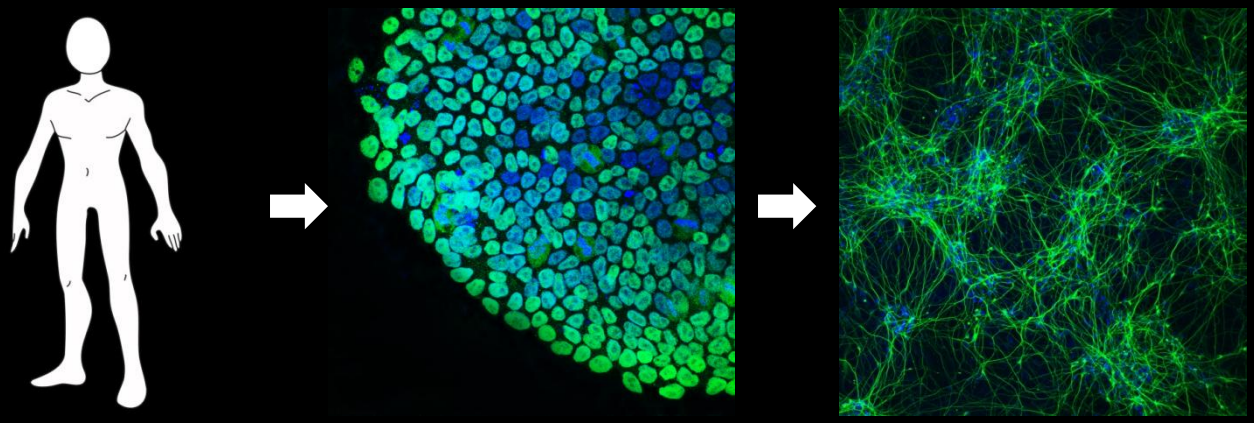
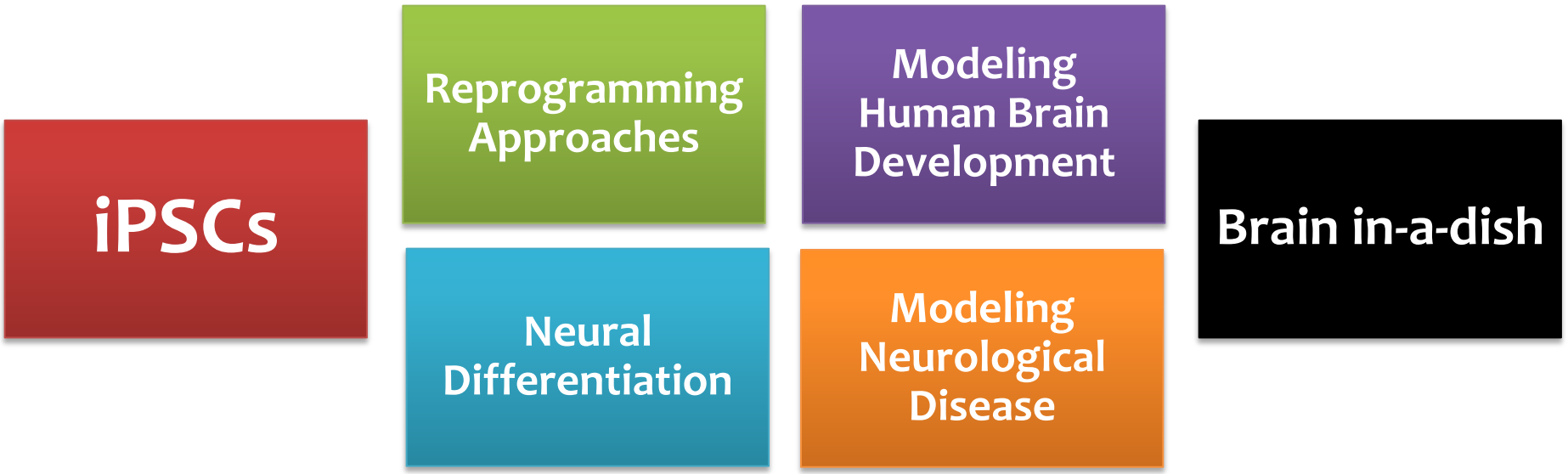
Investigating human brain development and neurological diseases using induced pluripotent stem cells (iPSCs)

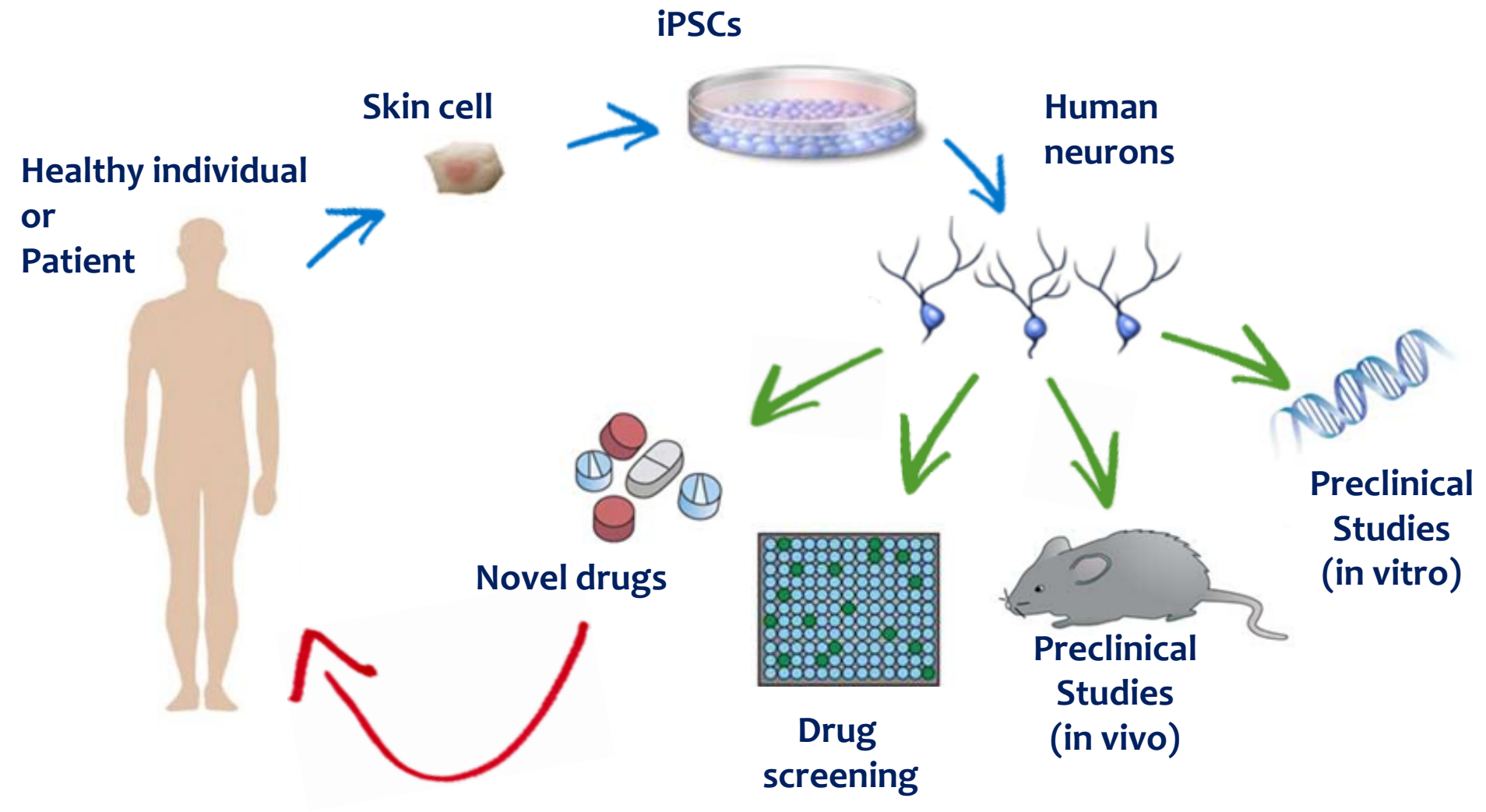


Georgia Kouroupi
Laboratory of Cellular and Molecular Neurobiology
Hellenic Pasteur Institute

Overview


iPSCs
Reprogramming Approaches
Modeling Human Brain Development
Neural Differentiation
Modeling Neurological Disease
Brain in-a-dish

Overview


iPSCs
Skin cell
Healthy individual
or
Patient
Human
neurons
Preclinical
Studies
(in vitro)
Novel drugs
Drug
screening
Preclinical
Studies
(in vivo)

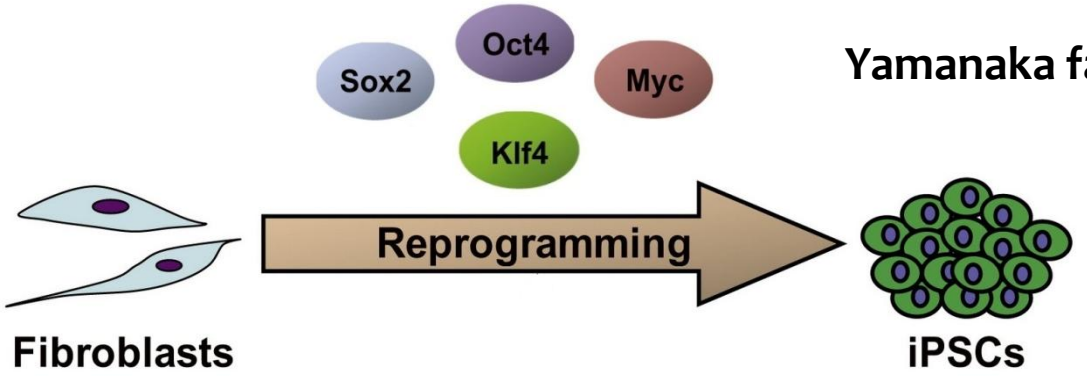

Sir John Gurdon


Shinya Yamanaka

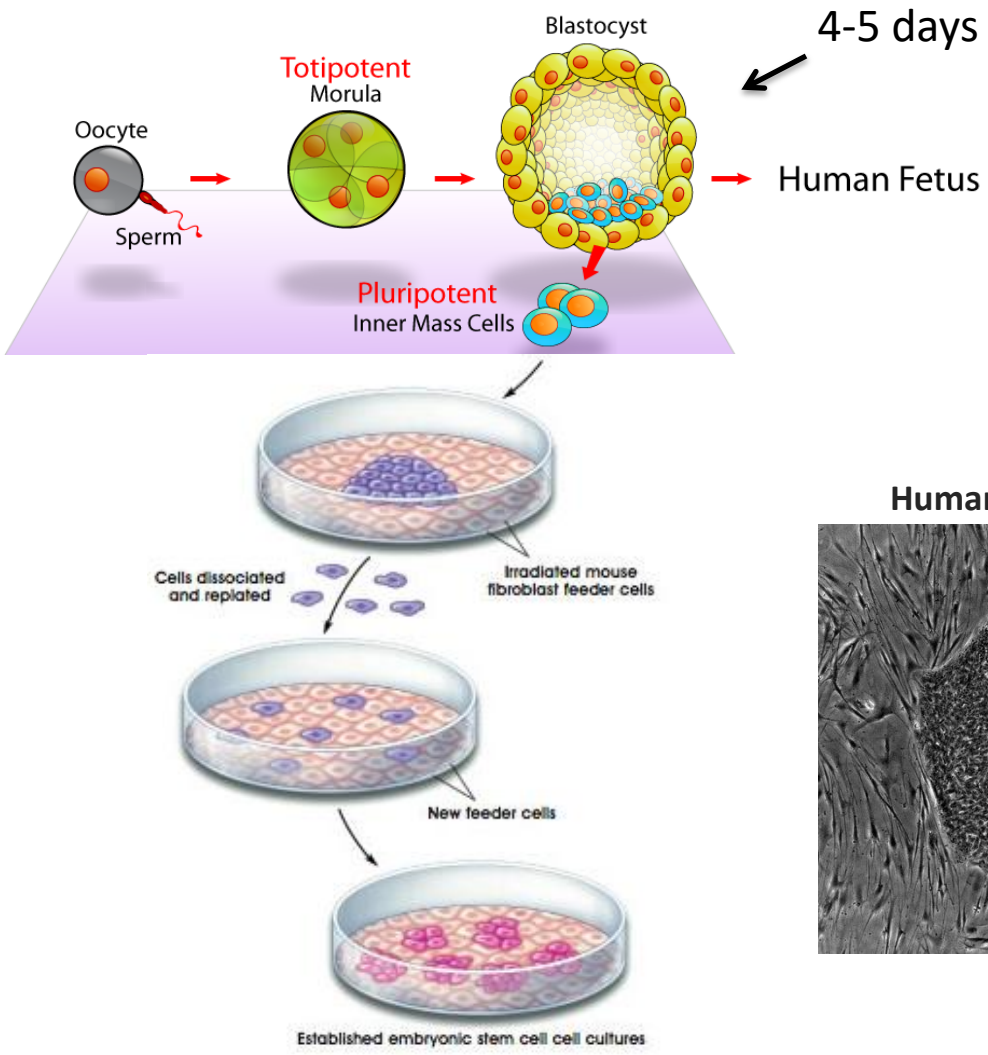
The Nobel Prize in Physiology or Medicine 2012

iPSC generation

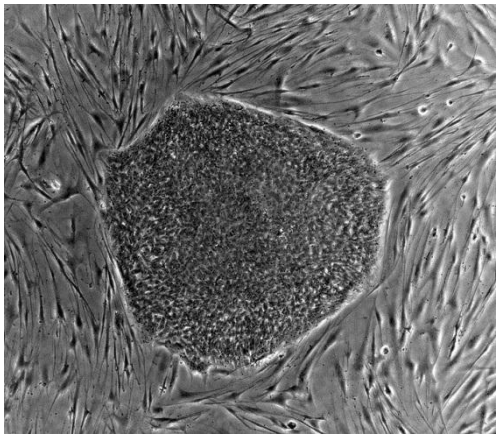
iPSCs are **pluripotent stem cells artificially derived** from a non-pluripotent cell, typically an adult somatic cell, by inducing a 'forced' expression of specific genes



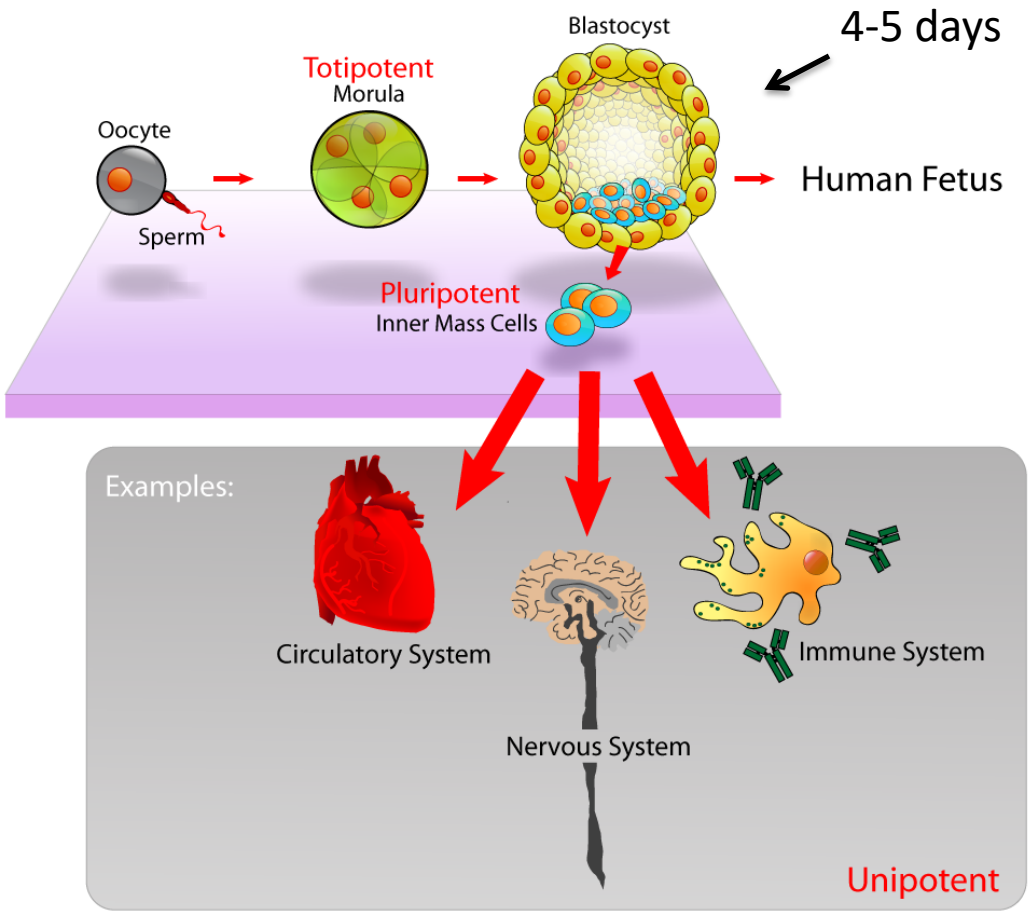
Embryonic Stem Cells



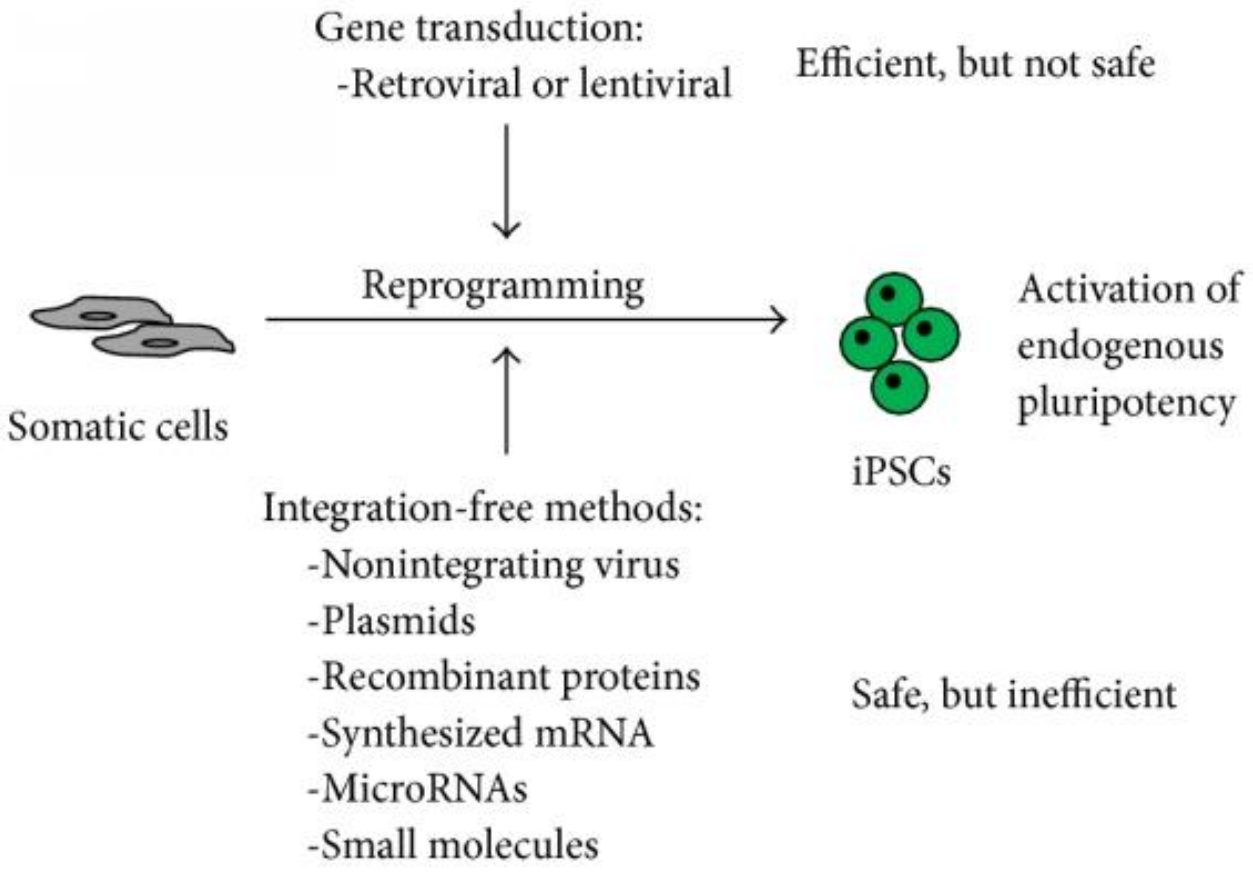
Human ESCs in culture



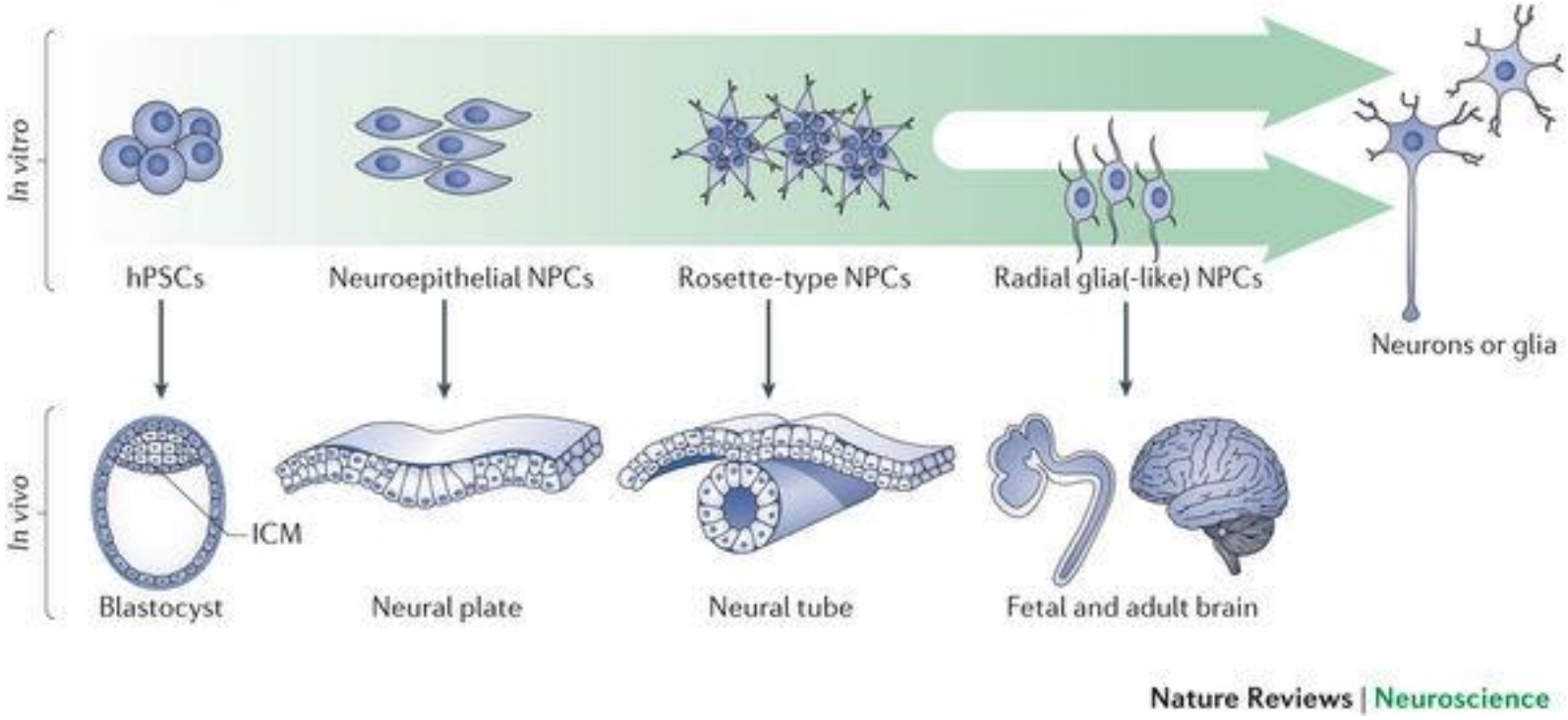
Embryonic Stem Cells



Reprogramming approaches



Neural differentiation

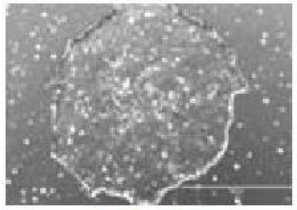
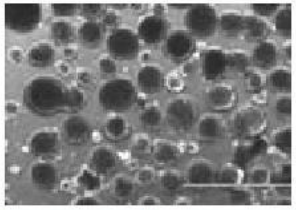
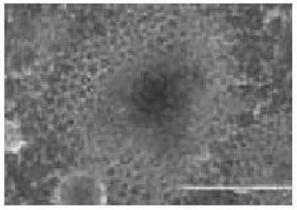
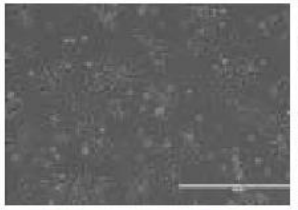
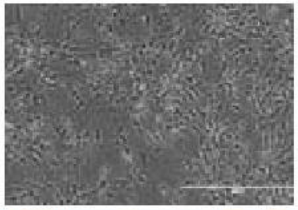
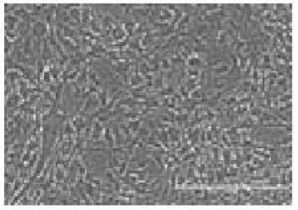


Stages of neural differentiation *in vitro* and *in vivo*

- hPSCs transit through defined stages during which they resemble distinct **neural progenitor cell (NPC) populations** present during *in vivo* neurogenesis
- hPSCs differentiate into neuroepithelial stem cells *in vitro*, corresponding to the **neuroepithelial NPCs** that form the neural plate *in vivo*
- **Rosette-type NPCs** derived from hPSCs resemble NPCs that populate the early neural tube
- **Radial glia-like NPCs** generated from the rosette-type NPCs give rise to postmitotic neurons

Mertens et al, Nat Rev Neurosci 2016

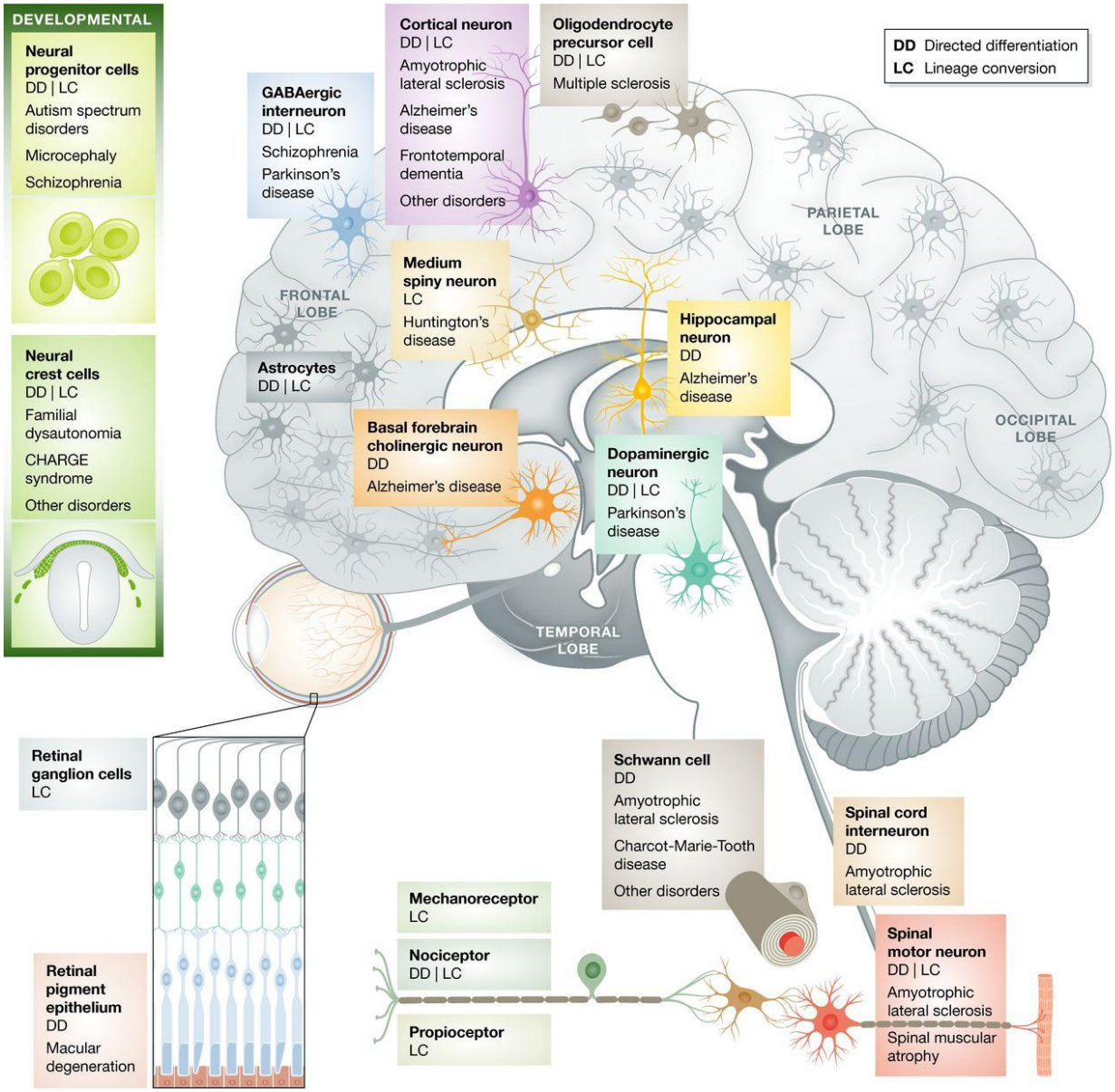
Neural differentiation

iPSC	EBs	Rosettes	NPCs	Neurons	Astrocytes
					
iPSC are a type of pliripotent cells morphologically similar to embryonic cells that can be generated directly from adult tissues and can be used in many studies as disease modeling and drug discovery Markers: SSEA-1, SSEA-3, SSEA-4, Lin28, TRA-1-60, TRA-1-81, Oct3/4, Nanog, Sox2	EBs are three-dimensional structures formed by multicellular aggregates that recapitulate aspects of cell differentiation during early embryogenesis and play an important role in the differentiation of ES cells into a variety of cell types *in vitro* Markers: Sox1, Otx2 (ectoderm), Brachyury, HAND1 (mesoderm), GATA-4, SOX17 (endoderm)	Neural rosette is the developmental signature of neuroprogenitors in cultures of differentiating embryonic stem cells. Rosettes are structures of columnar cells that express proteins present in neuroepithelial cells in the neural tube. Neuroprogenitors cells within neural rosettes are able to differentiate into neurons, oligodendrocytes, and astrocytes Markers: Pax6, Sox1, PSA-NCAM	NPCs are capable of self-renewing and differentiating into neural cell lineage. These cells hold promise for both biomedical research and potential research and potential cell therapy Markers: Nestin, Sox1 Sox2, Pax6, Ki67, Musashi 1	Neurons are cells that process and transmit information through electrical and chemical signal *via* synapses. The nervous system is composed of a network of neurons and others cells (such as glial cells) Markers: MAP2, SYN1, Homer 1, PSD95, TUJ1	Astrocytes are the most diverse neuroglial cells and exert many functions as organize the architecture of the brain, control emergence and function of the blood brain barrier, control neurogenesis, homeostatic regulatin, support and nurture, synaptogenesis, participate in neurotransmission and cytokine secretion Markers: GFAP, CD44, Aquaporin 4, S100β, Vimentin

Russo et al, World J Transplant. 2015

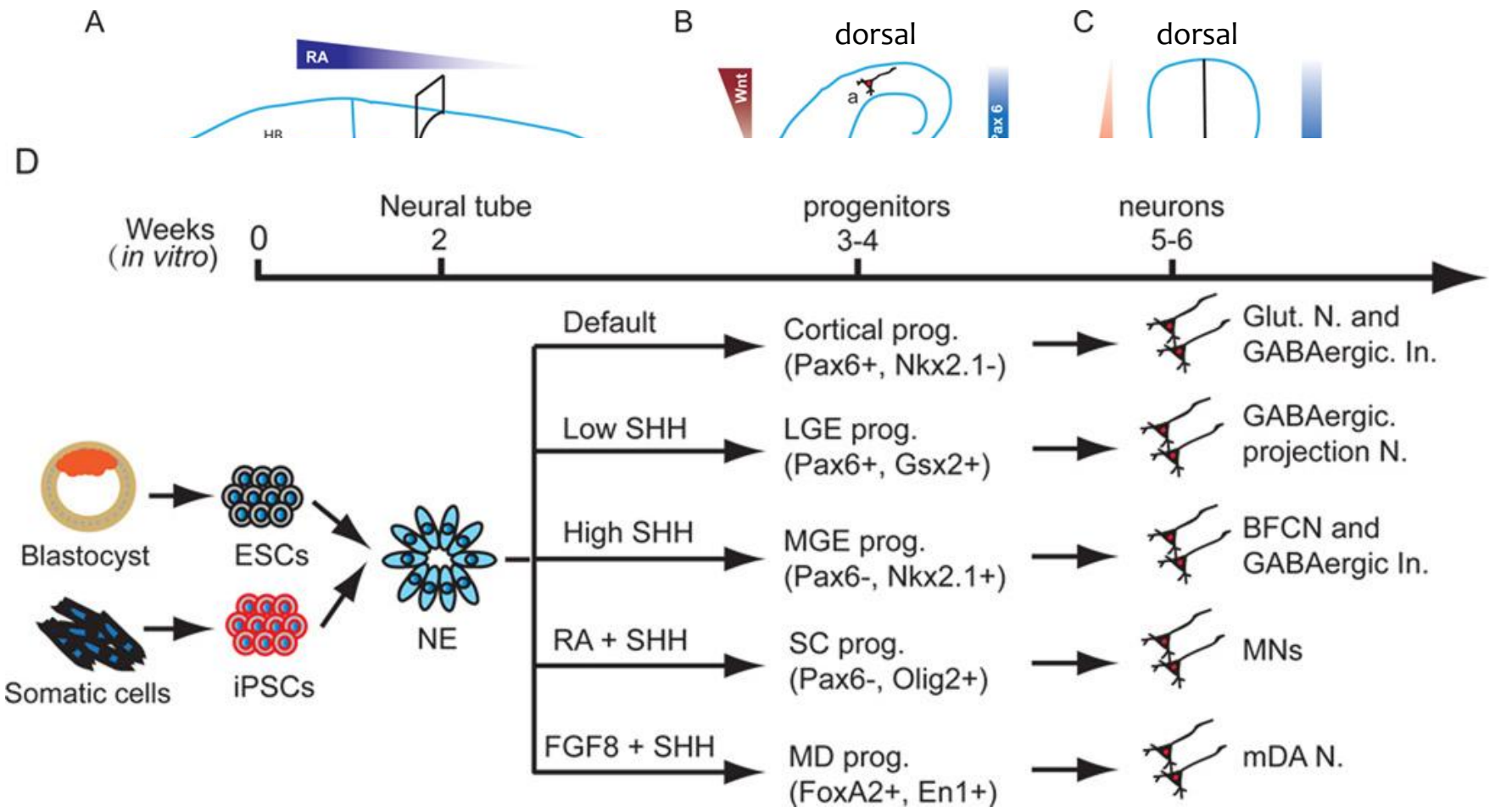
You can study only what you can make

A number of different **human neural subtypes** can be efficiently generated by **directed differentiation** from pluripotent stem cells

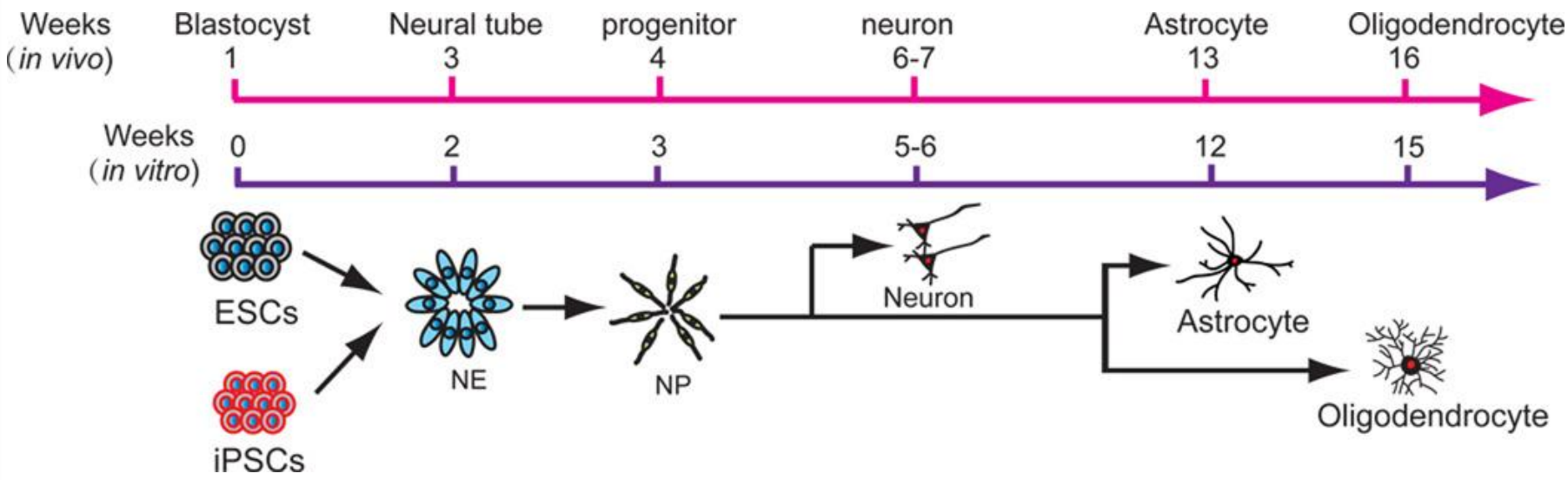


Ichida & Kiskinis, EMBO J 2015

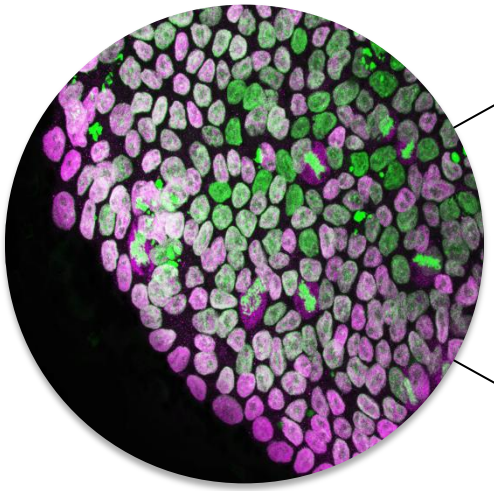
Neuronal subtype specification



Temporal course of glial differentiation



more than 100 days *in vitro*…



Modeling Human Brain Development

Modeling Neurological Disease

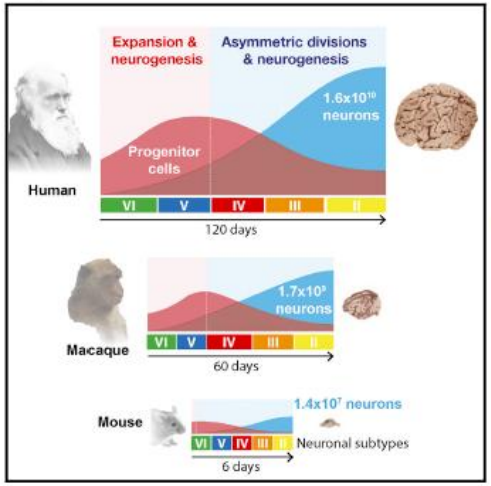
Modeling Human Brain Development

Article

Cell Stem Cell

2D and 3D Stem Cell Models of Primate Cortical Development Identify Species-Specific Differences in Progenitor Behavior Contributing to Brain Size

Graphical Abstract



Authors

Tomoki Otani, Maria C. Marchetto, Fred H. Gage, Benjamin D. Simons, Frederick J. Livesey

Correspondence

rick@gurdon.cam.ac.uk

In Brief

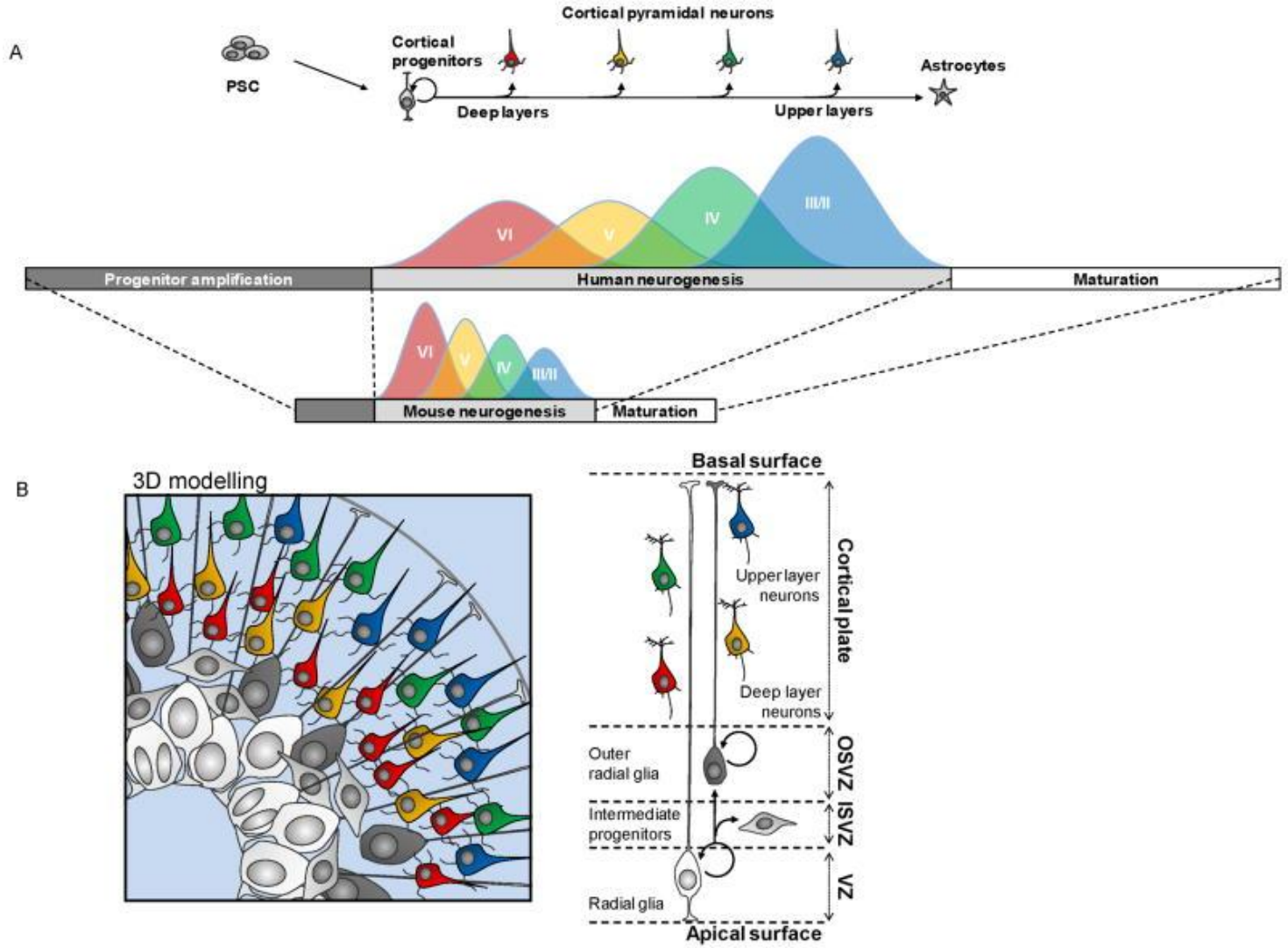
Based on modeling of cortical neurogenesis with pluripotent cells in 2D and organoid systems, Otani et al. suggest that species-specific differences in cortical size and cognitive ability between human and other animals result at least in part from cell-autonomous differences in cortical progenitor proliferation before neurogenic differentiation.

Highlights

- Human and primate PSCs can replicate cortical development in culture
- PSC-derived cortical progenitors from different species expand to different degrees
- Clonal analysis reveals marked difference in neurogenesis output over time
- Species-specific timing differences in neurogenesis are regulated cell autonomously

Otani et al., 2016, Cell Stem Cell 18, 467–480
April 7, 2016 ©2016 The Authors
http://dx.doi.org/10.1016/j.stem.2016.03.003

Modeling temporal and spatial patterning of cortical neurogenesis



Anderson & Vanderhaeghen, Curr Opin Neurobiol 2014

Modeling cortical development

- **the cortex of humans and other primates appears to follow different scaling rules** than that of other mammals, including mouse, in terms of the relationship between cortical volume and cell number and overall body size

- In this study, they extended the **use of stem cell systems to compare human, macaque, and chimpanzee cortical neurogenesis to understand the developmental mechanisms** regulating increased cortical size in different primates

macaque ESCs

Macaca nemestrina

Macaca fascicularis

chimpanzee iPSCs

Pan Troglodytes

human ESCs & iPSCs

Modeling cortical development

ESCs/iPSCs
(Dispase)

NE sheet
(Dispase)

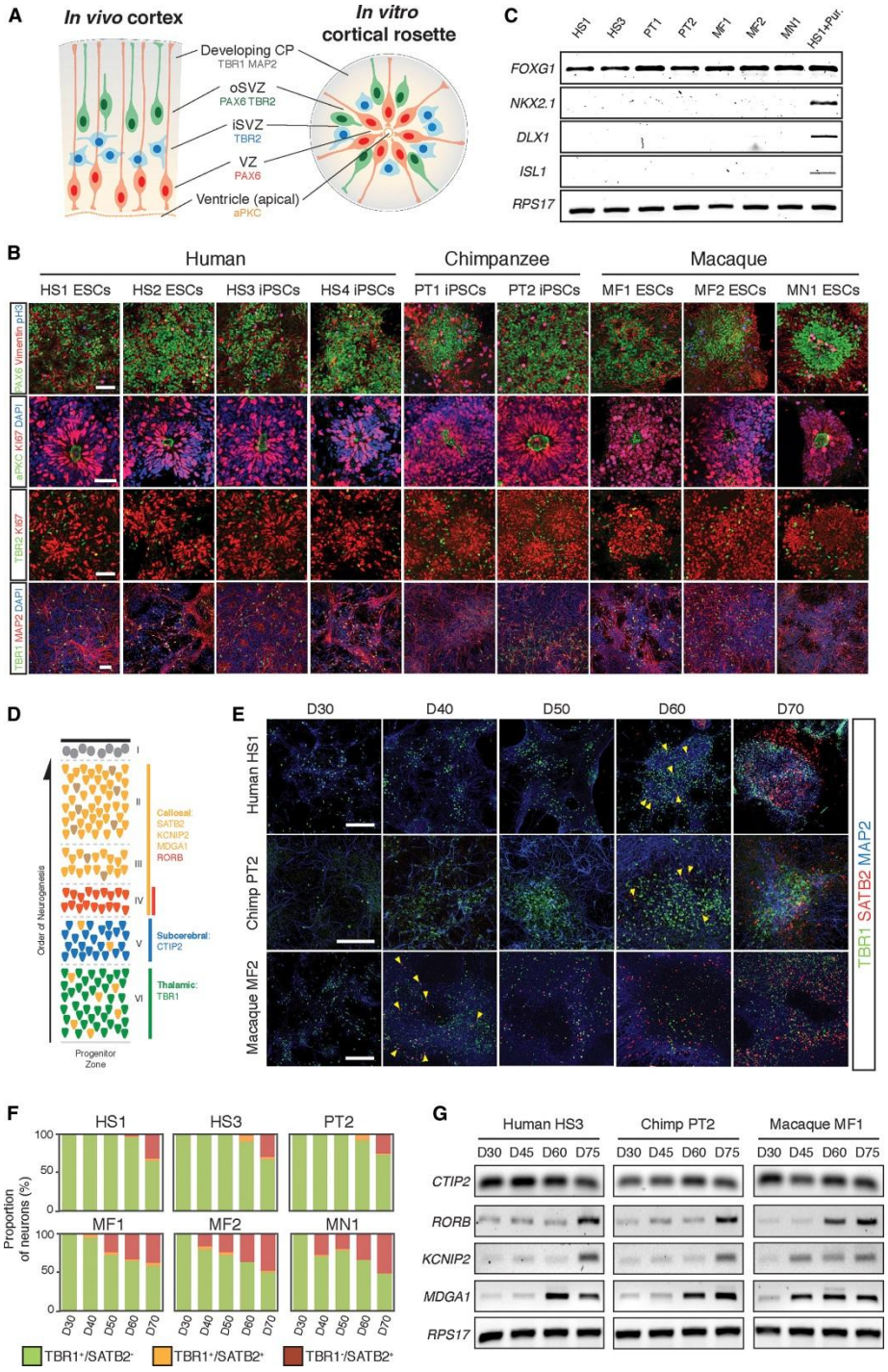
Day 20-35
early-born deep-layer neurons Tbr1+ and/or CTIP2+

After day 70
Late-born upper-layer neurons
Satb2+, Cux1+ and Brn2+

3-months
~ equal proportions of
deep- and upper-layer neurons

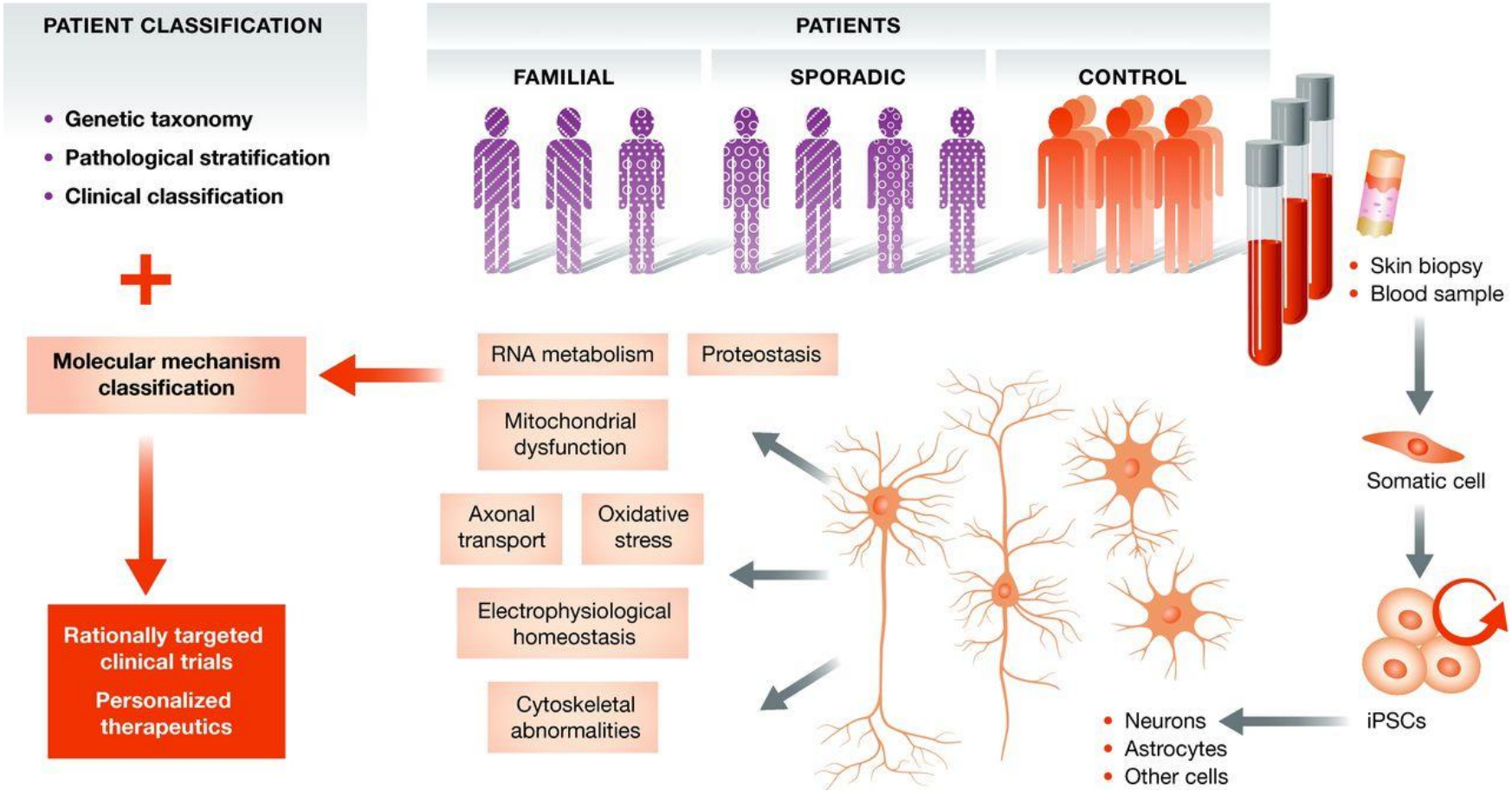
Shi et al, Nature Protocols 2012

Species-appropriate timing of major developmental events in cortical development is maintained *in vitro*



Cell Stem Cell 2016 18, 467-480DOI: (10.1016/j.stem.2016.03.003)

Modeling neurological diseases



Ichida & Kiskinis, EMBO J 2015

Modeling neurological diseases

List of human neurological diseases with published iPSC studies

Alzheimer's Disease
Amyotrophic Lateral Sclerosis (ALS)
Angelman & Prader–Willi Syndrome
Ataxia Telangiectasia
Best Disease
Dravet Syndrome
Familial Dysautonomia
Fragile X Syndrome
Friedreich's Ataxia
Frontotemporal Dementia
Gaucher's Disease
Gyrate Atrophy
Hereditary Spastic Paraplegia
Huntington's Disease
Lesch–Nyhan Syndrome
Microcephaly
Neuronal ceroid lipofuscinosis
Niemann–Pick type C1 disease
Parkinson's Disease
Phelan–McDermid Syndrome
Retinitis Pigmentosa
Rett Syndrome
Schizophrenia
Spinal Muscular Atrophy
Tauopathy
Timothy Syndrome

26 Diseases
>200 Publications

Ichida & Kiskinis, EMBO J 2015

Amyotrophic Lateral Sclerosis (ALS)

First report or patient-specific neurons

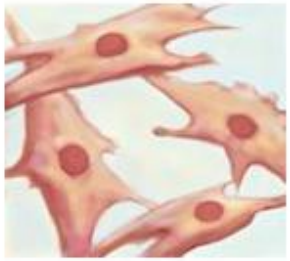
Science
AAAS

Induced Pluripotent Stem Cells Generated from Patients with ALS Can Be Differentiated into Motor Neurons

John T. Dimos, *et al.*

Science **321**, 1218 (2008);
DOI: 10.1126/science.1158799

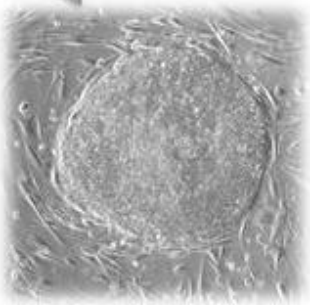
Amyotrophic Lateral Sclerosis (ALS)

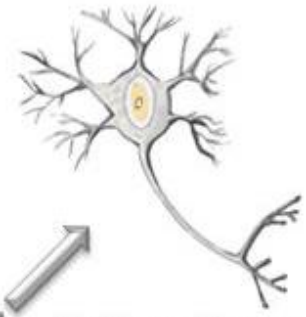


Skin cells from ALS patients
(82-year-old woman)

Yamanaka method
Oct4
Sox2
Klf4
C-Myc

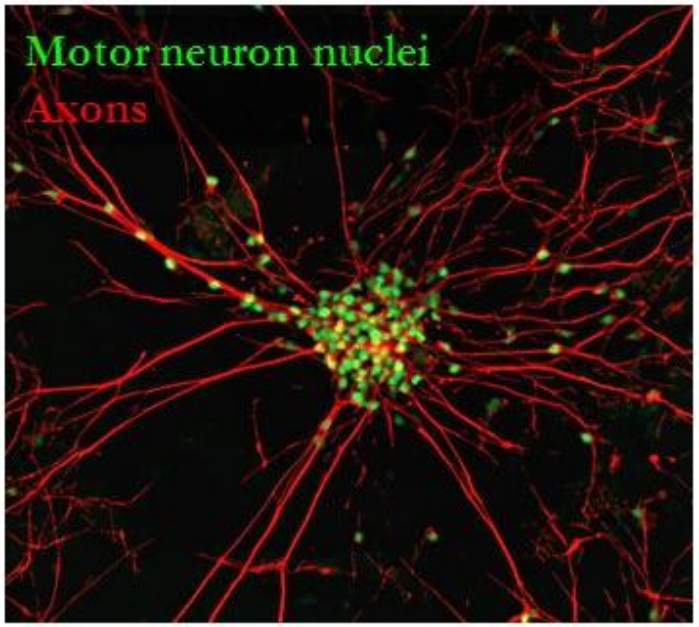
iPS cells
induced pluripotent stem cells





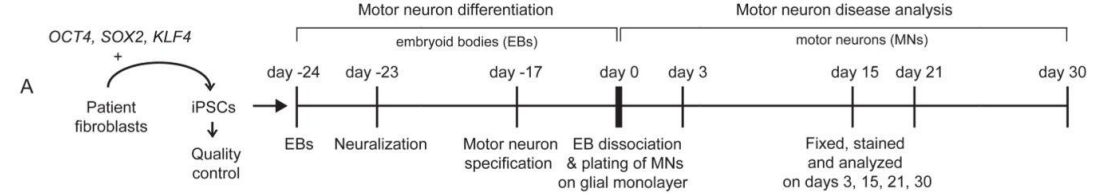
ALS motor neurons

Dimos, JT et al. (2008). Induced Pluripotent Stem Cells Generated from Patients with ALS Can Be Differentiated into Motor Neurons. Science 321: 1218-21.



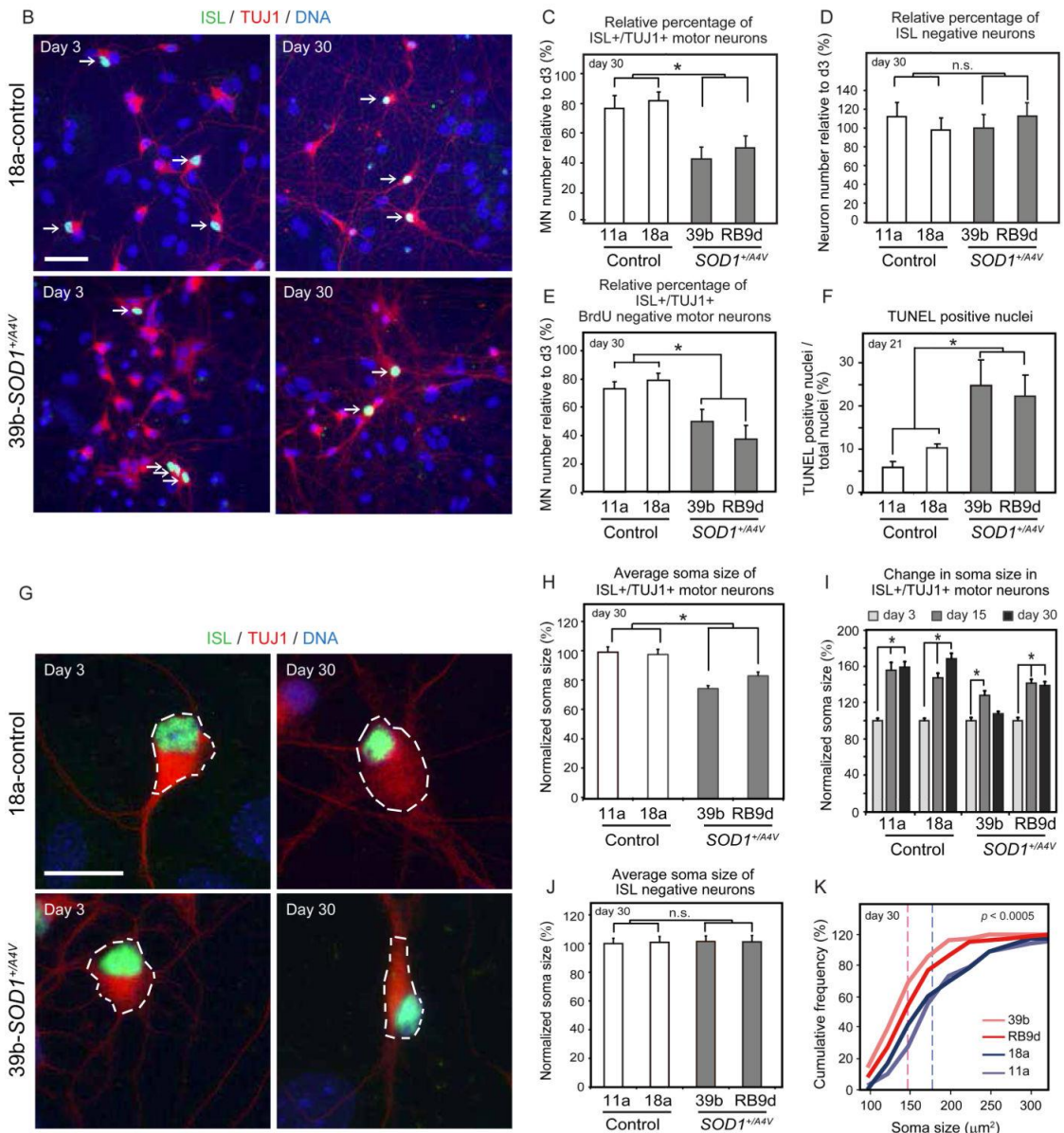
Amyotrophic Lateral Sclerosis (ALS)

ALS
patient-derived neurons

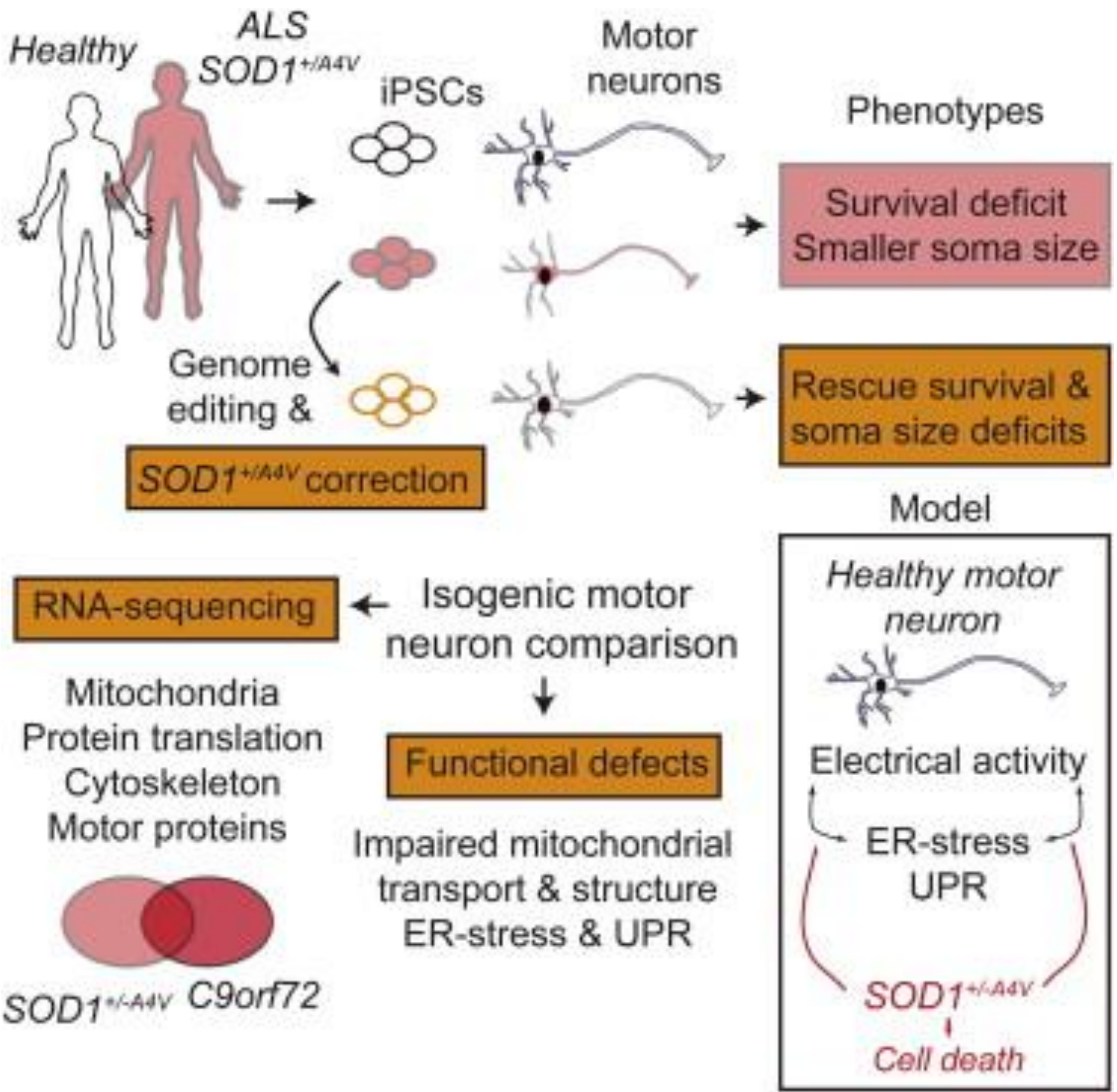


SURVIVAL DEFICIT

SMALLER SOMA SIZE



Amyotrophic Lateral Sclerosis (ALS)



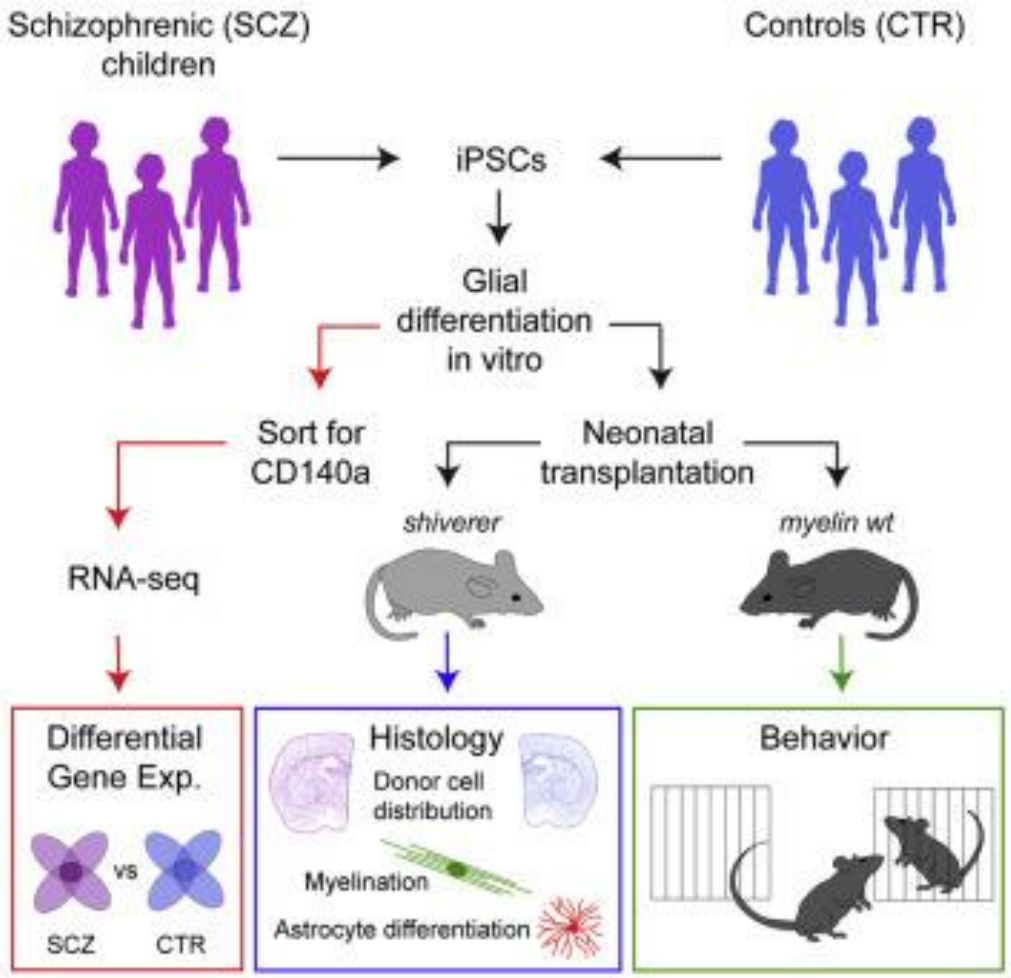
Modeling Parkinson's Disease

Schizophrenia (SCZ)

Cell Stem Cell

Human iPSC Glial Mouse Chimeras Reveal Glial Contributions to Schizophrenia

Graphical Abstract



Authors

Martha S. Windrem, Mikhail Osipovitch, Zhengshan Liu, ..., Robert L. Findling, Paul J. Tesar, Steven A. Goldman

Correspondence

steven_goldman@urmc.rochester.edu or goldman@sund.ku.dk

In Brief

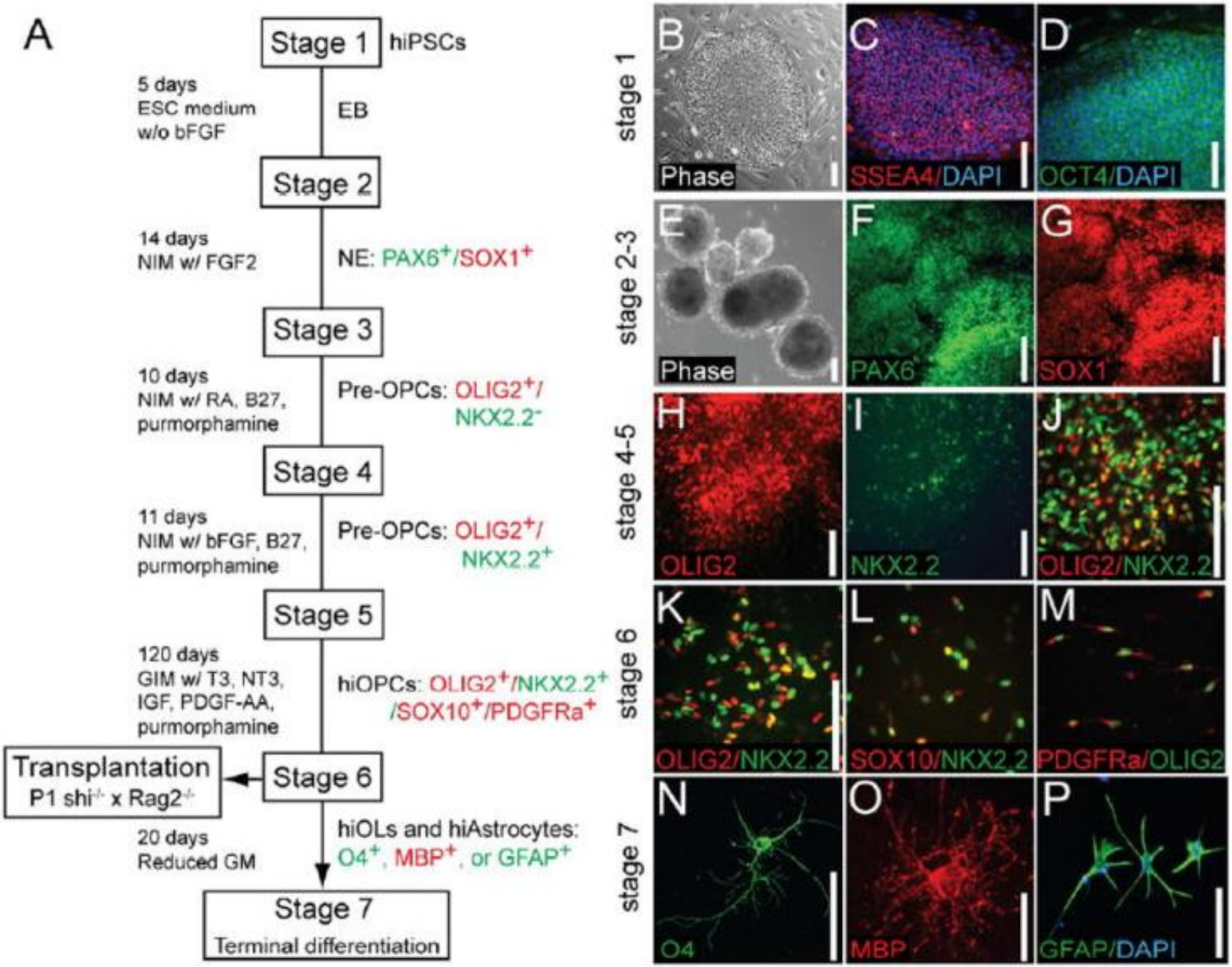
Goldman and colleagues use mice chimerized with human patient-derived glial progenitor cells to find out whether glia contribute to childhood-onset schizophrenia. The defects in cell differentiation, myelination, and behavior they see strongly suggest that glial cells do, in fact, have a previously unappreciated role in the pathogenesis of this disease.

Windrem et al., 2017, Cell Stem Cell 21, 195–208
August 3, 2017
http://dx.doi.org/10.1016/j.stem.2017.06.012

CellPress

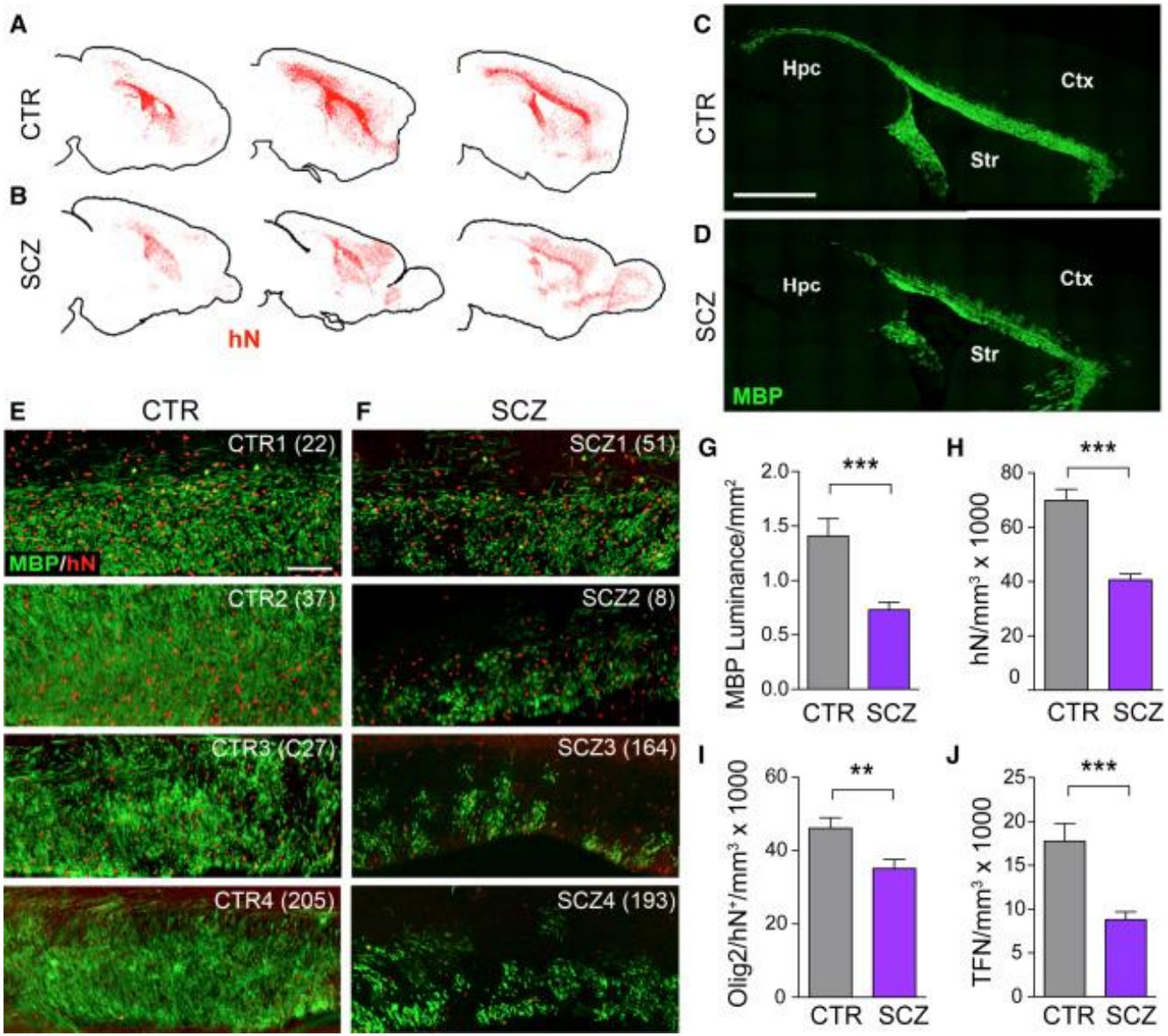
Schizophrenia (SCZ)

Human iPSCs can be directed into OPC fate

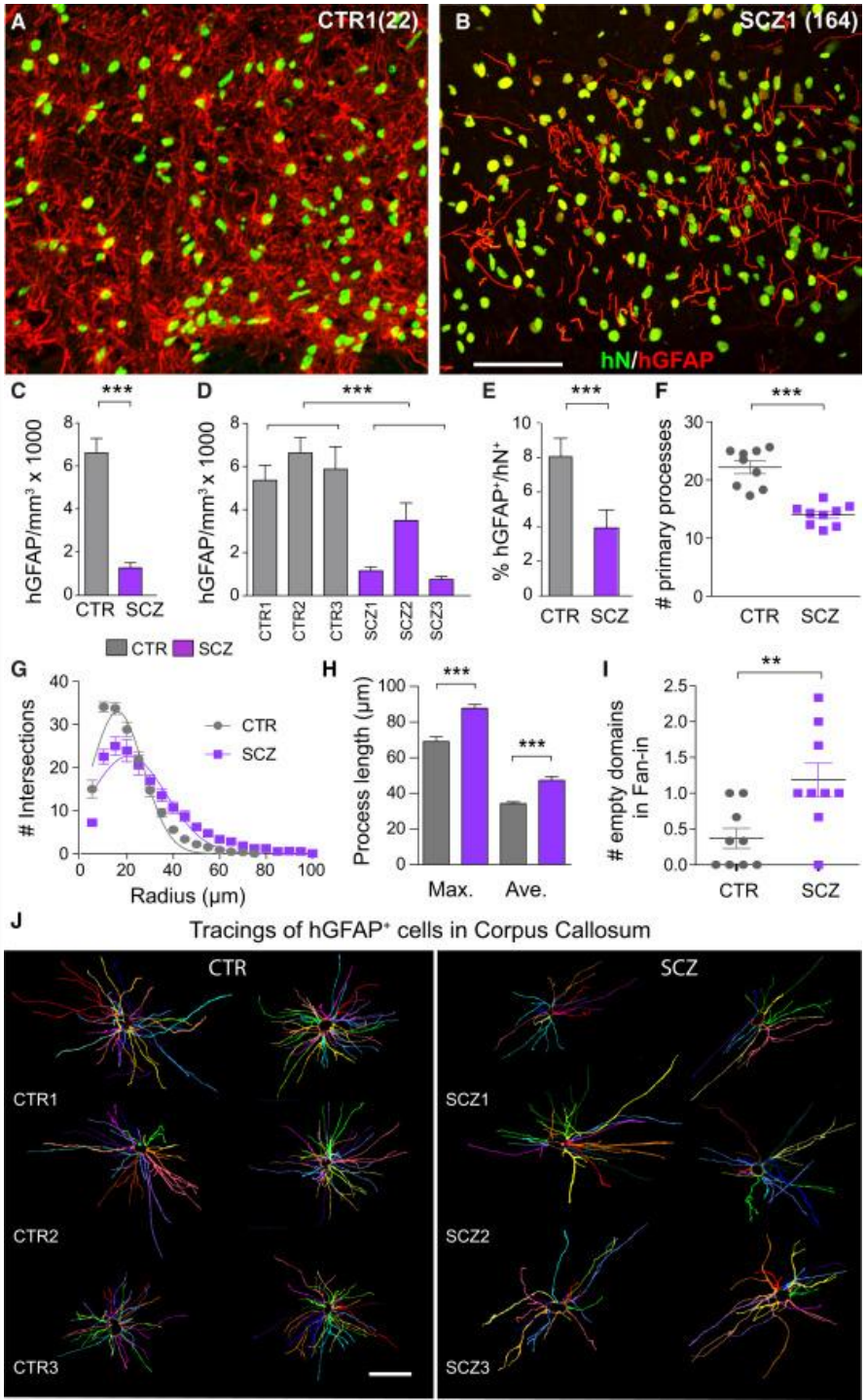


Schizophrenia (SCZ)

SCZ-derived hGPCs exhibit aberrant dispersal and relative hypomyelination



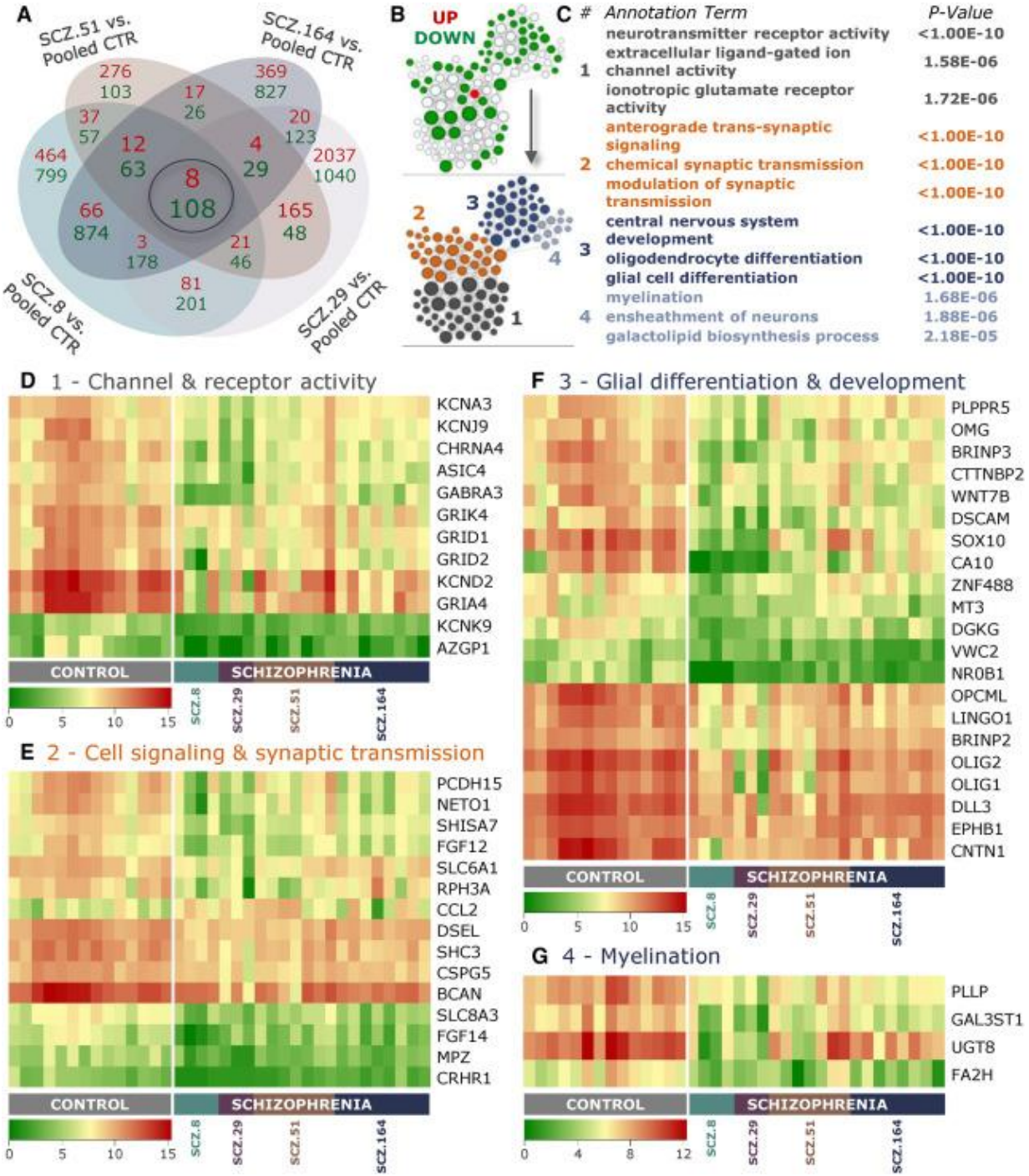
Schizophrenia (SCZ)



Astrocytic differentiation is impaired in SCZ hGPC chimeric brain

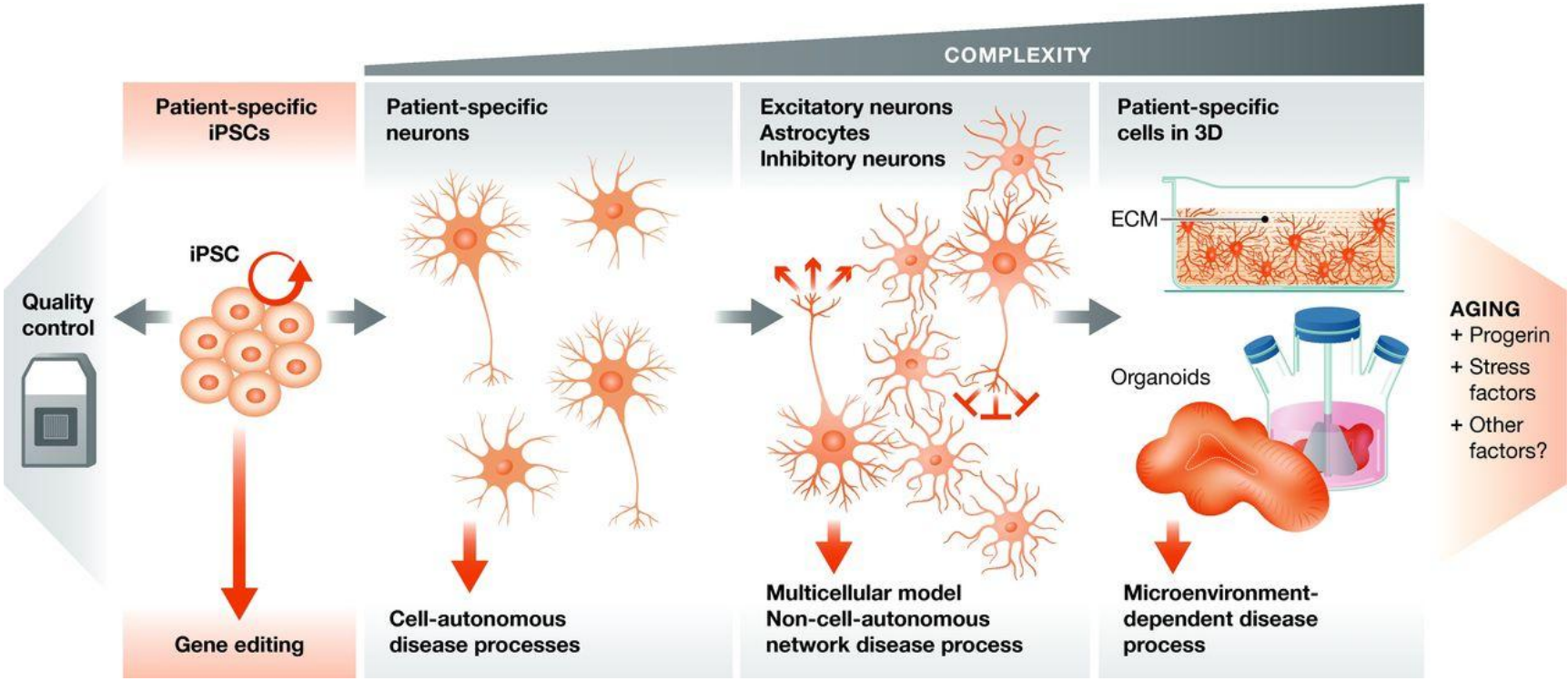
Schizophrenia (SCZ)

SCZ-derived hGPCs suppress glial differentiation-associated gene expression



From cell autonomy
to
more sophisticated systems

3D stem cell-based models



Organoids

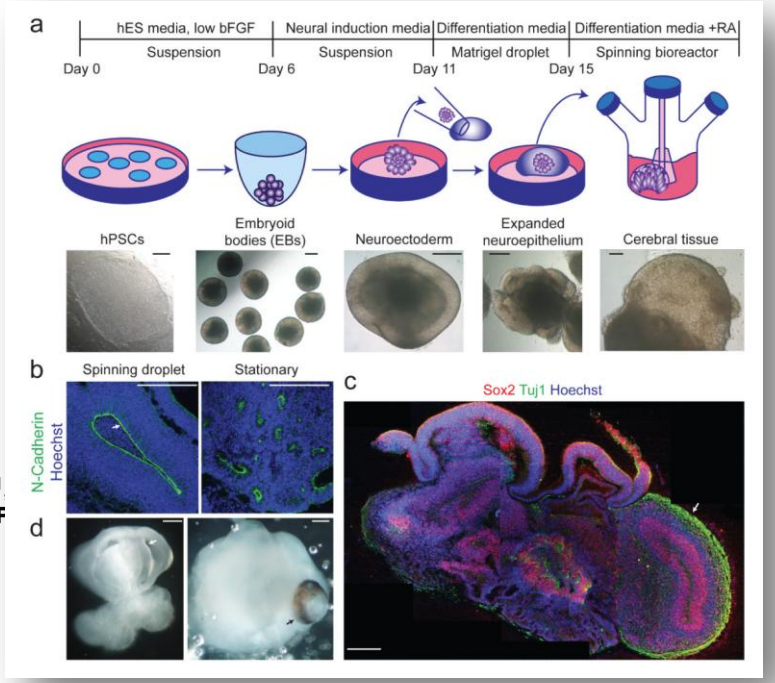
Ichida & Kiskinis, EMBO J 2015

Brain organoids

Nature. 2013 September 19; 501(7467): . doi:10.1038/nature12517.

Cerebral organoids model human brain development and microcephaly

Madeline A. Lancaster[1], **Magdalena Renner**[1], **Carol-Anne Martin**[2], **Daniel Wenzel**[1], **S. Bicknell**[2], **Matthew E. Hurles**[3], **Tessa Homfray**[4], **Josef M. Penninger**[1], **Andrew P Jackson**[2], and **Juergen A. Knoblich**[1]

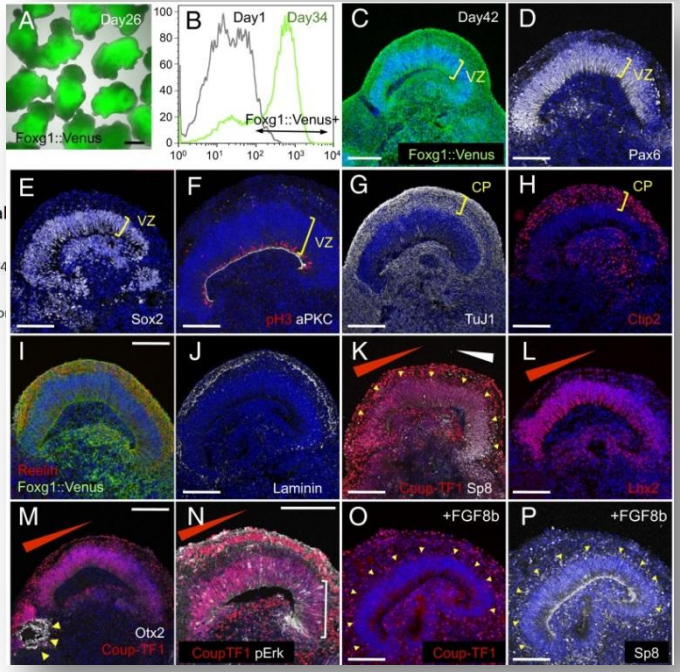


Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell–derived neocortex

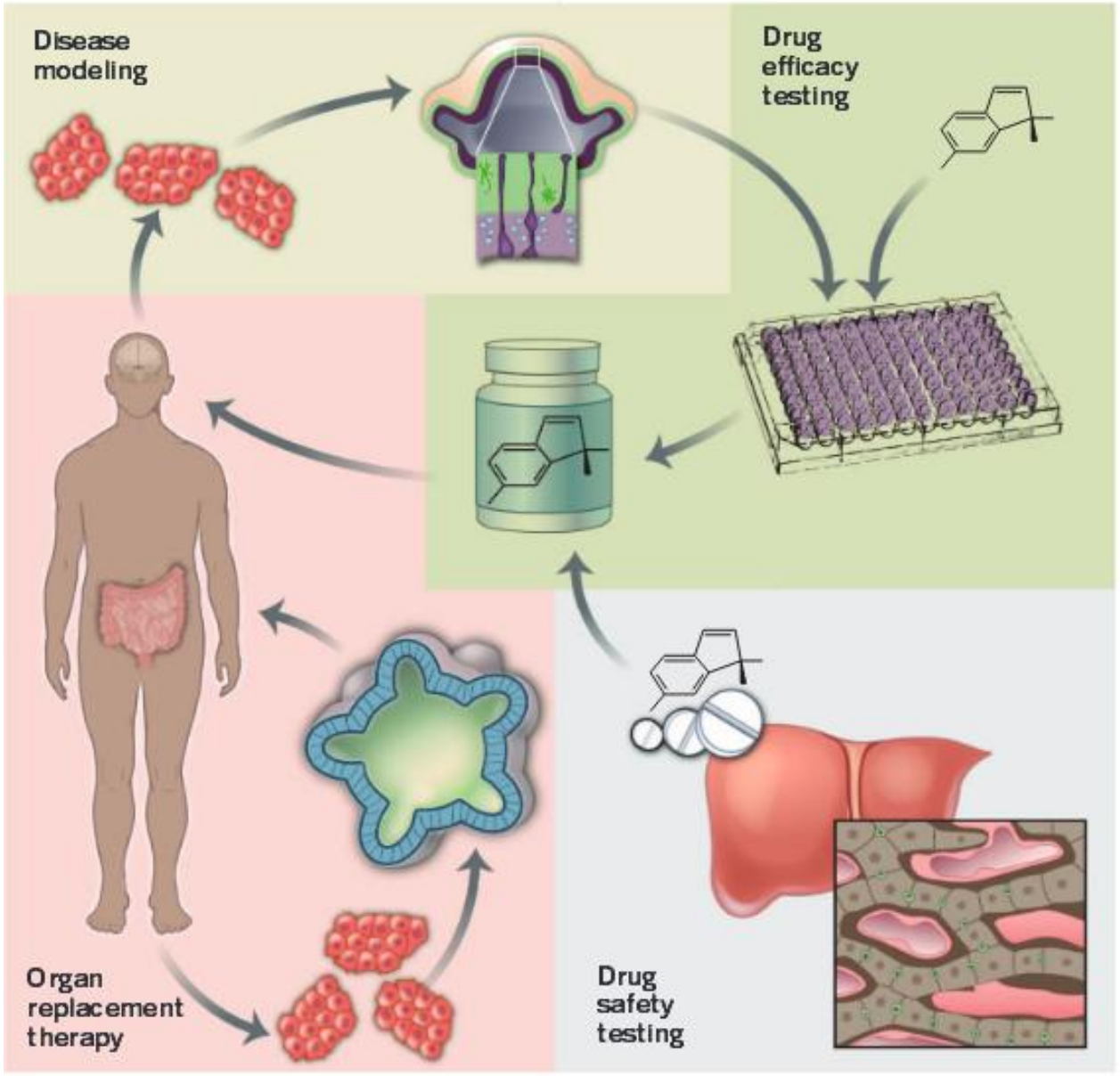
Taisuke Kadoshima[a,b,1], **Hideya Sakaguchi**[a,b], **Tokushige Nakano**[a,2], **Mika Soen**[a], **Satoshi Ando**[a,2], **Mototsugu Eiraku**[c] and **Yoshiki Sasai**[a,b,3]

[a]Laboratory of Organogenesis and Neurogenesis and [c]Four-Dimensional Tissue Analysis Unit, RIKEN Center for Developmental Biology, Kobe 650-0047 and [b]Department of Medical Embryology, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan

Edited by Chen-Ming Fan, Carnegie Institution of Washington, Baltimore, MD, and accepted by the Editorial Board October 17, 2013 (received for August 21, 2013)



Therapeutic potential of organoids



Lancaster & Knoblich, Science 2014

Take home points

iPSC-based models: study disease mechanisms in the context of human neurons and in the context of each patient's own unique genetic background

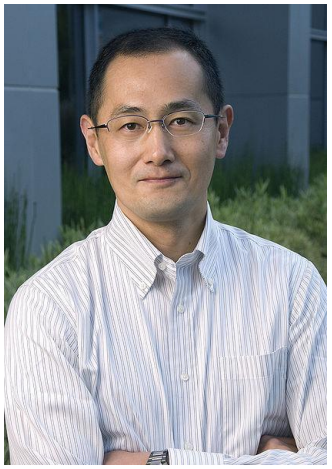
- What is the **right cell type** to make and study?
- What are the **right controls** to use when assessing a **disease-related phenotype**?
- How do **phenotypes** identified *in vitro* relate to the **clinical presentation of patients**?

- Can we match an ***in vivo* clinical trial** with an ***in vitro* iPSC-based clinical trial** to monitor the correlation of outcome measures?
- Can we predict how patients will respond to a **potential therapeutic treatment** by studying their stem cell-derived neurons?

Perhaps the seemingly biggest advantage of this approach - **the ability to study disease in the genetic background of the patient** - has created the **biggest challenge**, as genetic background contributes to **high variability in the properties of the patient-derived cells.** This variability is a reality that neurologists have been facing for years, as often, two patients diagnosed with the same condition might present with **very different clinical profiles.**

The technology of **cellular reprogramming** has brought this reality of **clinical heterogeneity** seen in patients **from the bedside to the lab bench.**

The answers to these questions will help us conclude what are the **capabilities** and **limitations** of this **promising technological tool.**



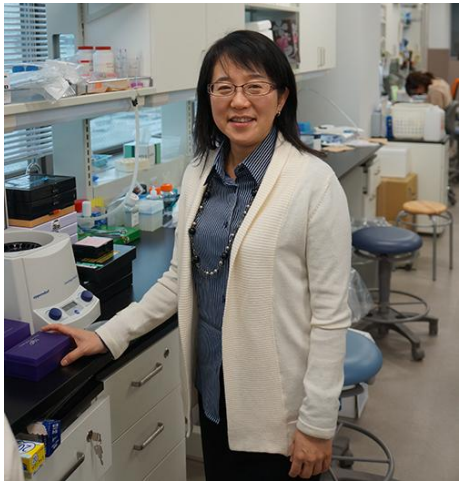
Telephone interview with **Shinya Yamanaka** following the announcement of the **2012 Nobel Prize in Physiology or Medicine**, 8 October 2012

[AS] But I just wanted ask you one final question, which was what your greatest hopes for stem cells technologies are at the moment? What do you hope will be the first benefit?

[SY] Well, I will bring this technology to clinics. I really want to help as many patients as possible. As you may know, I started my career as a surgeon 25 years ago. But it turned out that I am not talented as a surgeon. So I decided to change my career, from clinics to laboratories. But I still feel that I am a doctor, I am a physician, so I really want to help patients. **So my goal, all my life, is to bring this technology, stem cell technology to the bedside, to patients, to clinics.**

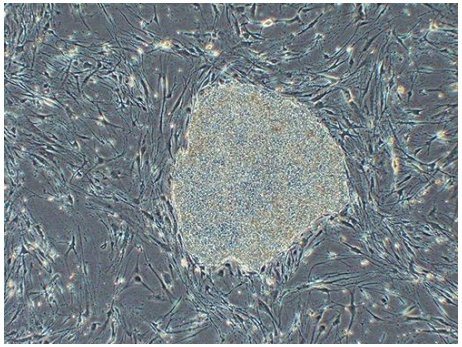
Thank you

Pilot clinical study into iPS cell therapy for eye disease in Japan

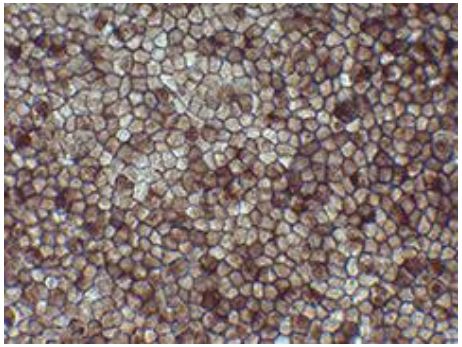


Masayo Takahashi M.D., Ph. D.
Laboratory for Retinal Regeneration
RIKEN Center for Developmental Biology

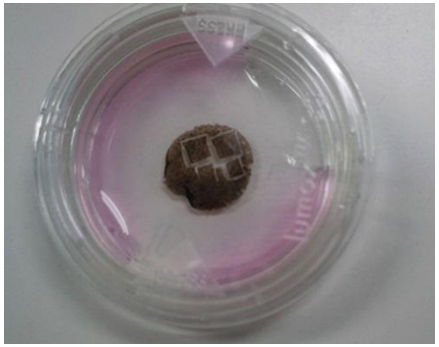
First patient
to receive iPSC-derived implant:
70 year old Japanese woman
age-related macular degeneration



Human iPSCs

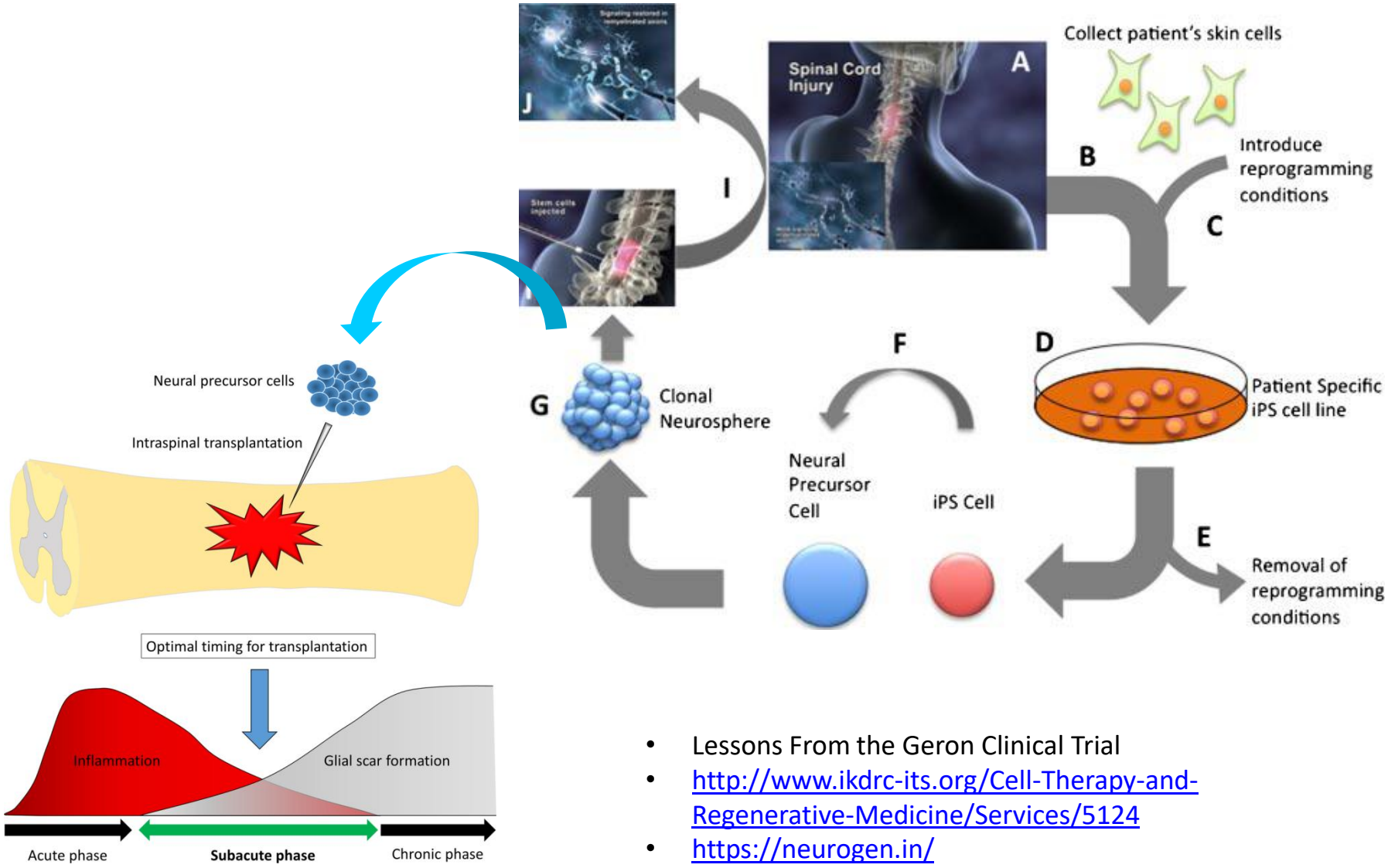


Human iPSC-derived
Retinal Pigment Epithelium (RPE)



RPE cell sheet

Applications of iPSC technologies in spinal cord injury



- Lessons From the Geron Clinical Trial
- http://www.ikdrc-its.org/Cell-Therapy-and-Regenerative-Medicine/Services/5124
- https://neurogen.in/

Nagoshi & Okano, J Neurochemistry 2017

Applications of iPSC technologies

Table 1 Planned clinical trials of iPS cell-based therapies

Principal investigator (Institute/Location)	*Cell type to transplant*	*Target disorders*
Masayo Takahashi, (RIKEN)	Retinal Pigment Epithelium (sheet)	Age-related macular degeneration (wet type)
Alfred Lane, Anthony Oro, Marius Wernig (Stanford University)	Keratinocytes	Recessive dystrophic epidermolysis bullosa (RDEB)
Mahendra Rao (NIH)	DA neurons	Parkinson's disease
Koji Eto (Kyoto University)	Megakaryocyte	Thrombocytopenia
Jun Takahashi (Kyoto University)	DA neurons	Parkinson's disease
Steve Goldman, (University of Rochester)	Oligodendrocyte precursor cell	Multiple Sclerosis
Hideyuki Okano, Masaya Nakamura (Keio University)	Neural stem/progenitor cells	Spinal Cord Injury
Shigeto Shimmura (Keio University)	Corneal endothelial cells	Corneal endothelial dysfunction
Koji Nishida (Osaka University)	Corneal epithelial cells (sheet)	Corneal epithelial dysfunction and trauma (e.g. Stevens–Johnson syndrome)
Yoshiki Sawa (Osaka University)	Cardiomyocytes (sheet)	Heart Failure
Keiichi Fukuda (Keio University)	Cardiomyocytes (sphere)	Heart Failure
Yoshiki Sasai and Masayo Takahashi (RIKEN)	Neuroretinal sheet including photoreceptor cells	Retinitis pigmentosa
Advanced Cell Technology	Megakaryocytes	Refractory thrombocytopenia

Representative studies of iPS-based cell therapy with planned clinical trials are listed.
References: [17,19-29].

Okano & Yamanaka, Mol Brain 2014