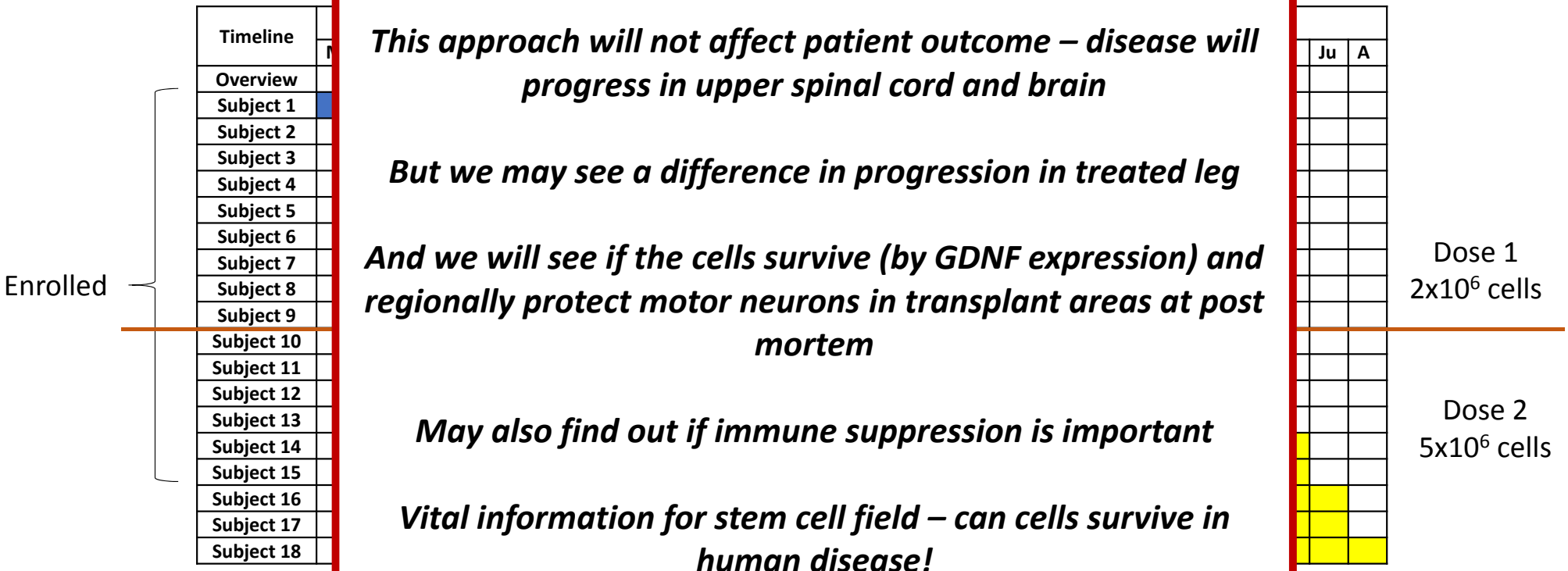
First patient dosed on May 3rd 2017







Beyond CIRM 2.0
now it's personal

One fetal brain neural progenitor product that makes astrocytes - many d

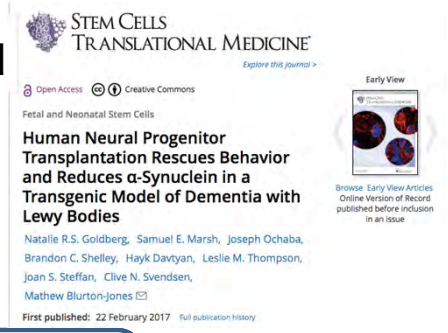

Gene Therapy (2006) 13, 379–388
© 2006 Nature Publishing Group All rights reserved 0969-7128/06 $30.00
www.nature.com/gt

ORIGINAL ARTICLE

Human neural progenitors deliver glial cell line-derived neurotrophic factor to parkinsonian rodents and aged primates

S Behrstock[1], A Ebert[1], J McHugh[1], S Vosberg[2], J Moore[1], B Schneider[1], E Capowski[1], D Hei[2], J Kordower[3], P Aebischer[4] and CN Svendsen[1]

[1]Waisman Center and Department of Anatomy, University of Wisconsin-Madison, WI, USA; [2]Waisman Center Clinical Biomanufacturing Facility, University of Wisconsin-Madison, WI, USA; [3]Rush University Medical Center, Chicago, IL, USA and [4]Swiss Federal Institute of Technology, Lausanne, Switzerland


STEM CELLS TRANSLATIONAL MEDICINE

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Fetal and Neonatal Stem Cells

Human Neural Progenitor Transplantation Rescues Behavior and Reduces α-Synuclein in a Transgenic Model of Dementia with Lewy Bodies

Natalie R.S. Goldberg, Samuel E. Marsh, Joseph Ochaba, Brandon C. Shelley, Hayk Davtyan, Leslie M. Thompson, Joan S. Steffan, Clive N. Svendsen, Mathew Blurton-Jones

First published: 22 February 2017 Full publication history

Early View

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Online Version of Record published before inclusion in an issue

Cedars-Sinai Regenerative Medicine Institute
CNS10-hNPC Research Bank

GMP Manufacturing
Master Cell Bank, Product
CBC – Biomanufacturing Cedars-Sinai

Alzheimer's
Blurton-Jones, Irvine

Parkinson's Disease
Bankiewicz, UCSF

Stroke
Steinberg, Stanford

Pre Clinical Animal Studies

Huntington's Disease
Svendsen, Cedars-Sinai

ALS
Svendsen, Cedars Sinai

Retinitus Pigmentosa
Wang, Cedars

Beyond CIRM 20 now it's personal

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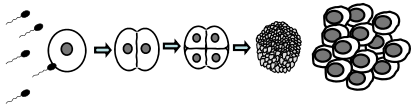

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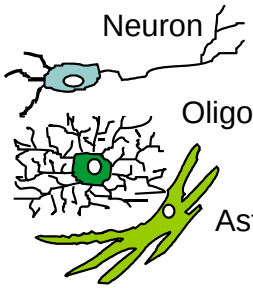

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The Cedars-Sinai iPSC core facility

Type "Cedars-Sinai iPSC core" into google…..

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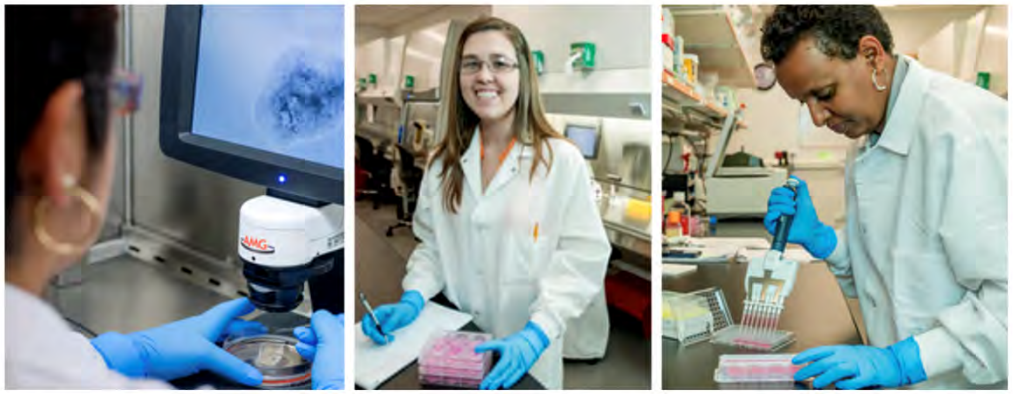
- Stem Cell Lines
- ALS iPSC Lines
- Answer ALS Project
- Staff
- FAQs

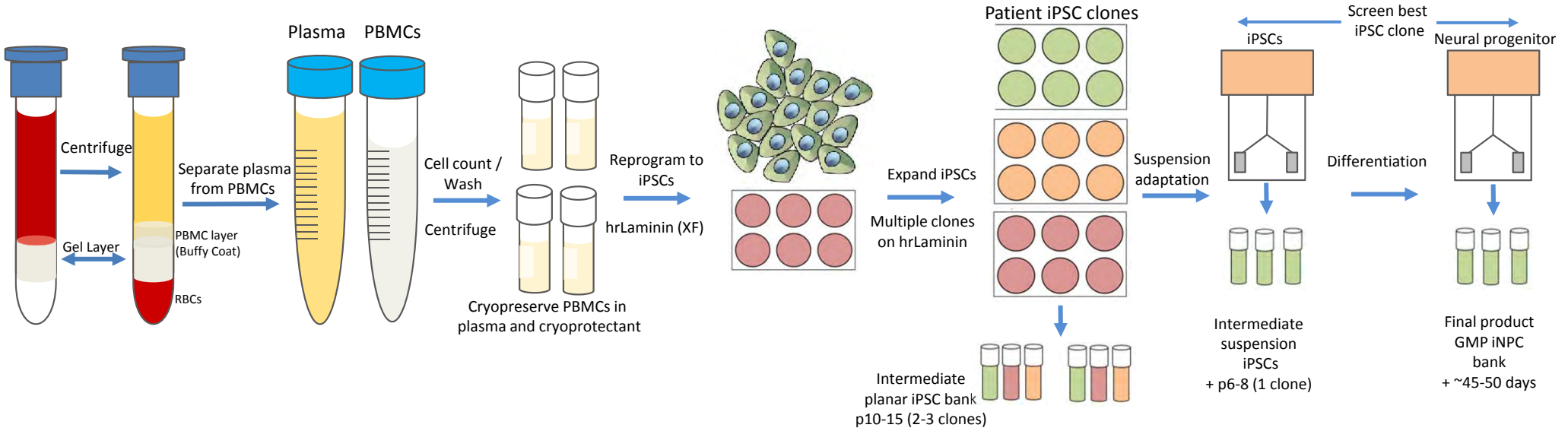


The Induced Pluripotent Stem Cell (iPSC) Core (David and Janet Polak Foundation Stem Cell Core Laboratory) at the Board of Governors Regenerative Medicine Institute uses the latest techniques to reprogram, expand and characterize human iPS cells from human skin or blood tissues of healthy subjects and diseased patients. We then turn the iPS cells into specific cells of the human body, including components of the nervous system, eyes, blood, bones, heart, gut, liver and pancreas, for use by researchers. Some applications of this technology includes human "disease modeling-in-a-dish," developing human reporter cell lines via genetic modification, drug screening on pathological human cell types and potentially developing cell replacement or regenerative therapies.

Internal Users - Please click HERE to access the internal iPSC Core web page

Dhruv Sareen, Loren Orlenas

Clinical-grade iPSC production and differentiation
Whole blood → PBMCs → iPSCs → Differentiated cell product



Cell type	*Whole blood*	*Isolated PBMCs*	*iPSCs (planar)*	*iPSCs (suspension)*	*i-differentiated cell product (suspension)*
Criteria	Clinical screening	Cell viability	Normal karyotype	>85% Oct4 (pluripotent stage)	>XX% of AA marker (early stage-specific)
	Serology tests	Number of vials	Clearance of episomal plasmids	Normal karyotype	>YY% of BB marker (mid-stage specific)
	OncoPanel		Pluripotency (Oct4)		>ZZ% of CC marker (late stage-specific)
			Sterility, endotoxin, & mycoplasma tests		< 0.1% Oct4 (by PCR at final stage)
			Adventitious agent testing (murine, bovine, porcine)		Functional Assay (final stage)
			Interspecies contamination		Viability
			Cell identity (16 human STR loci)		Sterility, endotoxin and mycoplasma tests

Cytogenetic normality key to stability - G-band karyotype

Cytogenetic stability of iPSC lines derived from non-expanded (PBMC) vs. expanded source tissue
G-band karyotype

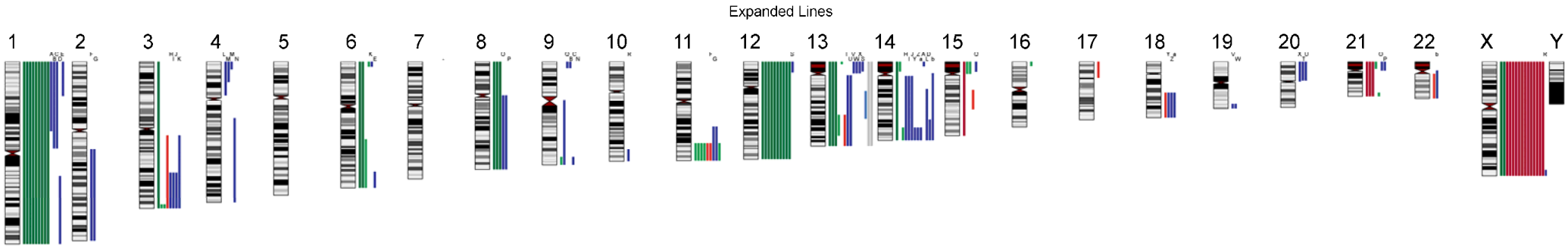
Non-expanded source tissue (PBMC)-iPSCs

Description	Numbers	% abnormal
Total iPSC lines	884	**5.2%**
Total abnormal	46	
First karyotype	40 / 518	7.7%
Repeat karyotype	6 / 366	**1.6%**
Excluding monosomy X	12 / 884	**3.7%**

Expanded source tissue-iPSCs

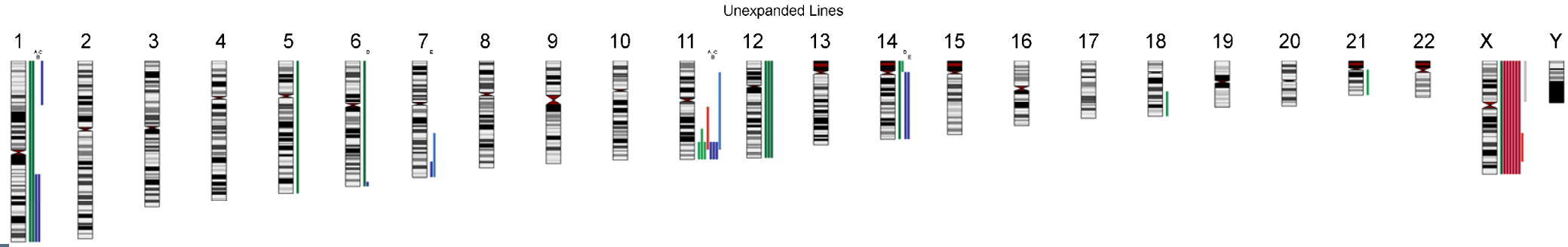
Description	Numbers	% abnormal
Total iPSC lines	423	**21.8%**
Total abnormal	92	
First karyotype	76 / 353	21.5%
Repeat karyotype	17 /70	**24.3%**

Expanded source tissue iPSC lines

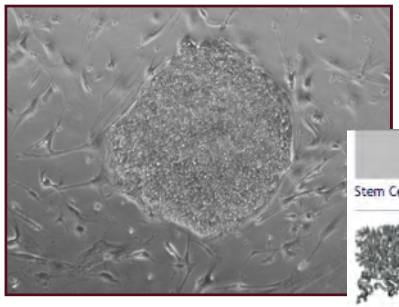


Expanded source tissue: Skin fibroblasts, 1° epithelial cells, LCLs

Non-expanded source tissue (PBMC) iPSC lines

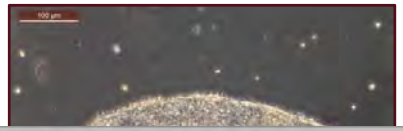


Generation of EZ-spheres and iNPCs from iPSC lines

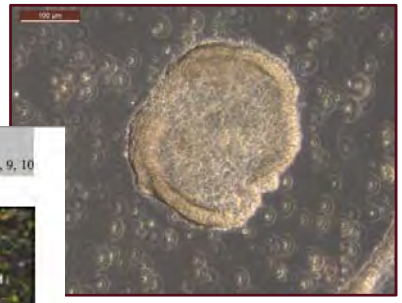


ES/iPS cells on MEF feed

Collagenase / Dispase

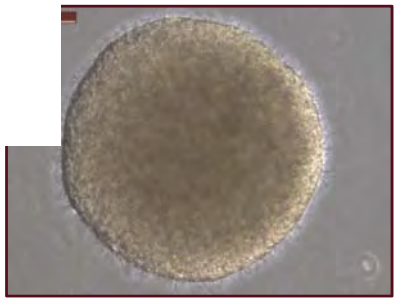


Gently lift



loating colony folding into a new EZ sphere

2 weeks



EZ sphere

of in

ARTICLE IN PRESS SCR-00272; No. of pages: 11; 4C: 4, 6, 8, 9, 10

Stem Cell Research (2013) xx, xxx–xxx

Available online at www.sciencedirect.com

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ELSEVIER

METHODS AND REAGENTS

EZ spheres: A stable and expandable culture system for the generation of pre-rosette multipotent stem cells from human ESCs and iPSCs

Allison D. Ebert [a,*], Brandon C. Shelley [b,1], Amanda M. Hurley [b,1], Marco Onorati [c,1], Valentina Castiglioni [c,1], Teresa N. Patitucci [a], Soshana P. Svendsen [b], Virginia B. Mattis [b], Jered V. McGivern [a], Andrew J. Schwab [a], Dhruv Sareen [b], Ho Won Kim [b], Elena Cattaneo [c], Clive N. Svendsen [b,**]

Cryopreservation

Banking & Distribution

Differentiation

Disease modeling

Drug screening

Transplantation

EGF, FGF-2, heparin media

Transition to iPSC from neural progenitors

Challenges for iPSC derived neural cell product:

1. ***Stability of iPSC lines (karyotype, maintain pluripotent state)***
2. ***Manufacturing at scale and at cGMP***
3. ***Constancy of differentiation***
4. ***Equivalency with other neural products in clinic (neural progenitors)***

Article first published online: 2 JUN 2015
DOI: 10.1002/stem.2032

inclusion in an issue)

Cells in space to reduce variation? Effects of zero-G on stem cell growth and differentiation *Collaboration with space tango and NASA*

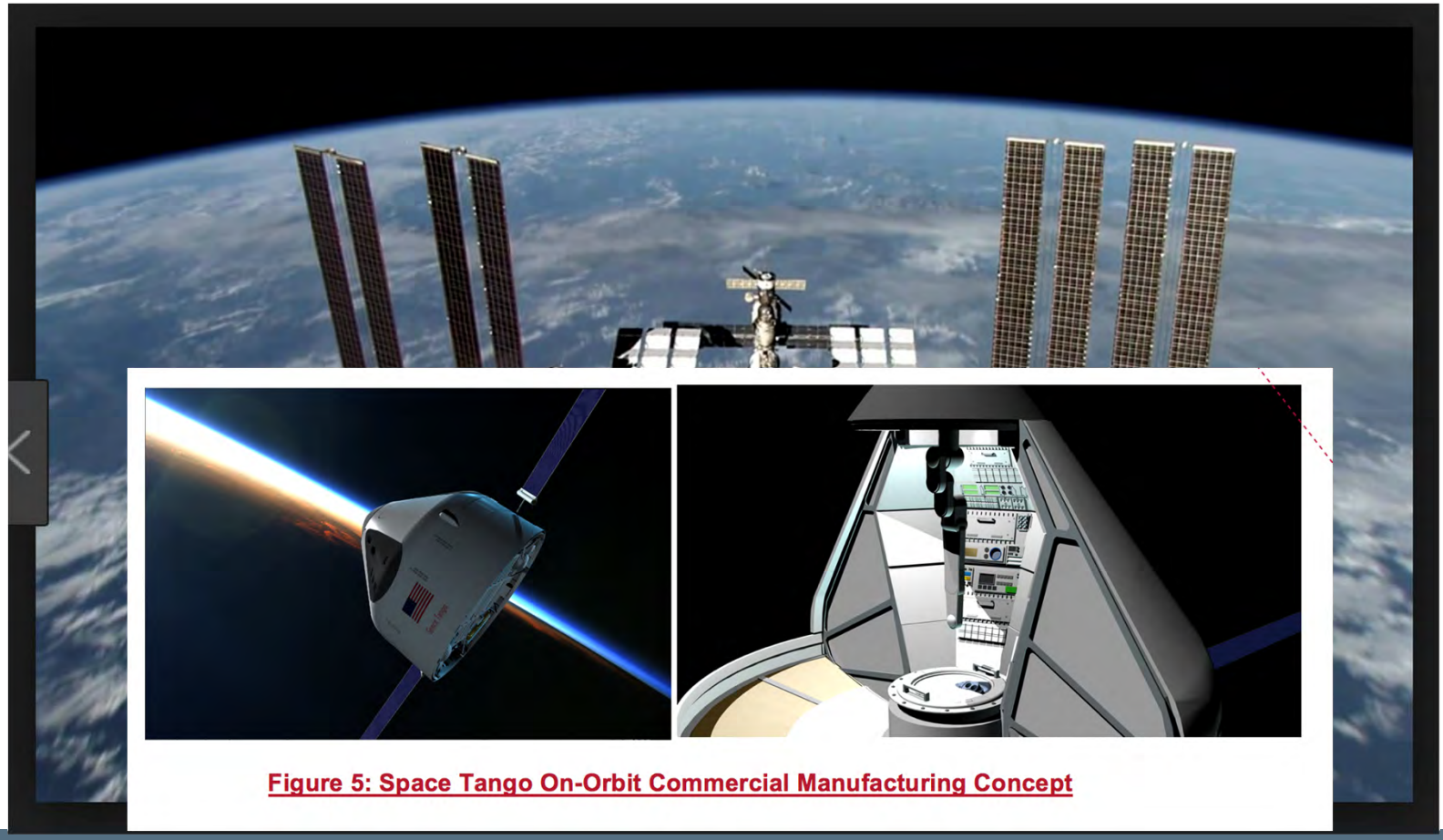

Figure 5: Space Tango On-Orbit Commercial Manufacturing Concept

Conclusions

- Developing a cell product from human fetal brain tissue requires strict SOP's and attention to every detail of the process
- Once cells proliferate in culture they are always changing in some small way. This needs constant attention
- Transition to iPSC technology is underway – challenges include stability of iPSC lines, variability between patients and clones and reliable differentiation protocols

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Translation of Stem Cells to the Clinic:
Challenges and Opportunities

December 2–4, 2018 — Cedars-Sinai, Los Angeles, CA, USA

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Hongkui Deng, *Peking University, China*

Sally Temple, *Neural Stem Cell Institute, USA*

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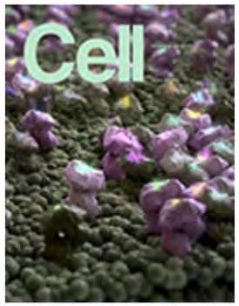

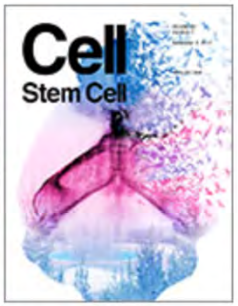

The Group….

CIRM Disease Team:
- Bob Baloh
- Pat Johnson
- Pablo Avalos
- Brandon Shelley
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- Larry Couture
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