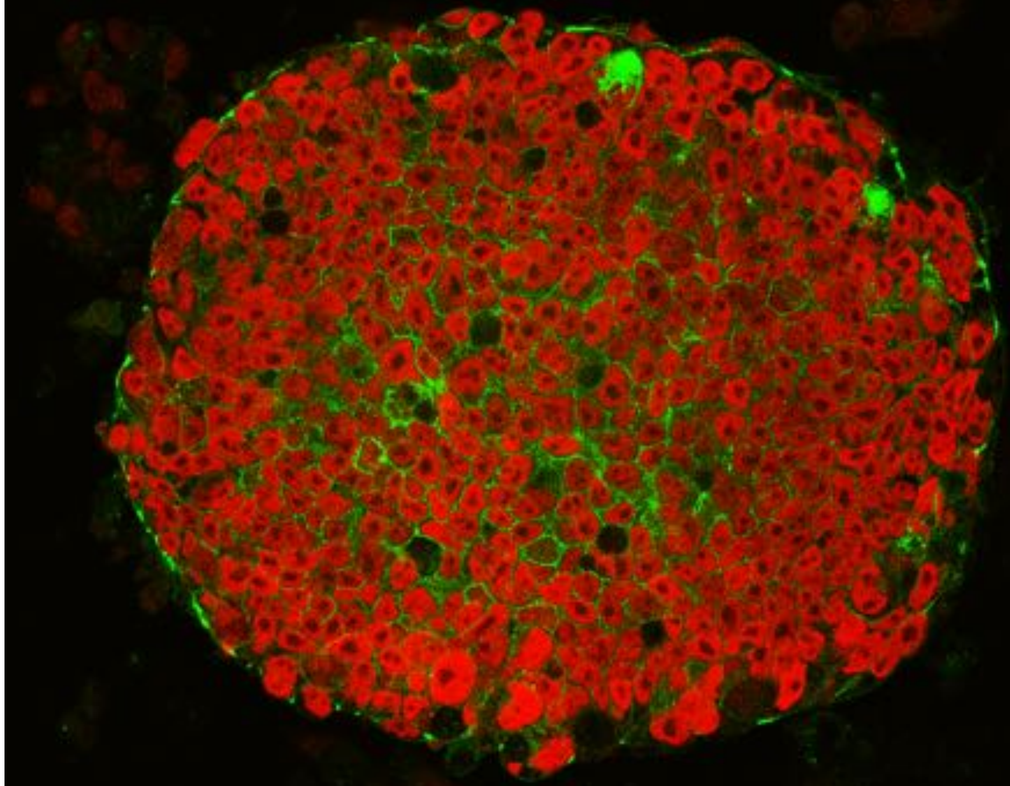
Stem cell systematics and potentials for modeling and therapy



Poul Hyttel
University of Copenhagen

The Anatomy Building
University of Copenhagen, Frederiksberg

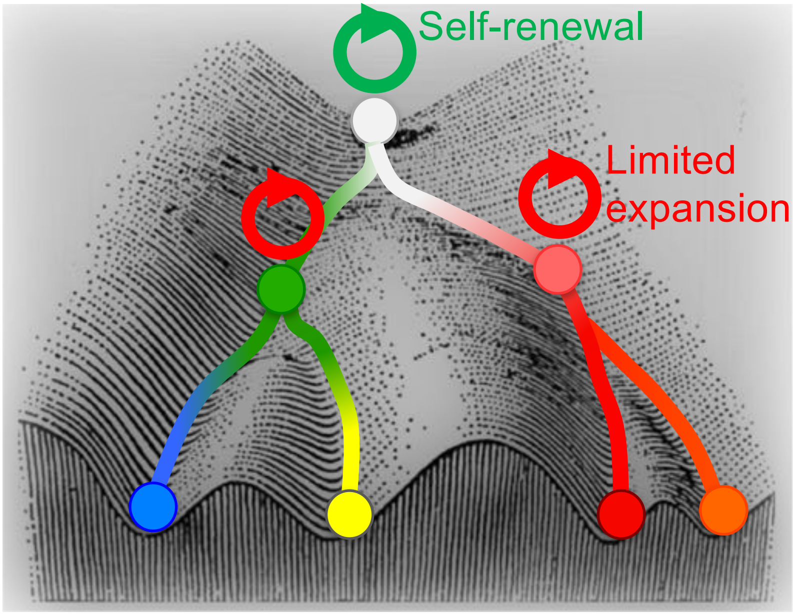
Contents

Contents

Stem cell definitions

- Self-renew (indefinite multiplication)
- Differentiate into specialized cell types (differentiation potency)

Conrad Waddington (1957)



Stem cell

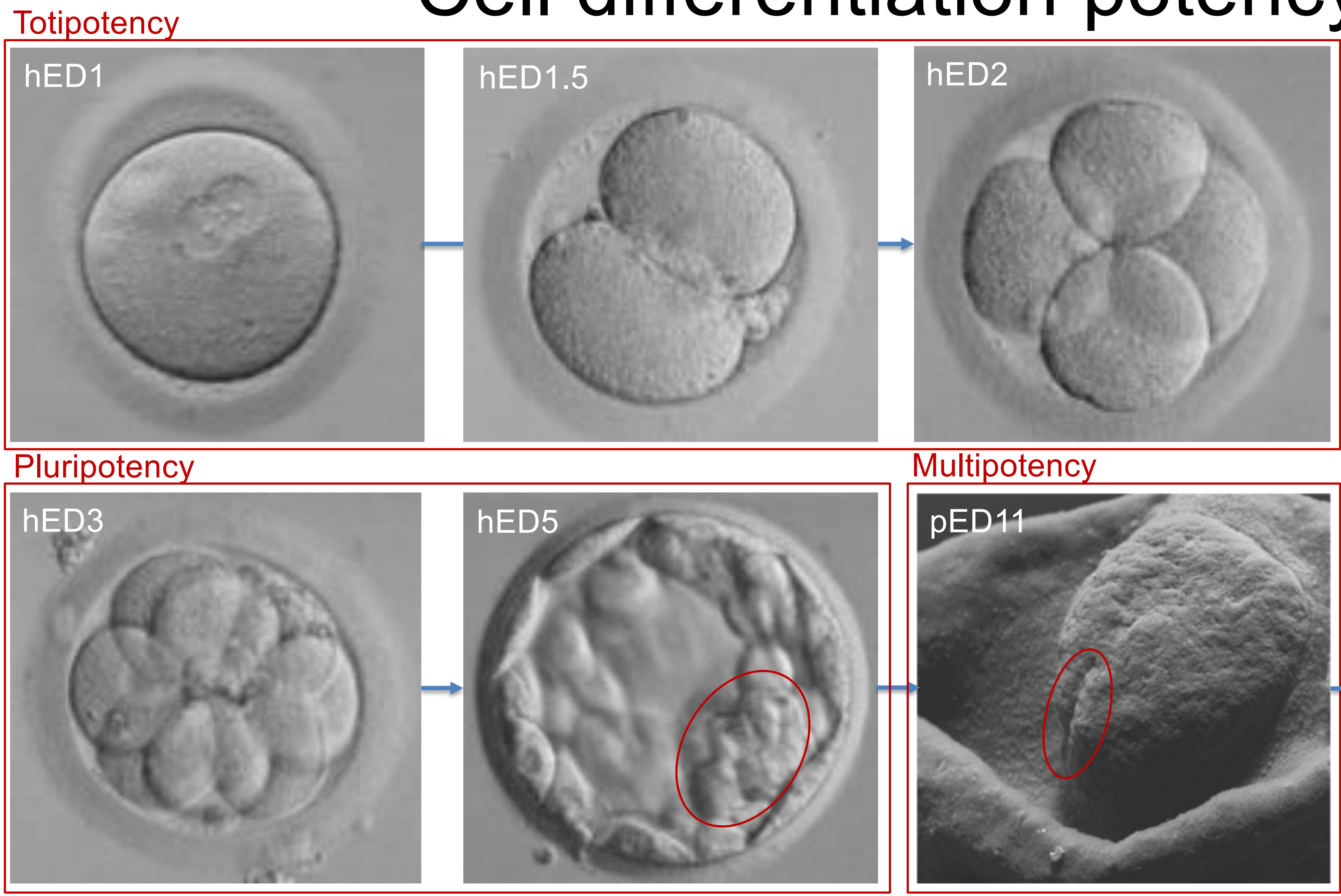
Intermediate progenitors

Terminally differentiated

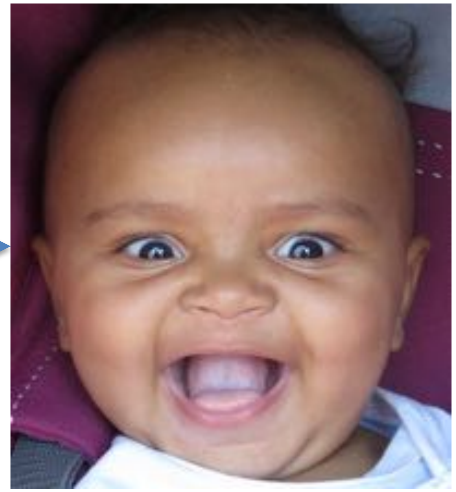
Contents

- Stem cell definitions
- Cell differentiation potency
- The stem cell hierarchy: Pluripotent, multipotent and unipotent stem cells
- Induced pluripotent stem cells for disease modeling
- Stem cell therapy
- Conclusions

Cell differentiation potency



- Totipotency: All embryomic and extra-embryonic cell types
- Pluripotency: All embryonic cell types
- Multipotency: Several cell types
- Unipotency: A single cell type



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Most common stem cell types

- Pluripotent stem cells (PSCs)
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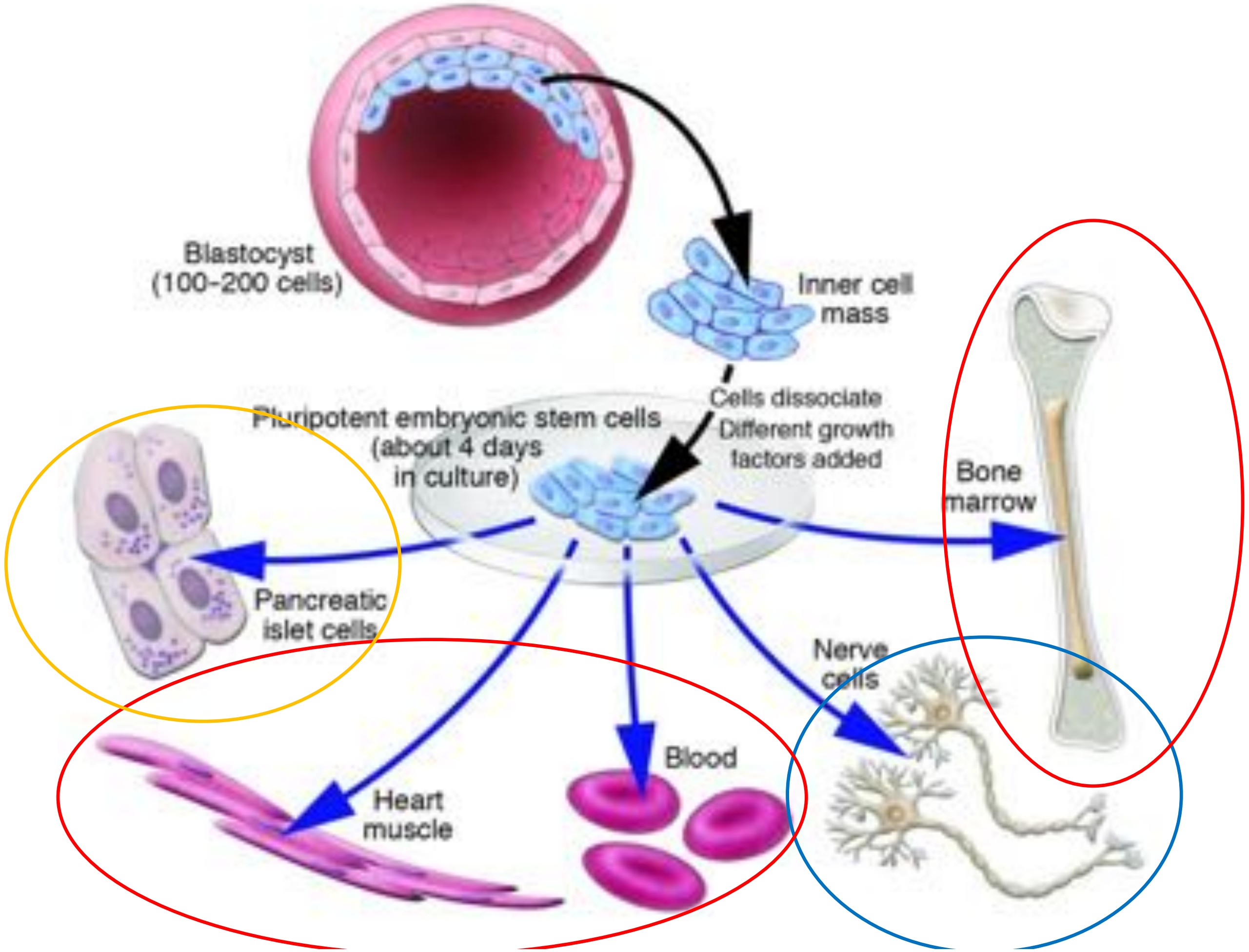
Embryonic stem cells (ESCs)

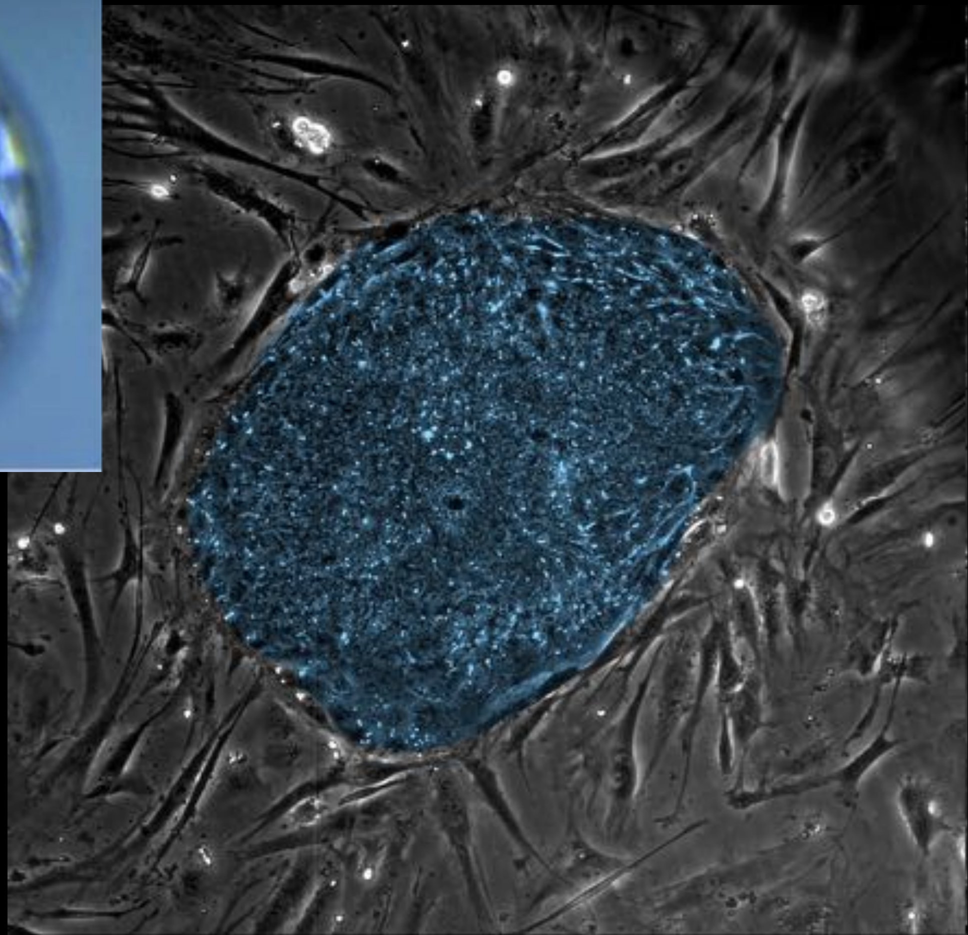


Ectoderm

Mesoderm

Endoderm

Human embryonic stem cells (hESCs) - 1998



Thompson et al. (1998)


THIS IS THE STEM
CELL RESEARCH LAB,
THE STEM CELL ETHICS
COMMITTEE IS
NEXT DOOR...

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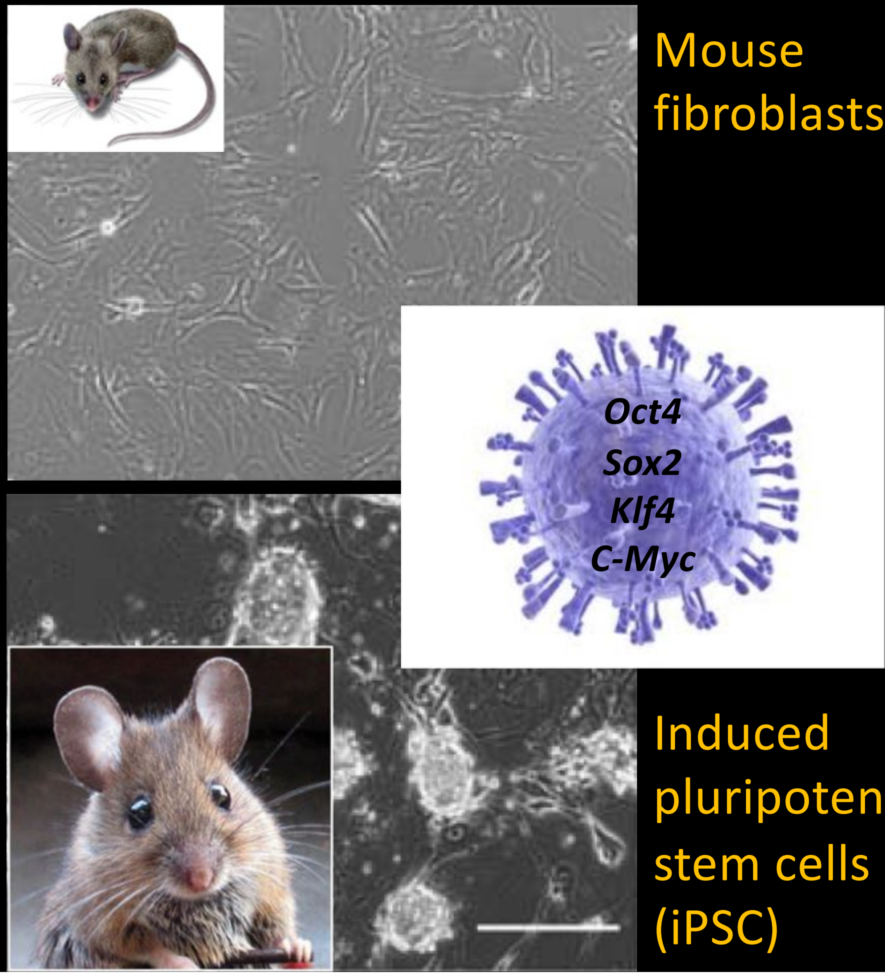
Induced pluripotent stem cells (iPSCs)

Shinya Yamanaka

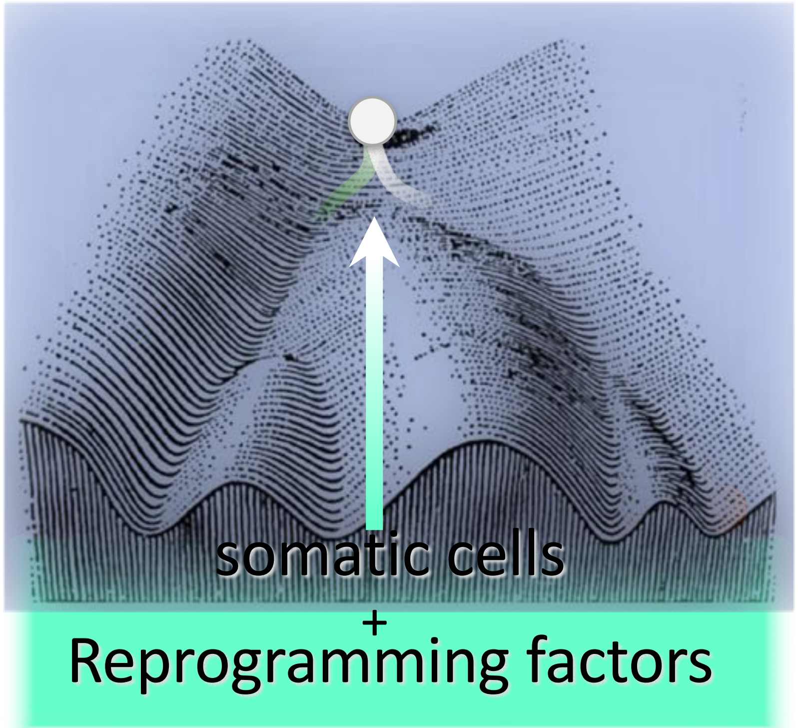


Mouse fibroblasts

Induced pluripotent stem cells (iPSC)

Cell differentiation potency

Conrad Waddington (1957)



Nobelprize 2012 in medicine

John B Gurdon

Shinya Yamanaka

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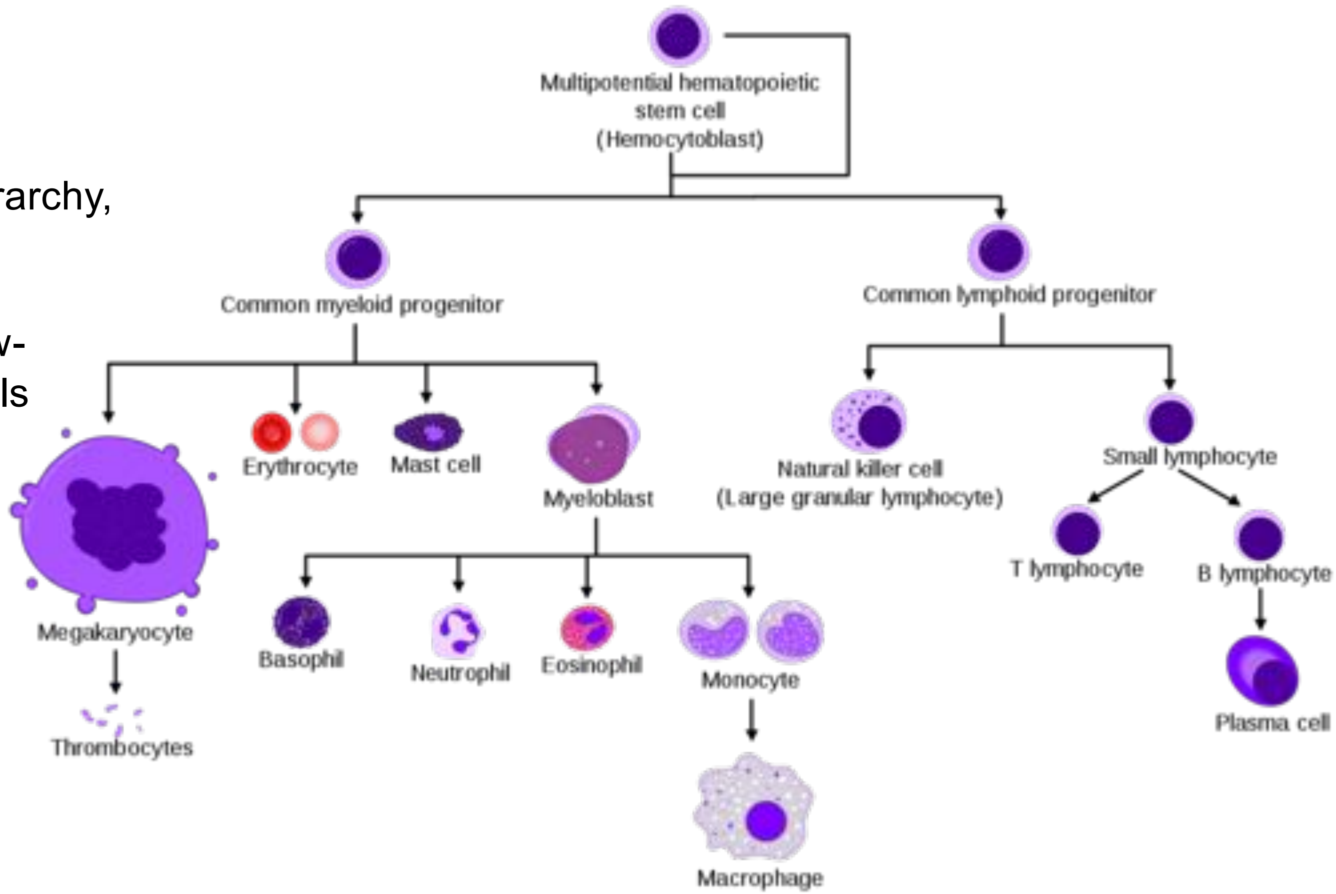
Hematopoietic stem cells (HSCs)

Reside in bone marrow

Most well defined stem cell hierarchy, but still developing

Transplantation of bone marrow-derived hematopoietic stem cells commonly used



Eaves (2015)

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Mesenchymal stem cells (MSCs)

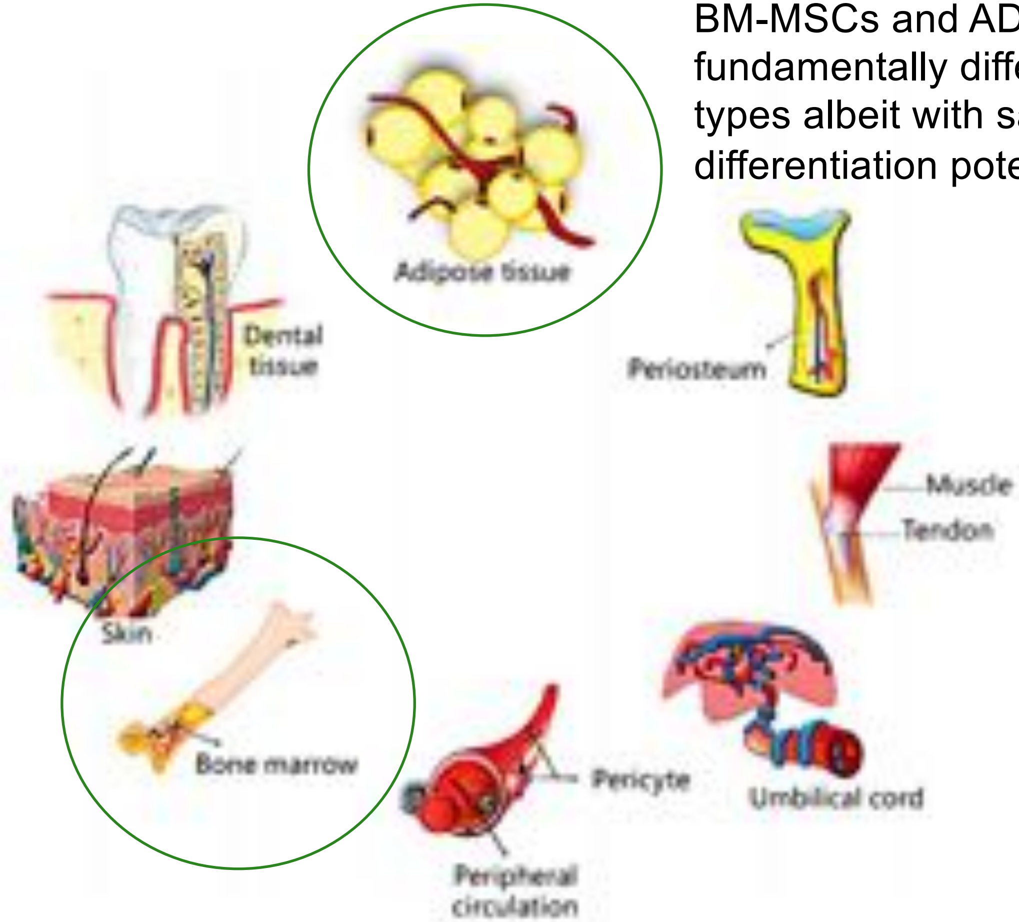
Reside in different organs

Definition

- Adhere to plastic
- Positive for CD73, CD90 and CD105
- Negative for CD14, CD34, CD45 and HLA-DR
- In vitro differentiation into
    - Chondroblasts
    - Osteoblasts
    - Adipocytes

- Further differentiation potentials
    - Myocytes
    - Neurons
    - Oligodendrocytes
    - Hepatocytes
    - Pancreocytes

BM-MSCs and AD-MSCs fundamentally different cells types albeit with same differentiation potentials



Oryan et al. (2017); Ullah et al. (2015); Noël et al. (2008)

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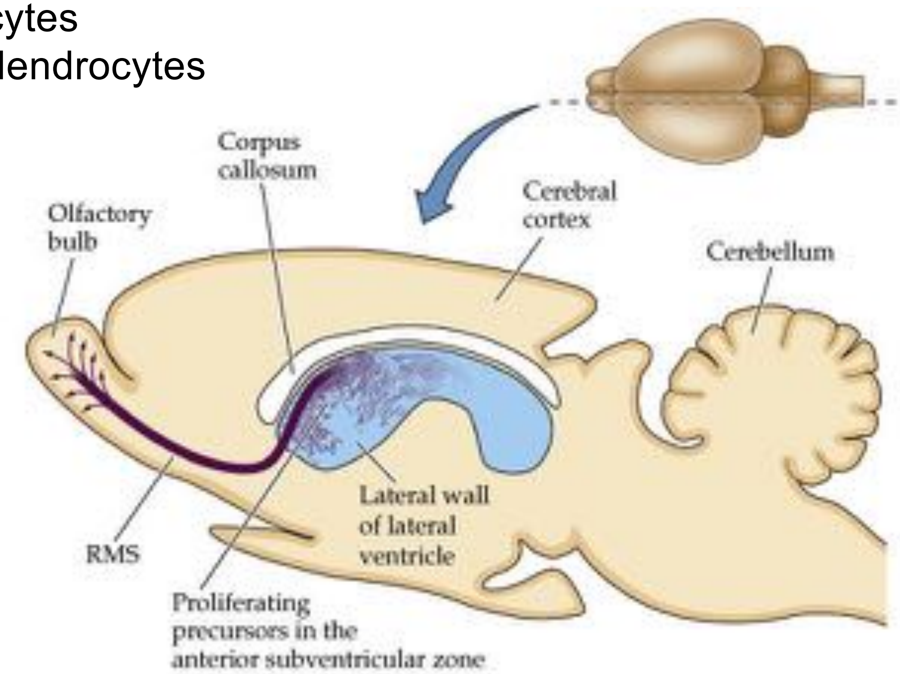
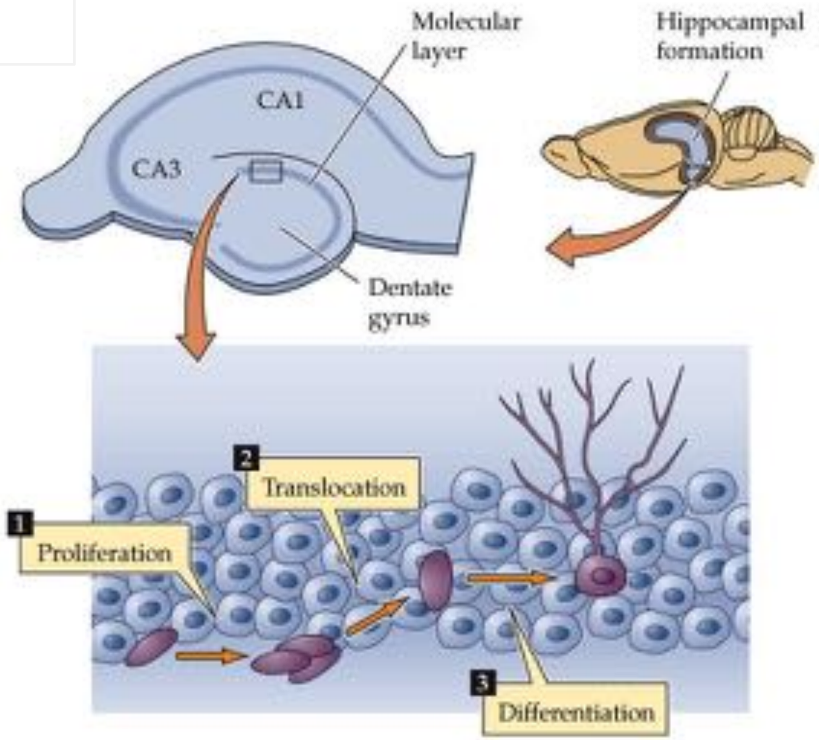
Neural stem cells (NSCs)

Reside in CNS

Differentiate into
- Neuronal subtypes
- Astrocytes
- Oligodendrocytes





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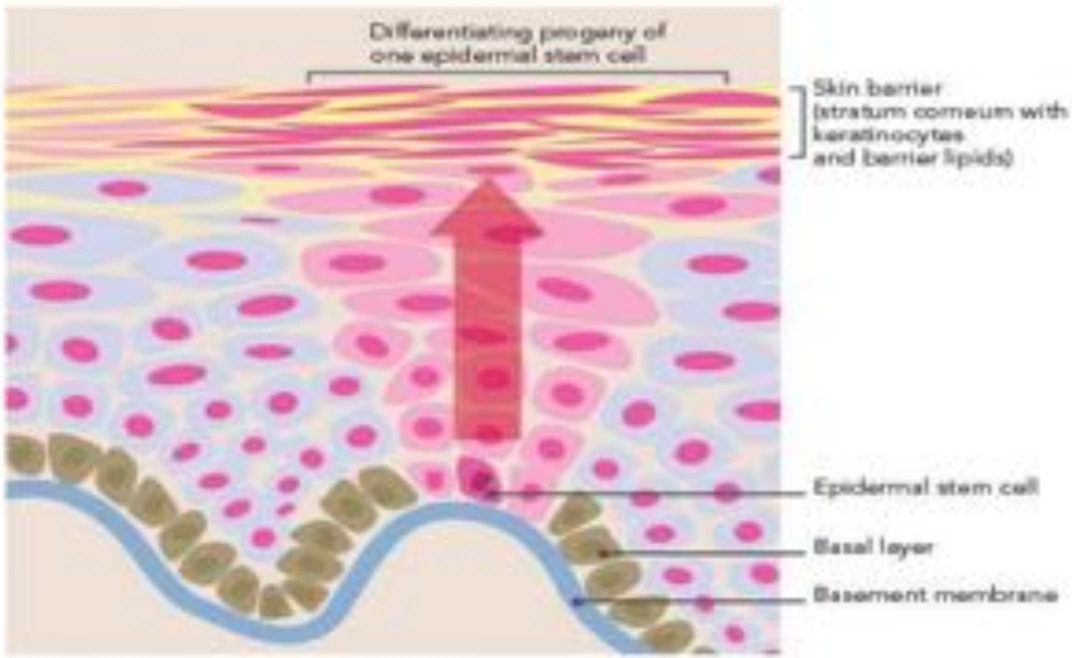
Epidermal stem cells (EpiSC)

Reside in skin

Differentiate into
- Keratinocytes



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How can iPSCs help modeling dementia?

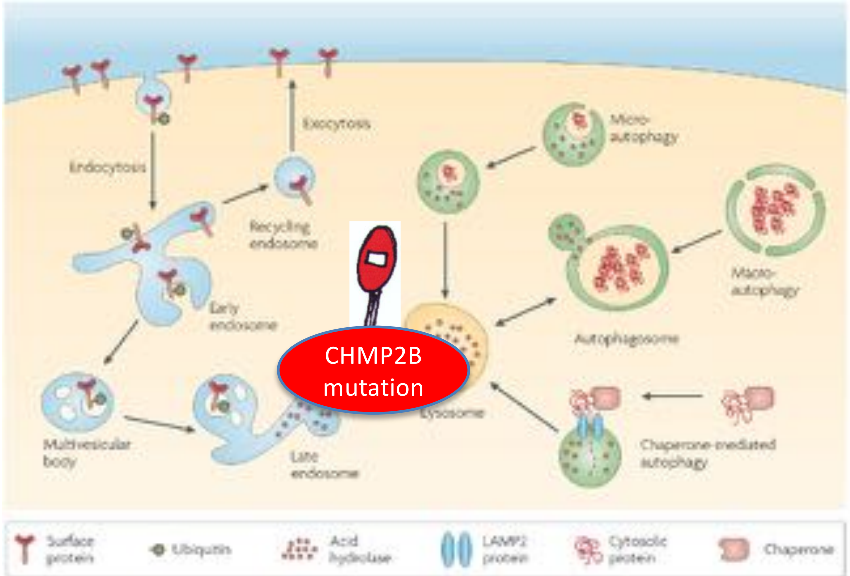
Access to the human target: iPSC-derived patient-specific neurons

Frontotemporal dementia localized to chromosome 3 (FTD3)

Mutation in *CHMP2B* on chromosome 3 resulting in dementia with early onset

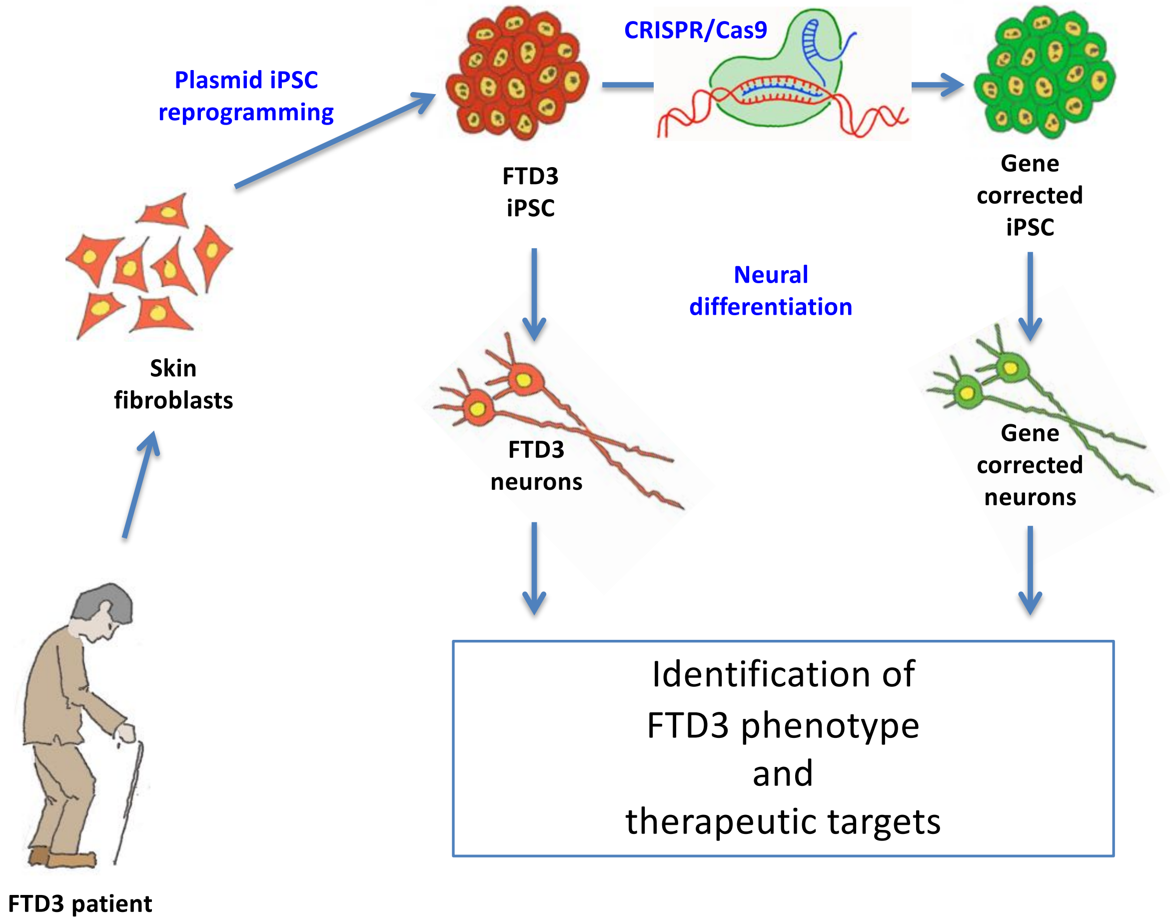
DANISH DEMENTIA RESEARCH CENTRE

FReJa Consortium

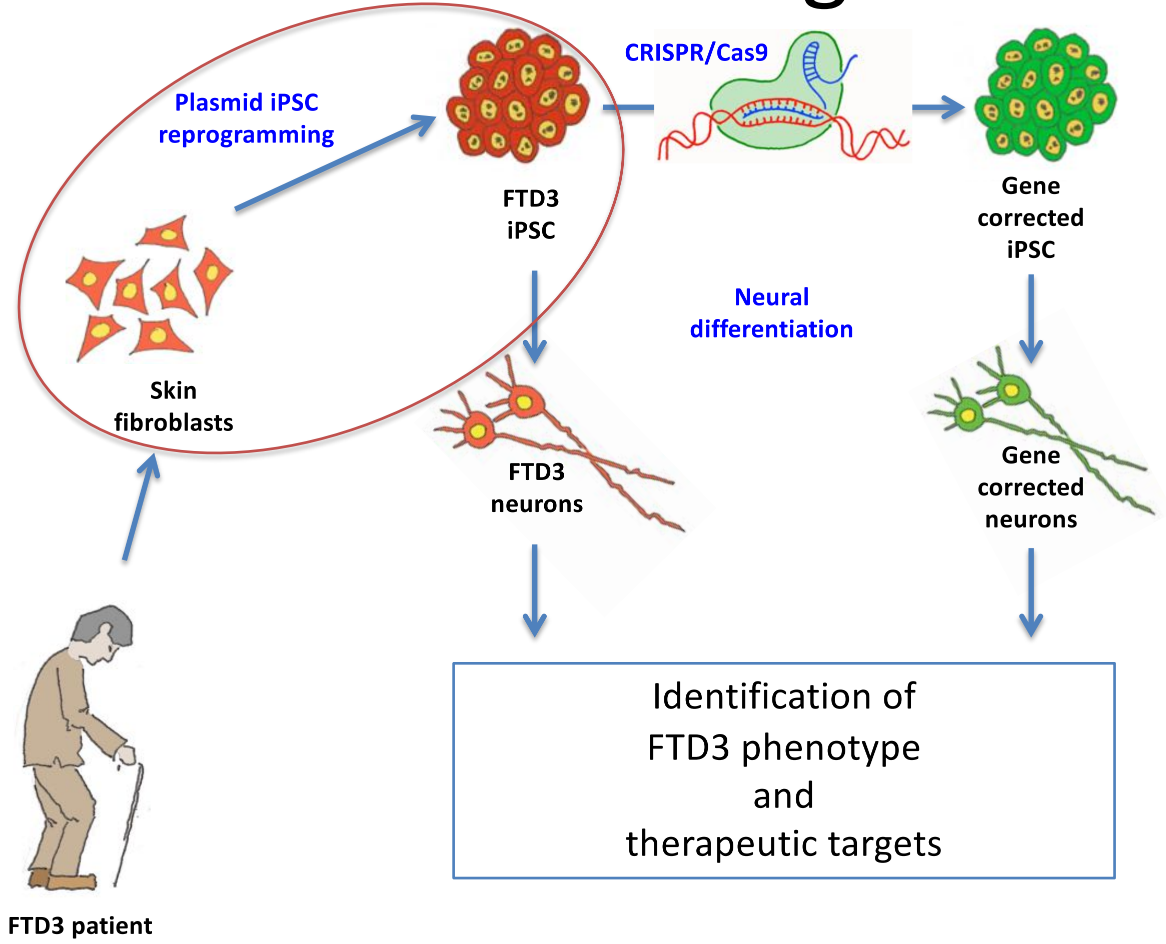


Nature Reviews | Neuroscience

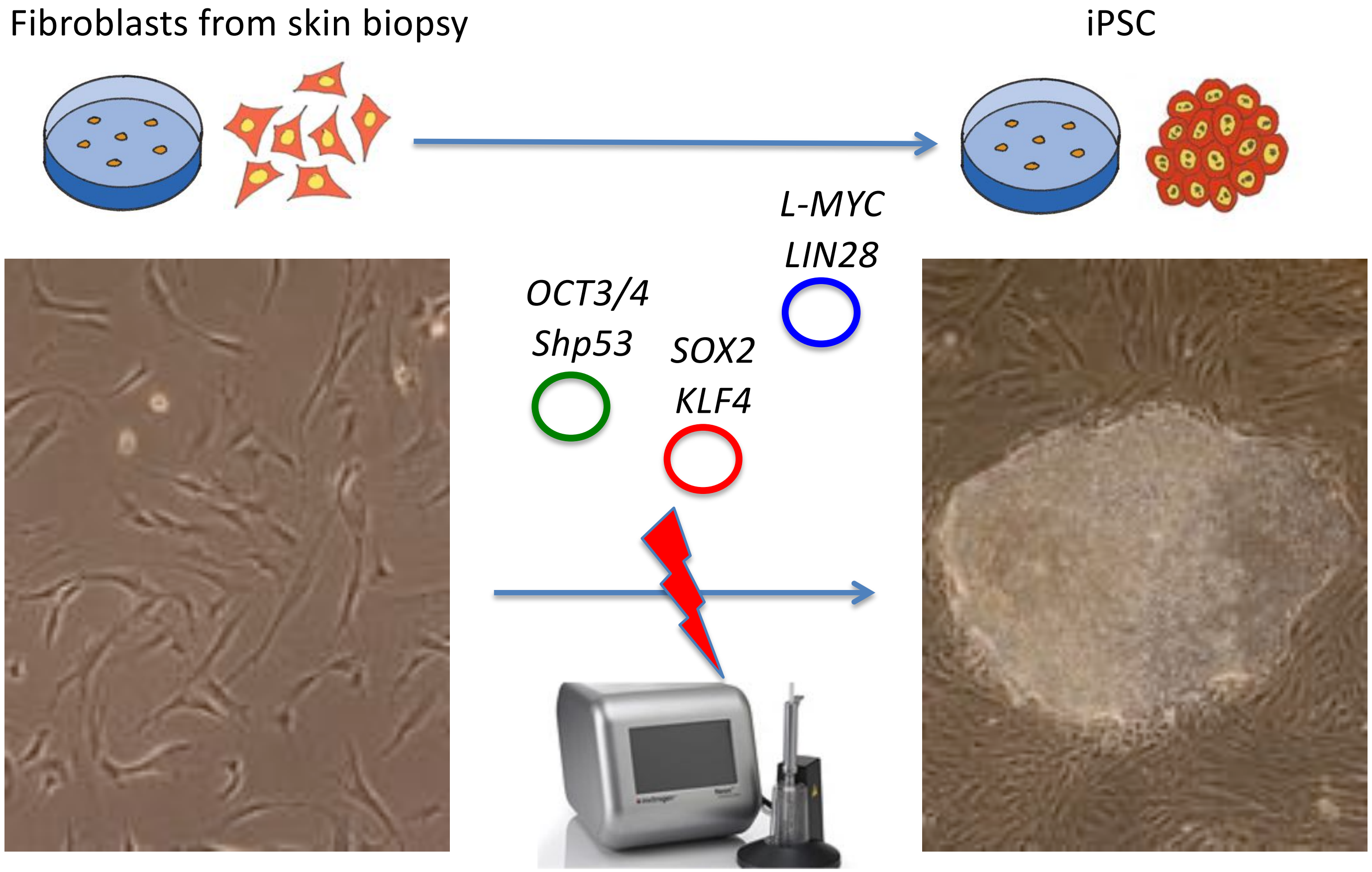
iPSC-based modeling of FTD3

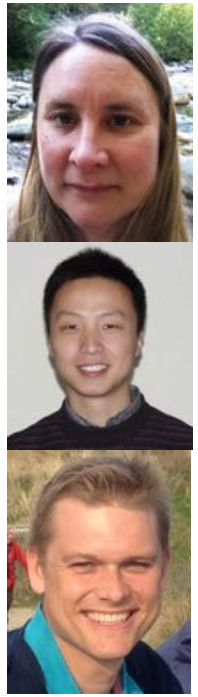


iPSC-based modeling of FTD3



Generation of integration-free patient-specific iPSCs

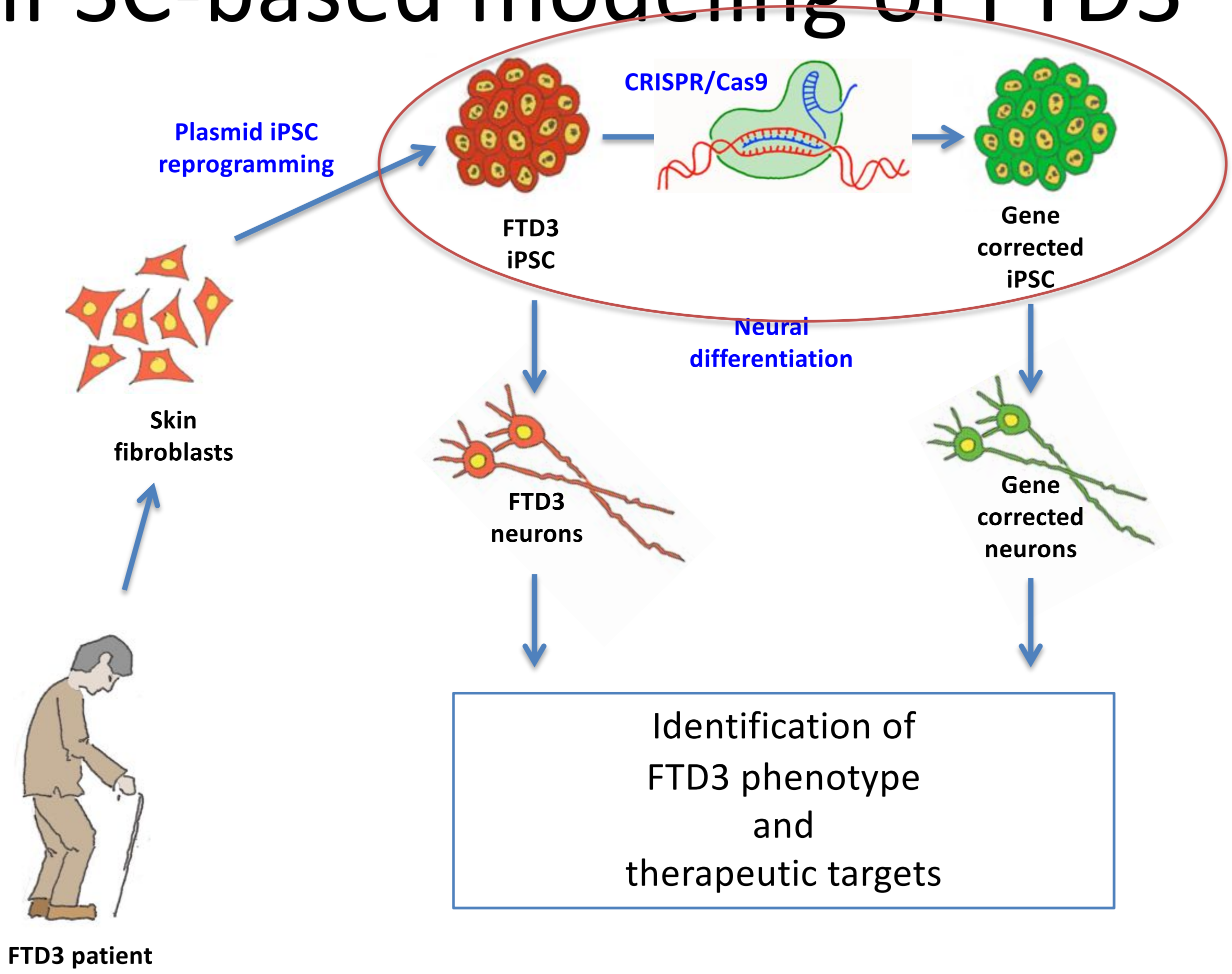




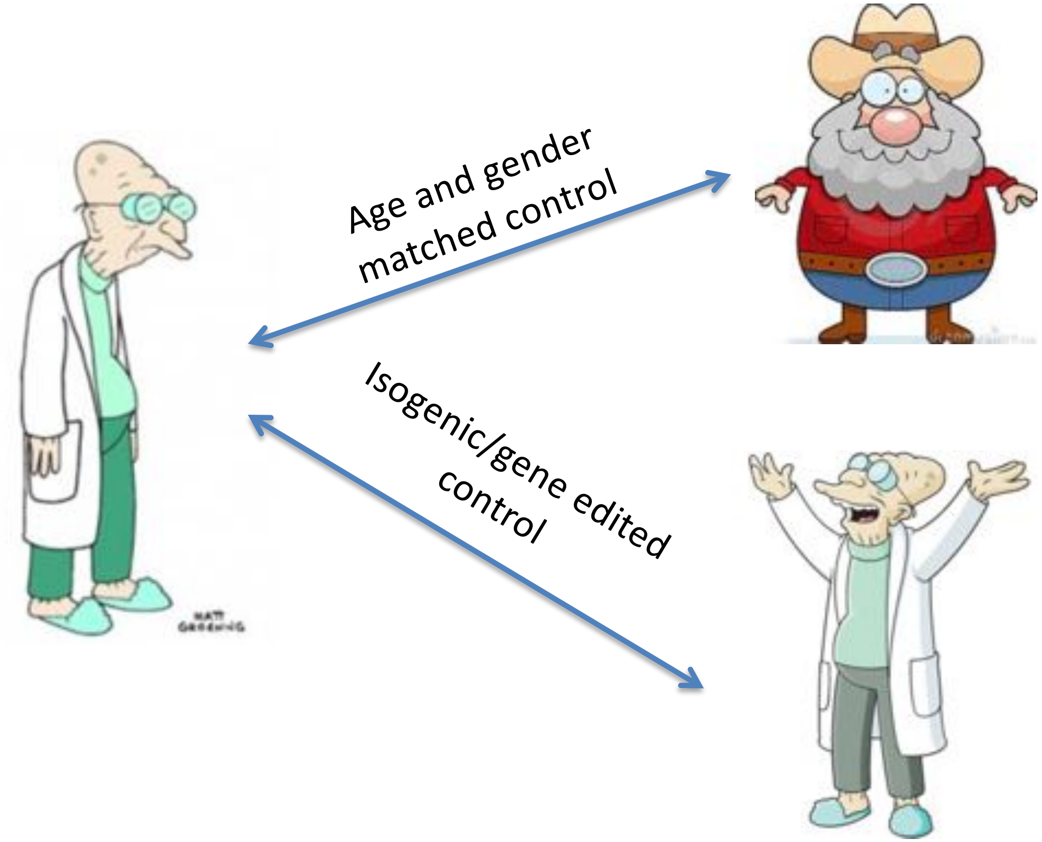
Okita et al. 2011
Rasmussen et al. 2011

Mikkel Rasmussen, Kristine Freude and Yu Zhang

iPSC-based modeling of FTD3

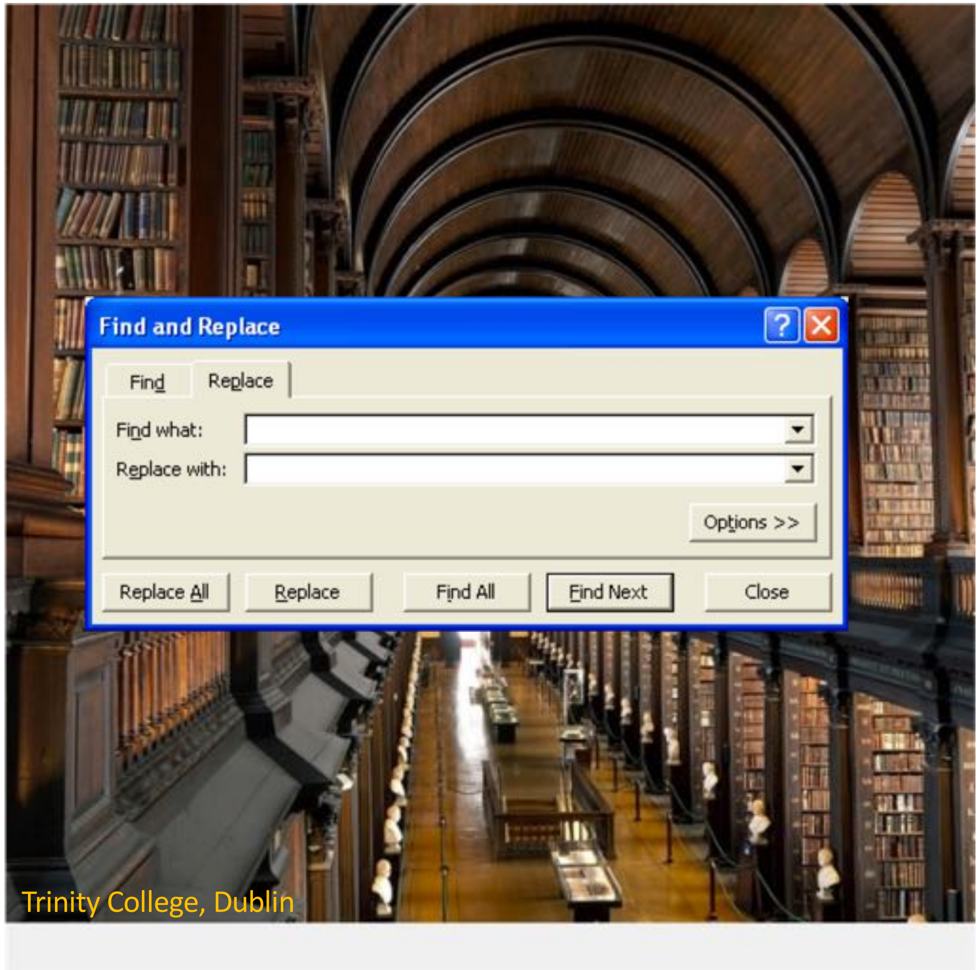


Importance for gene editing in iPSC disease modeling



Courtesy of Kristine Freude

Gene editing of iPSCs

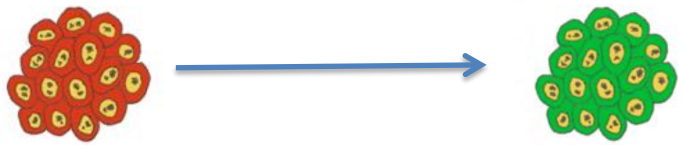


Courtesy of
Jacob Corn

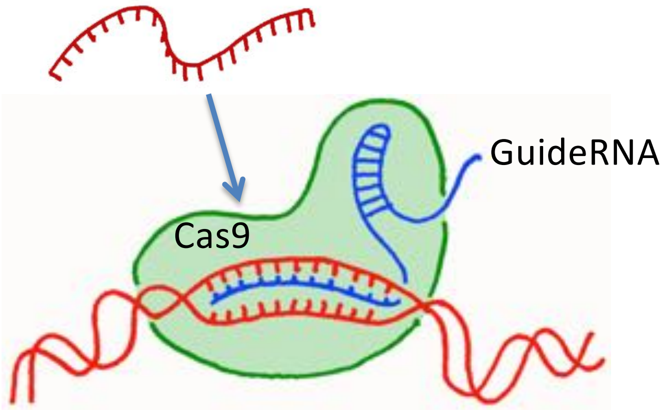
Gene editing of iPSCs

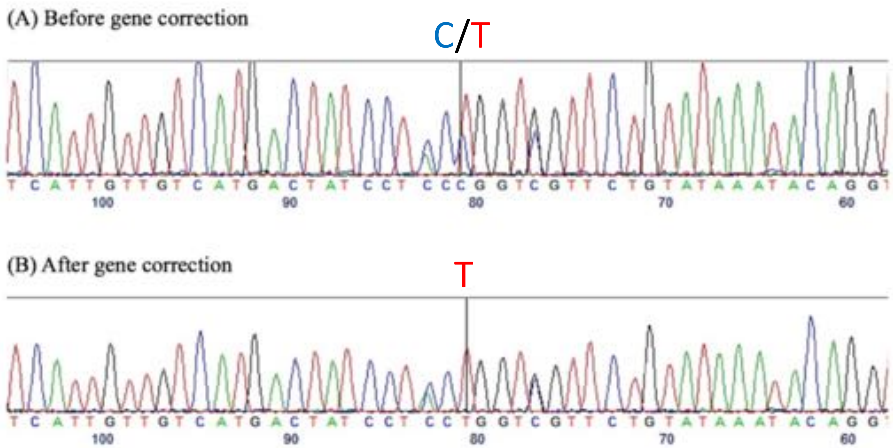
FTD3 iPSC

Gene-corrected iPSC



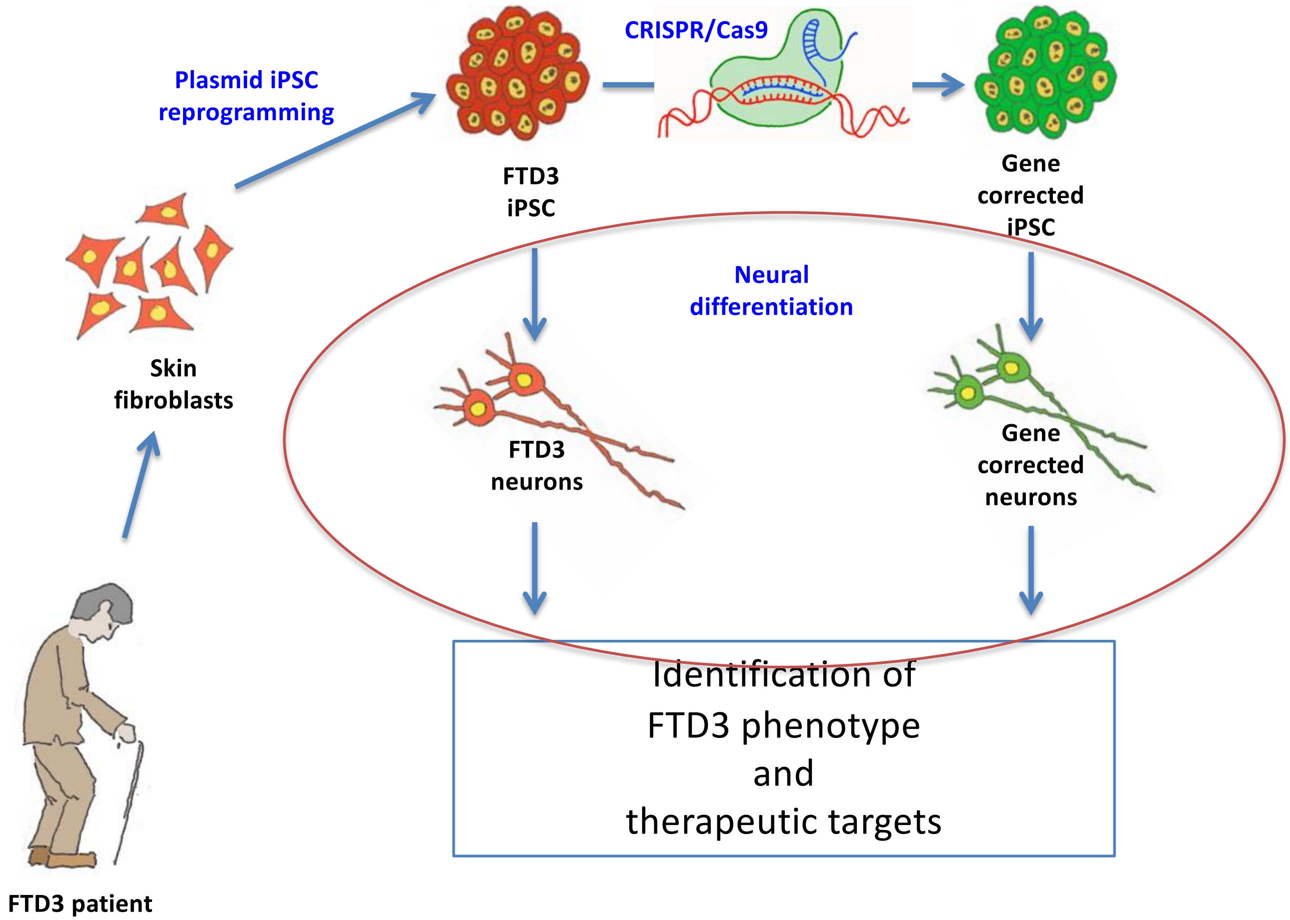
ssODN with correct template sequence





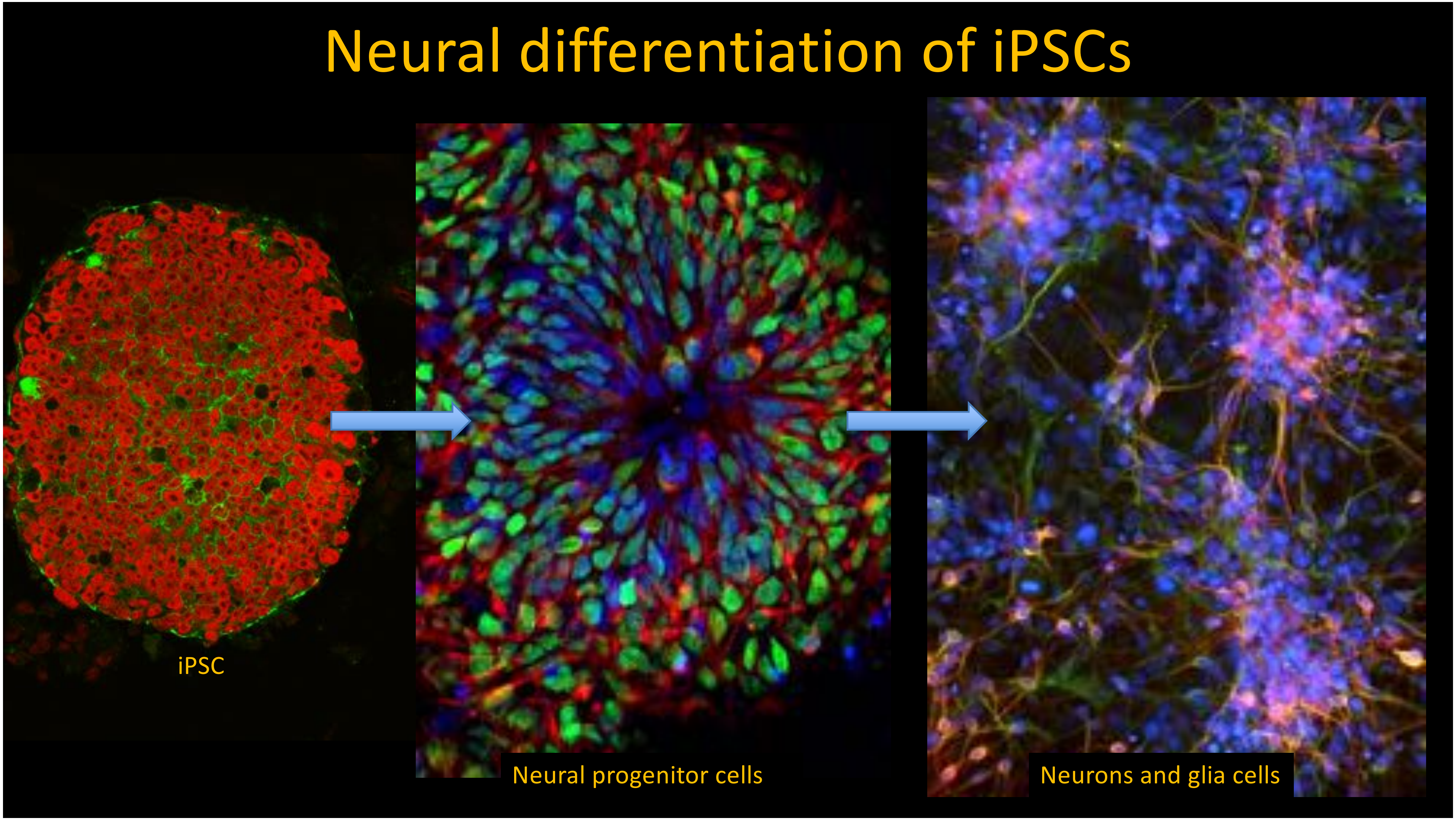
Anna Poon, Benjamin Schmid and Kristine Freude

iPSC-based modeling of FTD3

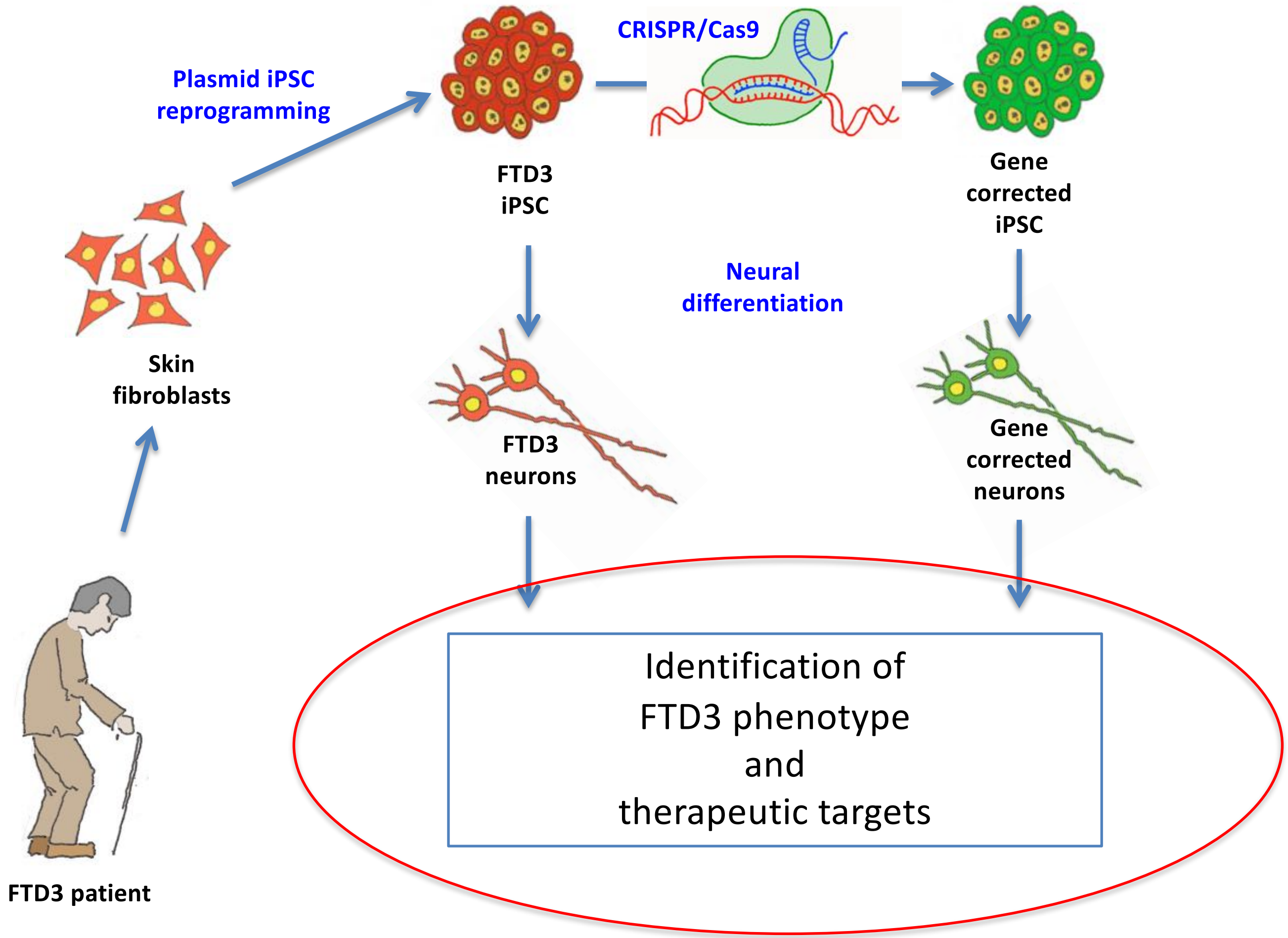



Blood

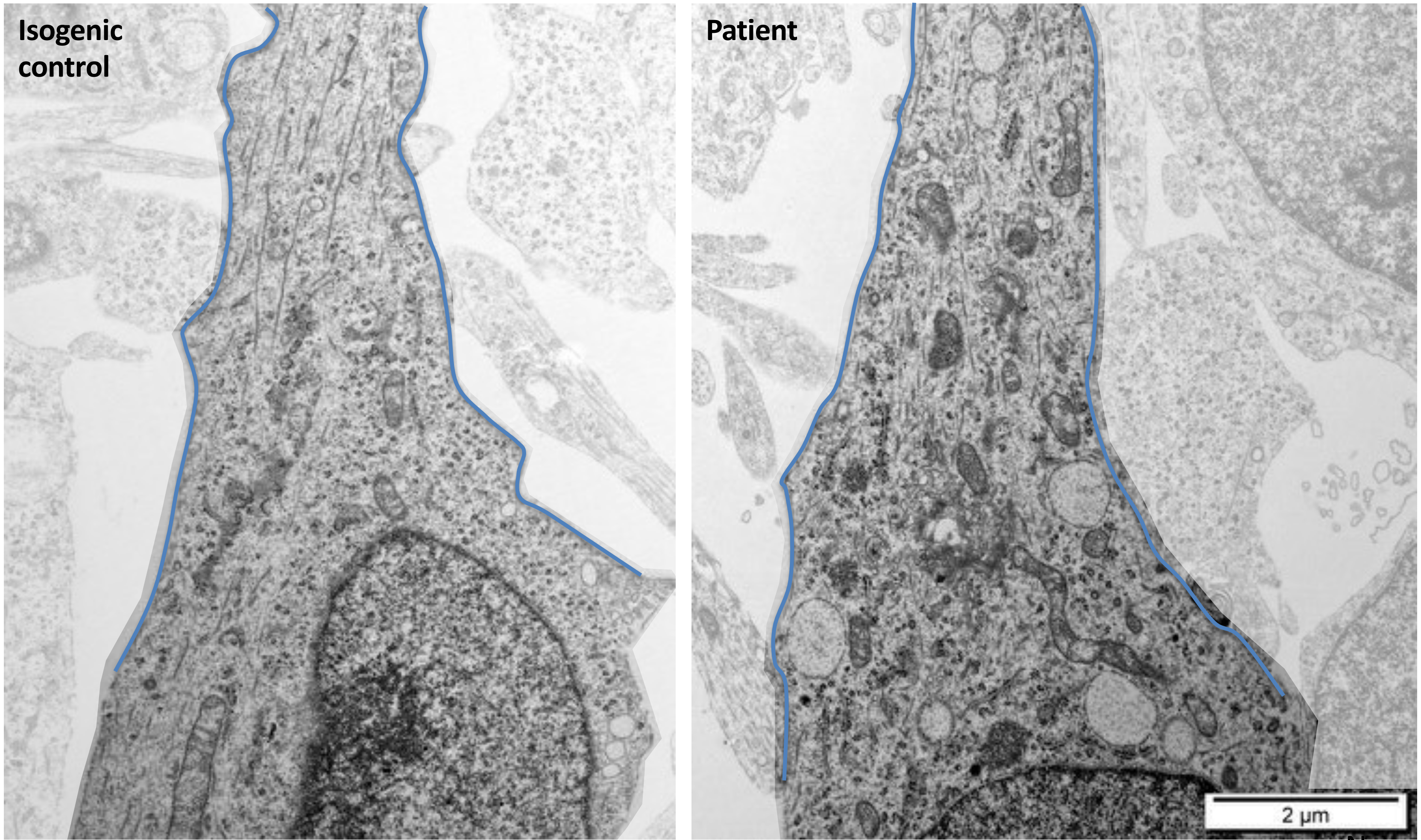
Neural differentiation of iPSCs


Neural differentiation of iPSCs
iPSC
Neural progenitor cells
Neurons and glia cells

iPSC-based modeling of FTD3

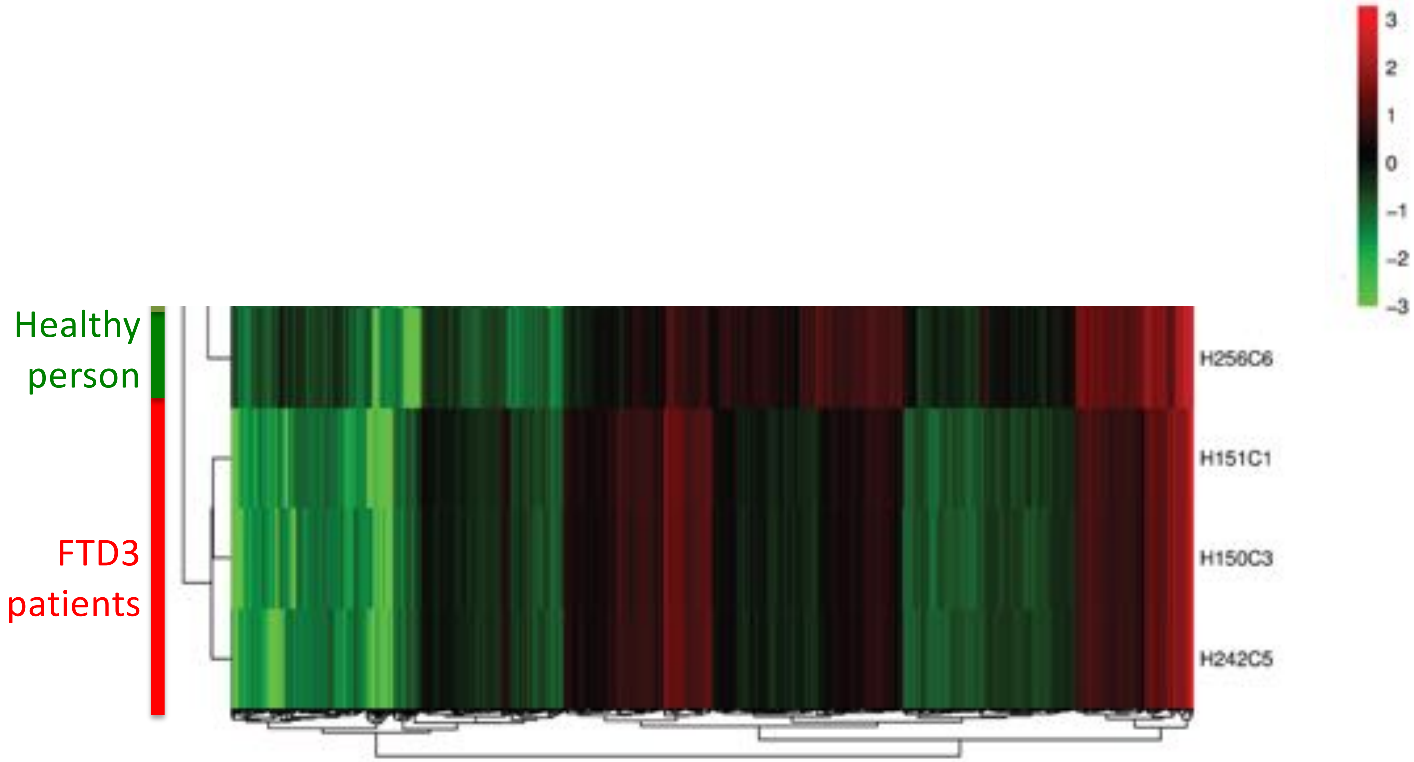


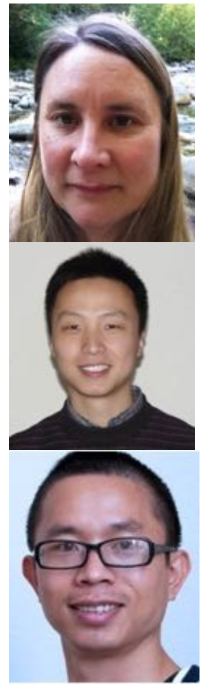
Electron microscopy of iPSC-derived neurons



Nanett K Nicolaisen

RNAseq analyses of iPSC-derived neurons





Yu Zhang, Alun and Kristine Freude

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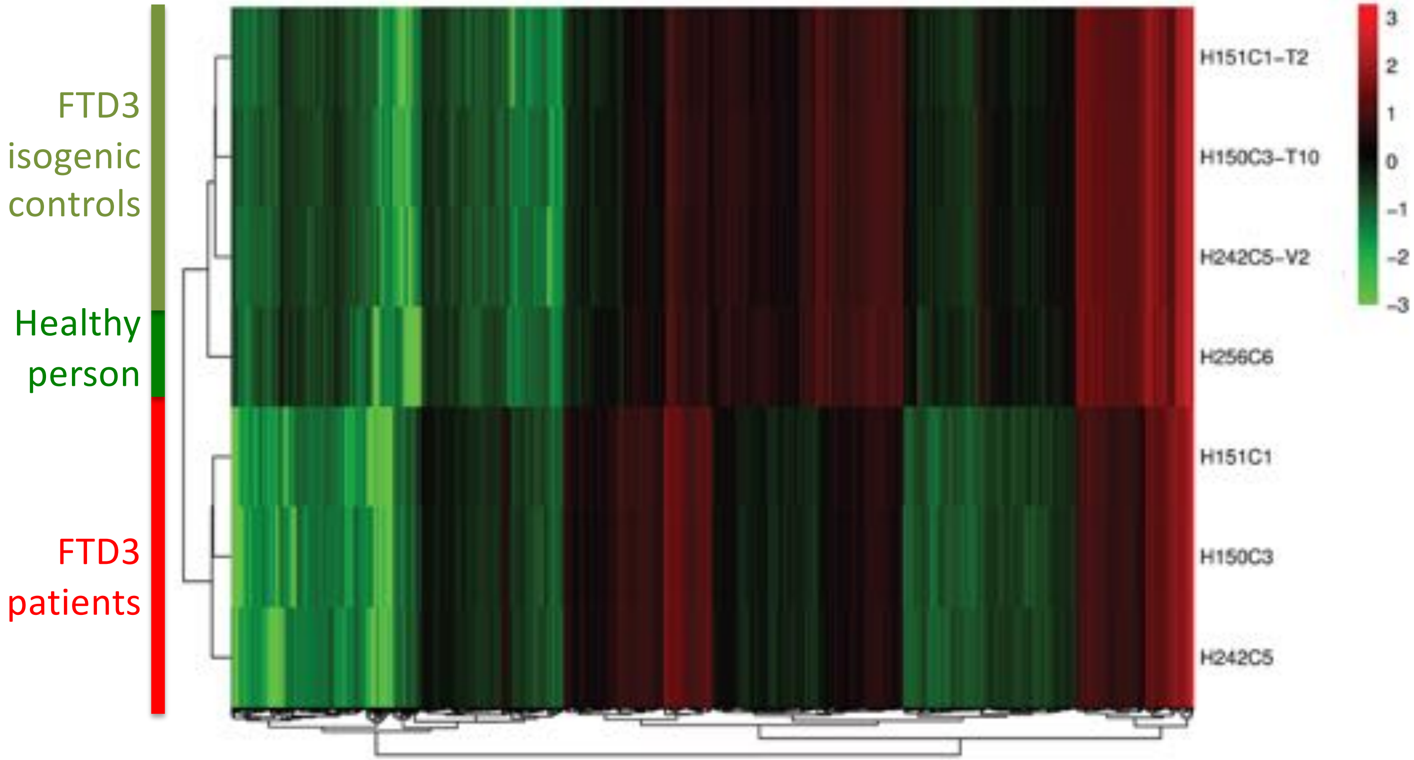
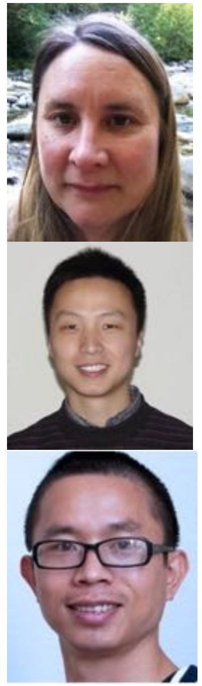


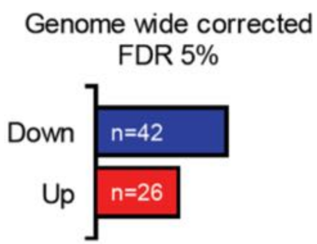


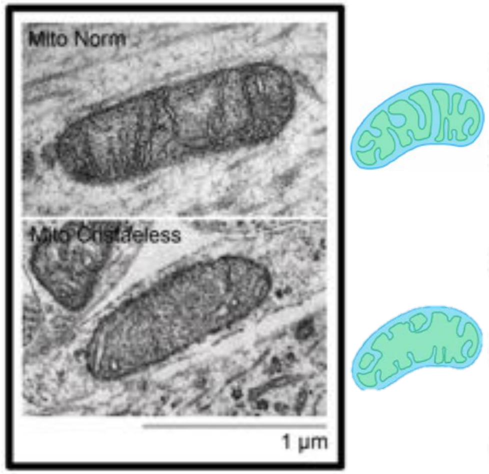
Major FTD3 misregulated genes: Endosomes, mitochondria and iron homeostasis

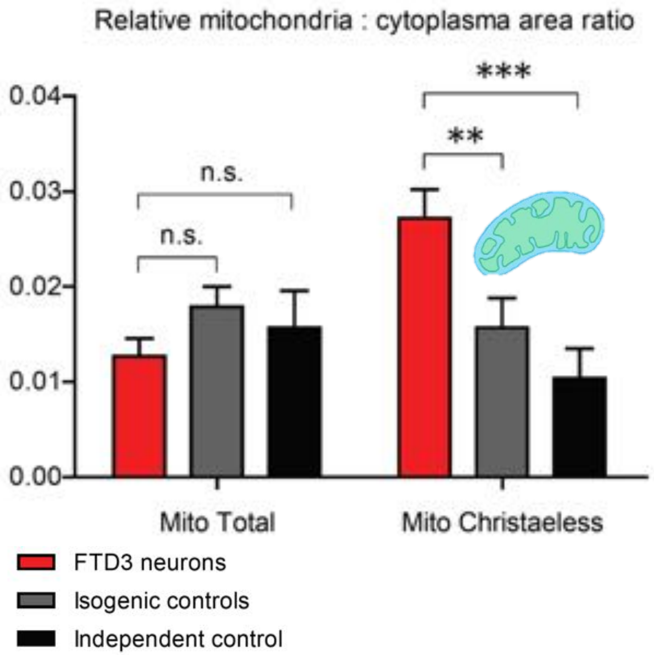
Yu Zhang, Alun and Kristine Freude

Mitochondrial defects

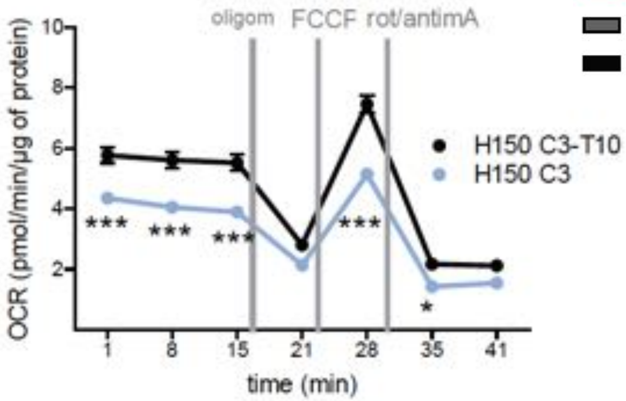
RNAseq
Mitochondrial genes
FTD3 vs isogenic control





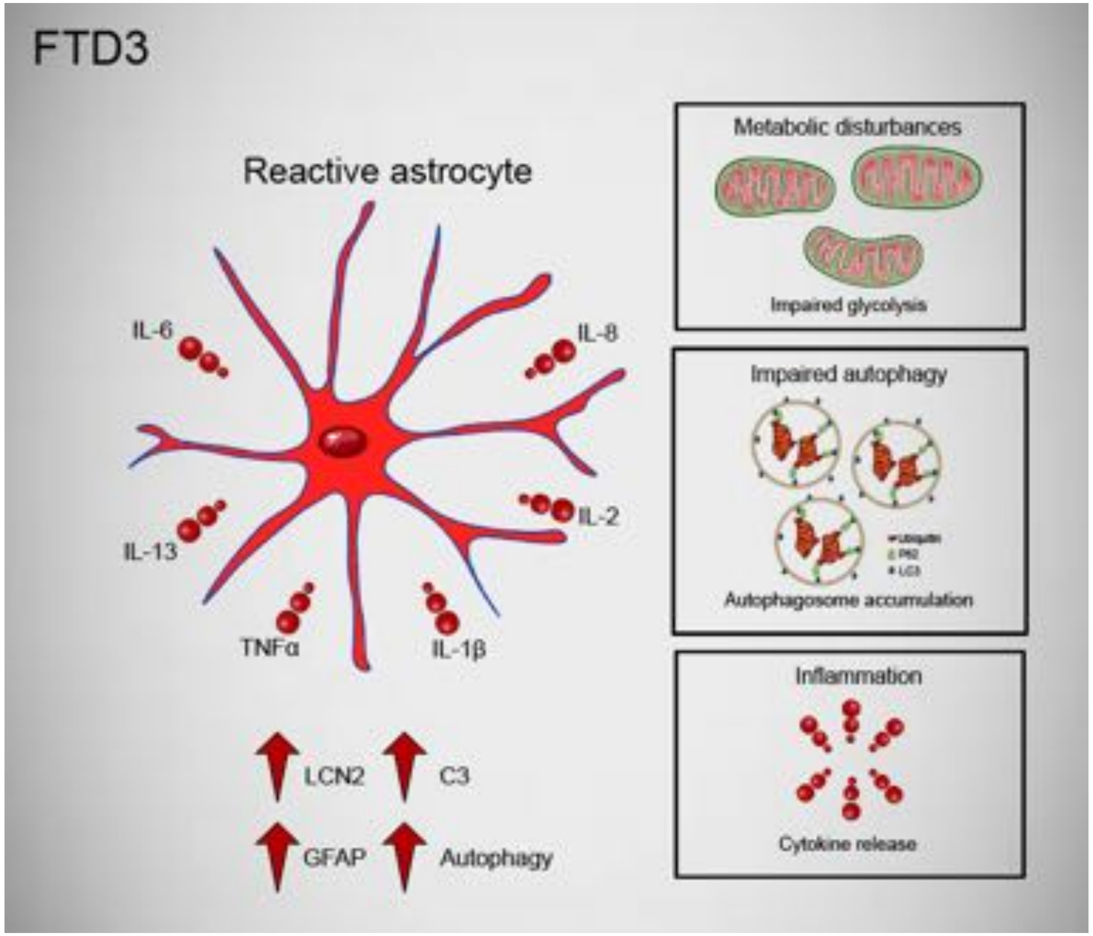


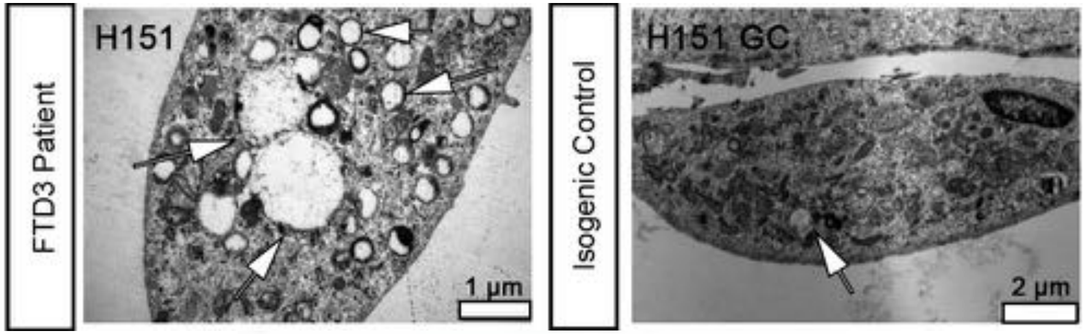
Seahorse Bioscience

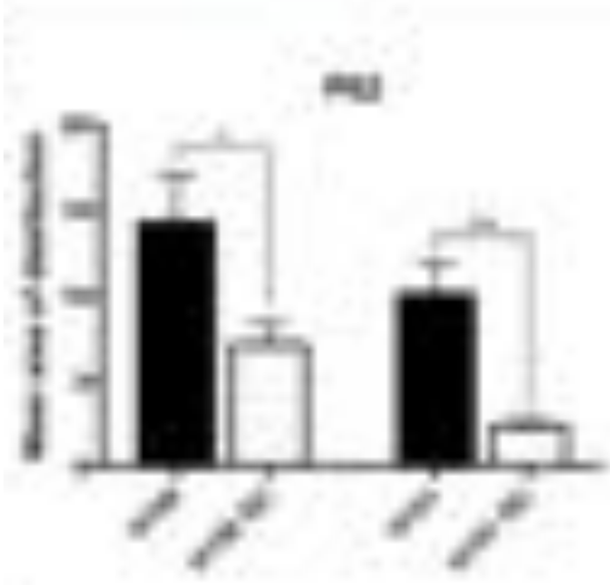


Nanett K Nicolaisen and Blanca Garcia

Astrocyte reactivity and impaired autophagy







Katarina Stoklund Dittlau

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“Stem cell tourism” – false hope for real money

“*one of the most dangerous elements of our culture: that we have forgotten how to die.*” (Jill Lepore)

Important issues in stem cell therapy

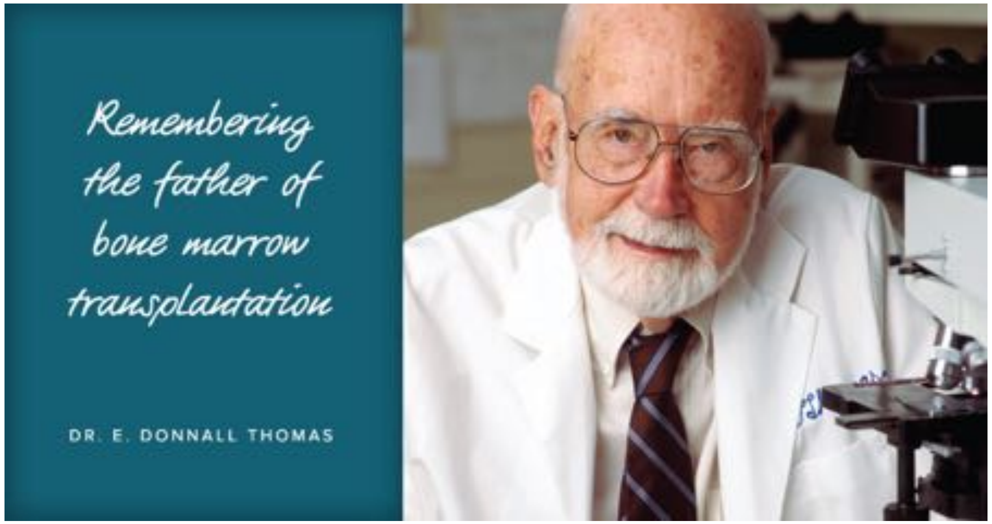
- Cell type
  - Multipotent (e.g. MSCs, NSCs)
  - Pluripotent (e.g. ESCs, iPSCs)
  - Autologous or allogenic cells
    - The “super donor” – homozygous for human leucocyte antigen (HLA) – will match heterozygous patients with just one match
      - ”Super donor” iPSC banks established in Japan, China and US
- Large scale manufacturing of cells
- Principle for administration
  - Scaffolds
  - Homing
  - Engraftment vs. stimulatory effects of secretome
- Legal regulation
  - Special “fast track” approval processes in US, EU and Japan

A brief overview of clinical trials in stem cell therapy categorized according to stem cell types

(Mid July) ClinicalTrials.gov; U.S. National Library of Medicine

Hematopoietic stem cells – the forerunner

Indication	#studies	Results	Reference
Bone marrow transplantation	3,309	Reestablishment of hematopoietic function First allogenic transplantation 1968 Dr. E Donnall Thomas Nobel Prize 1990	(Gatti et al., 1968)
Cancer	324	Chimeric antigen receptor (CAR) CAR T Cell therapy Several FDA approved treatments	(Androulla and Papadopoulou. 2018)



ClinicalTrials.gov; U.S. National Library of Medicine

Epidermal stem cells
– a superficial speciality

Indication	#studies	Results	Reference
Burns, chronic wounds, autoimmunity and urethral reconstruction	20	Restoration of normal skin function	(Jackson et al., 2017)

ClinicalTrials.gov; U.S. National Library of Medicine

Mesenchymal stem cells – a wide field

Indication	#studies	Results	Reference
Ischemic heart disease	244	Improved cardiac function, effect potentially due to secreted chemokines	(Jeong et al., 2018)
Bone and cartilage defects	194	Accelerated healing, mechanisms still controversial	(Oryan et al., 2017)
Degenerative disc disease	25	Significant pain relief	(Orozco et al., 2011)
Immunomodulation	58	Clear immunosuppressive properties, mechanisms still controversial	(Wang et al., 2018)
Diabetes	56	Positive effects, mechanisms still controversial	(Wehbe and Hawat, 2017)
Spinal cord injury	46	Functional repair, mechanisms still controversial	(Qu and Zhang, 2017)
Parkinson's disease	9	Lack of convincing results	(Palmer et al., 2016)
Retinopathy incl. macular degeneration	6	Some visual improvement but also side effects	(Öner, 2018)

ClinicalTrials.gov; U.S. National Library of Medicine

Neural stem cells – a niche

Indication	#studies	Results	Reference
Parkinson's disease	7	Some clinical improvement but inconsistent	(Palmer et al., 2016)
Spinal cord injury	4	Functional repair but inconsistent	(Wright et al., 2018)
Retinopathy incl. macular degeneration	1	Lack of updated results	(Tsukamoto et al., 2013)

ClinicalTrials.gov; U.S. National Library of Medicine

Embryonic stem cell – a potent challenge

Indication	#studies	Results	Reference
Retinopathy incl. macular degeneration	19	ESC-derived retinal pigmented epithelium visual improvement but not consistent	(Schwartz et al., 2015; Öner, 2018)
Diabetes	3	Lack of updated results	(Cheng et al., 2016)
Spinal cord injury	1	Significant clinical improvements	(Schroff, 2016)
Parkinson’s disease	1	Lack of updated results	(Palmer et al., 2016)
Ischemic heart disease	1	Improved cardiac function	(Menasché et al., 2015)

ClinicalTrials.gov; U.S. National Library of Medicine

Induced pluripotent stem cells – a new era

Indication	#studies	Results	Reference
Retinopathy incl. macular degeneration	2	No results posted, Japanese study discontinued due to safety reasons	(Öner, 2018)
Parkinson's disease	1	Lack of updated results	(Cheng et al., 2016)
Spinal cord injury	1	Significant clinical improvements	(Schroff, 2016)
Parkinson's disease	0	Projected	(Palmer et al., 2016)

ClinicalTrials.gov; U.S. National Library of Medicine

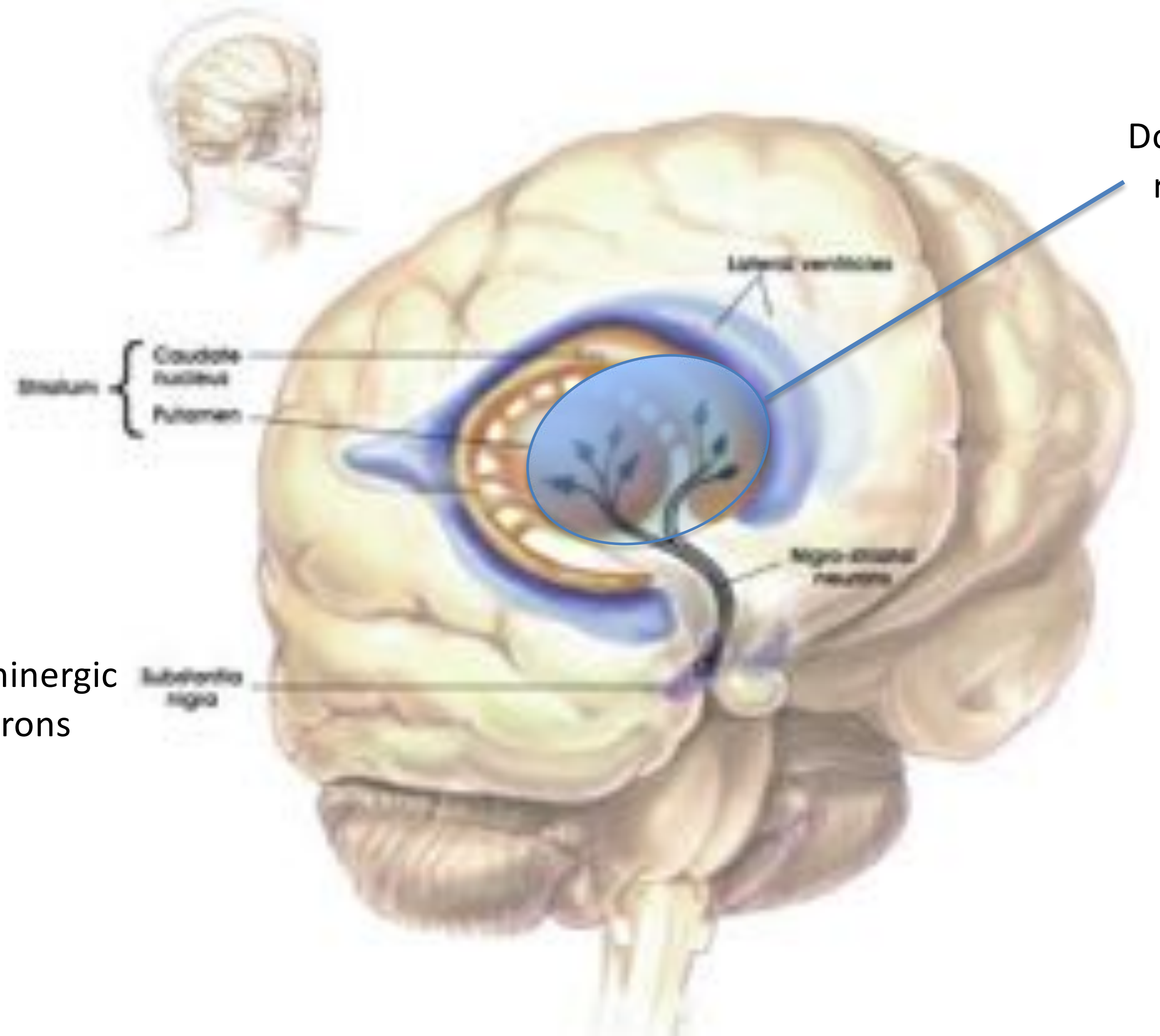
Parkinson's disease and iPSC

Jun Takahashi (left) and colleagues explained their plans for a trial in Parkinson's disease patients at a press conference at Kyoto University in Japan today. *THE YOMIURI SHIMBUN/AP IMAGES*

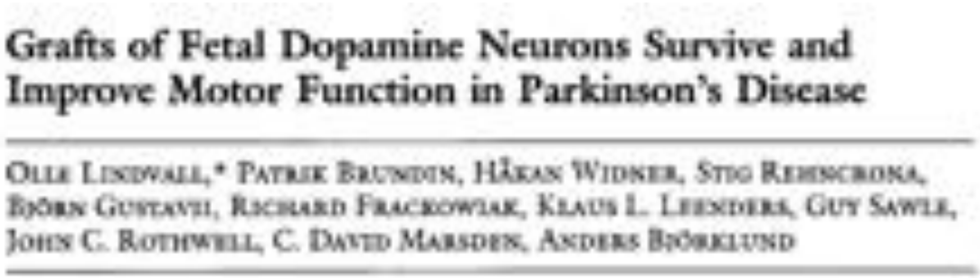
First-of-its-kind clinical trial will use reprogrammed adult stem cells to treat Parkinson's

By **Dennis Normile** | Jul. 30, 2018 , 3:35 PM

Parkinson's disease



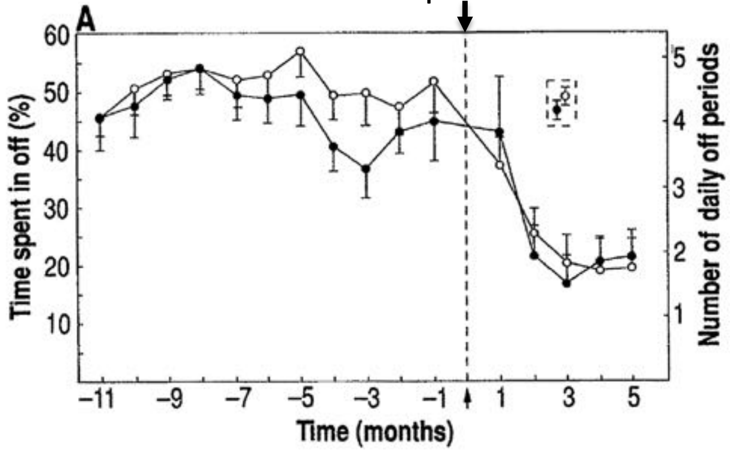
Parkinson's disease

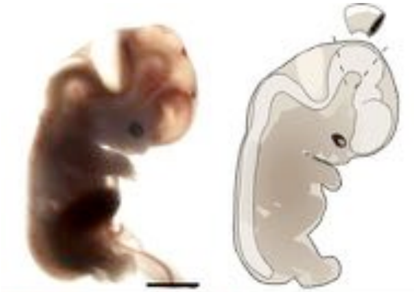

Grafts of Fetal Dopamine Neurons Survive and Improve Motor Function in Parkinson's Disease

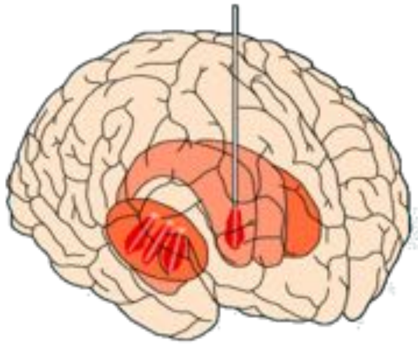
Olle Lindvall,* Patrik Brundin, Håkan Widner, Stig Rehncrona, Björn Gustavii, Richard Frackowiak, Klaus L. Leenders, Guy Sawle, John C. Rothwell, C. David Marsden, Anders Björklund

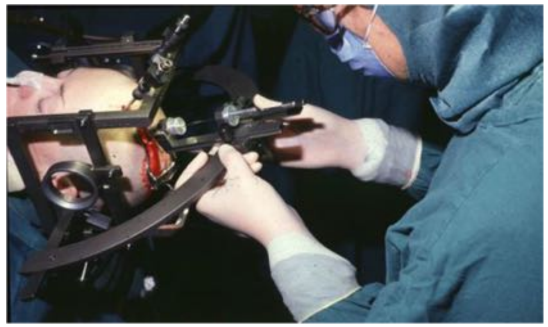
Science 1990

Transplantation









6-8 week fetal mesencephalon

Suspension of dopaminergic progenitor cells

Uni- or bilateral stereotaxic injections to immunosuppressed patients

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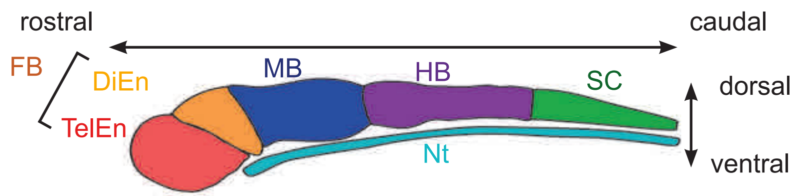
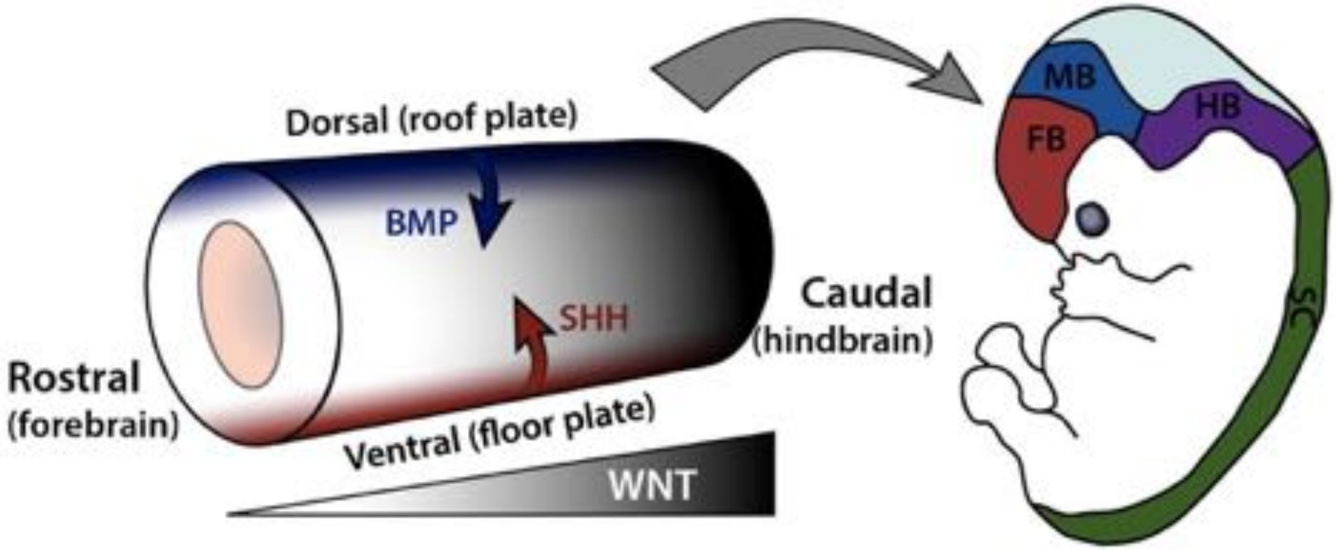
How to produce dopaminergic progenitors without aborted fetuses?





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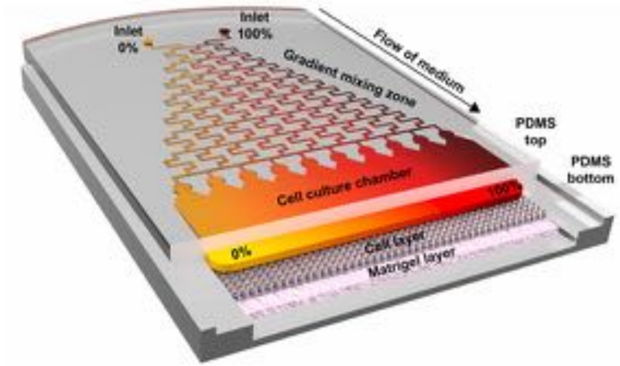
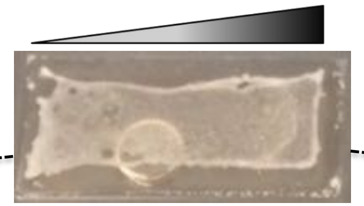
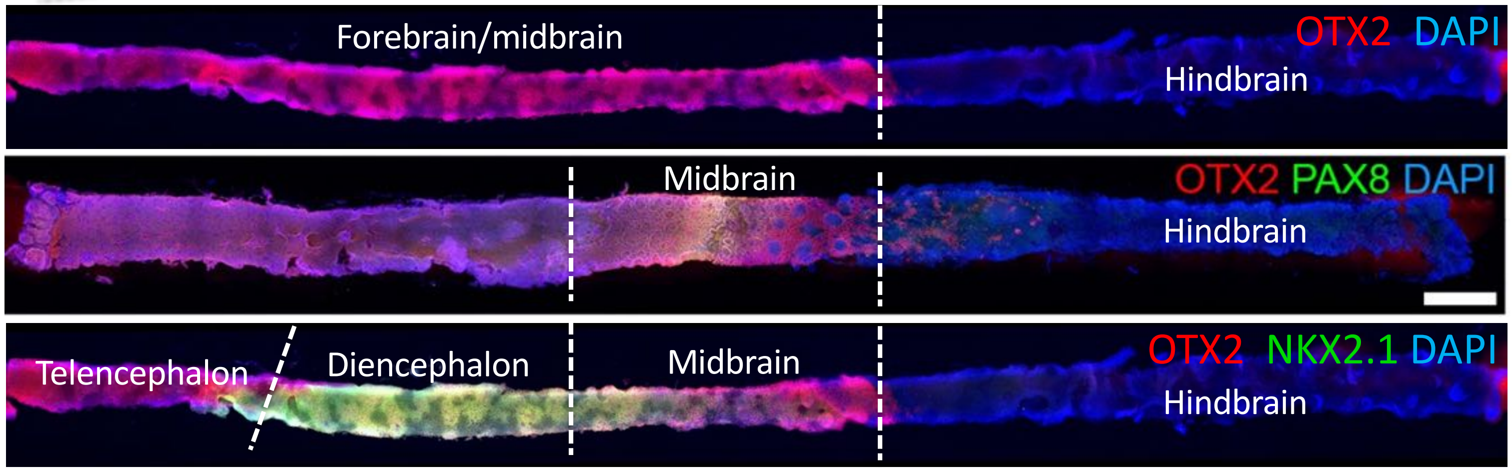
How to produce dopaminergic progenitors without aborted fetuses?



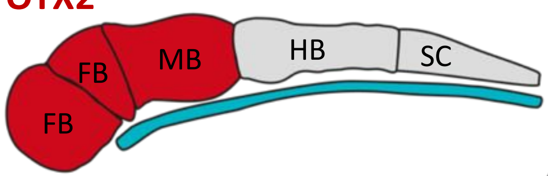
WNT activation (GSK3i)



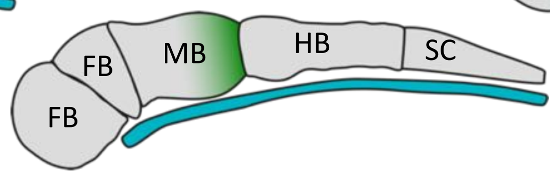
Day 14 tissue
(estimated equivalent to 4 wks pc)

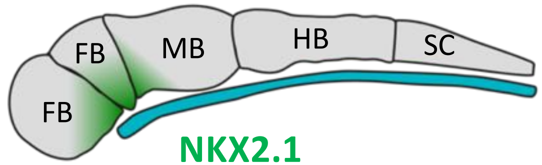


OTX2



PAX8





NKX2.1

Kirkeby lab, unpublished data

Agnete Kirkeby

Contents

- Stem cell definitions
- Cell differentiation potency
- The stem cell hierarchy: Pluripotent, multipotent and unipotent stem cells
- Induced pluripotent stem cells for disease modeling
- Stem cell therapy
- Conclusions

Conclusions

- Stem cells are defined by self-renewal and differentiation potency
- Stem cells are best categorized according to their differentiation potency (pluripotent, multipotent, unipotent), not by their origin in embryos or adults
- Induced pluripotent stem cells (iPSCs) allow for patient-specific disease modeling
- Numerous clinical trials are registered on multipotent stem cell therapy
- First clinical trials are registered on pluripotent (ESC, iPSC) stem cell therapy

BrainStem collaborating partners

- University of Copenhagen
- Danish Dementia Research Center
- University of Southern Denmark
- Aarhus University
- Bioneer
- Lundbeck
- Innovative Concepts in Drug Development (F)
- Lund University (S)
- Biotalentum (H)

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- Copenhagen Consortium for Designer Organisms

BrainStem

Stem cell center of excellence in neurology