Centre for Regenerative Medicine

Modelling Parkinson's Disease using dopaminergic neurons derived from human stem cells

Sophie Glendinning

Supervisor: Tilo Kunath

27/06/2020

The Cure Parkinson's Trust

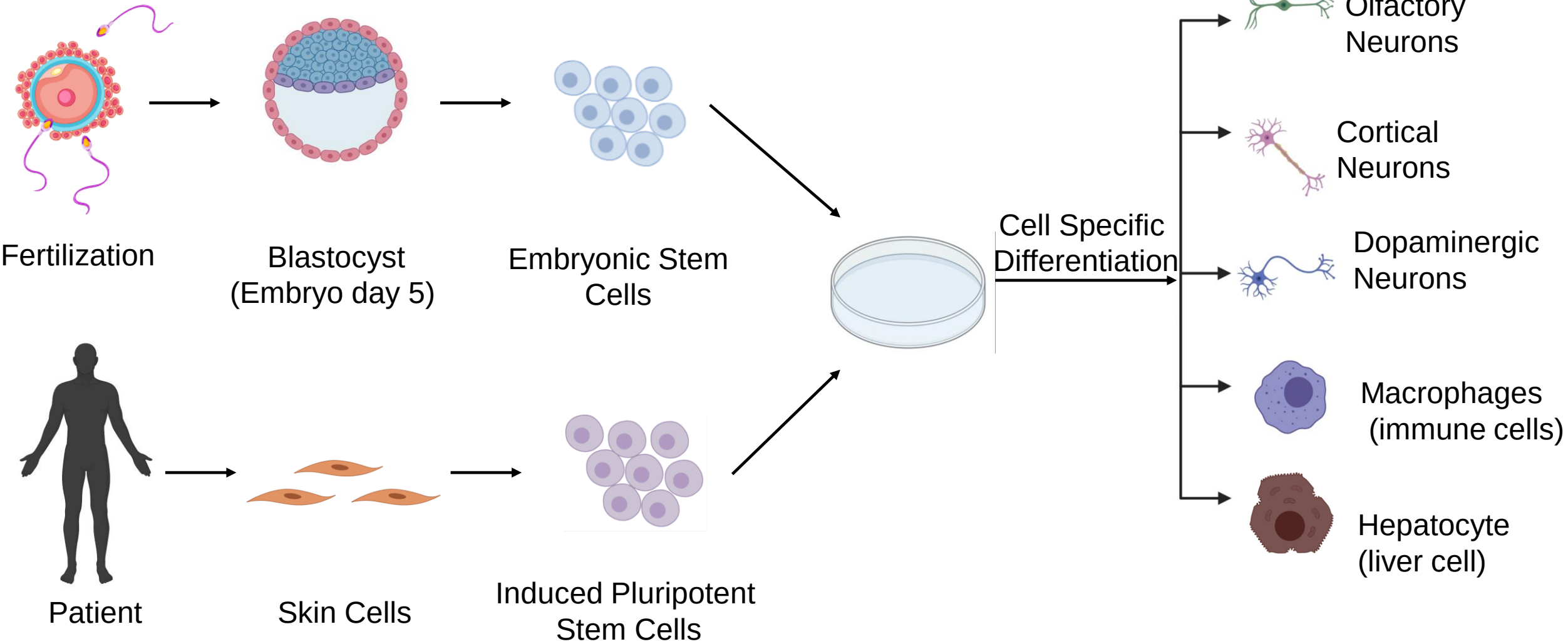
Anne Rowling Regenerative Neurology Clinic

MRC Medical Research Council

THE UNIVERSITY of EDINBURGH

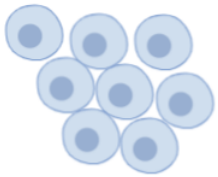
Human embryonic stem cells and induced pluripotent stem cells

- Human embryonic stem cells are derived from the blastocyst during early embryonic development
- Skin cells taken from patients can be converted into induced pluripotent stem cells
- Both embryonic stem cells and induced pluripotent stem cells have the potential to be differentiated into any cell type

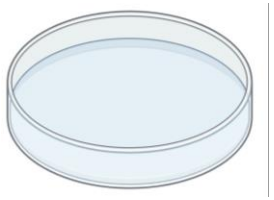


Human embryonic stem cells and induced pluripotent stem cells

- A hallmark of Parkinson's is the progressive neurodegeneration of dopaminergic neurons within the Substantia Nigra (SNpc)
- Human embryonic stem cells and induced pluripotent stem cells can be differentiated into dopaminergic neurons
- Differentiated cells can be used for disease-modelling, drug screening and transplantation



Embryonic Stem Cells



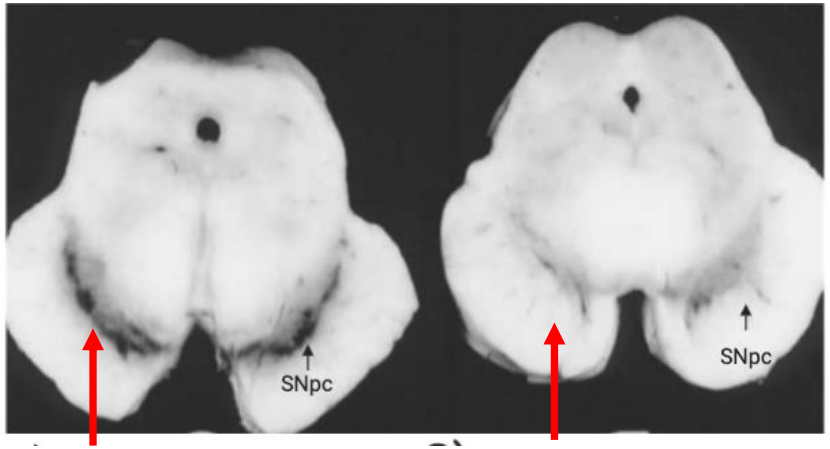
Induced Pluripotent Stem Cells

Cell Specific Differentiation

Dopaminergic Neurons

Non-Parkinson's Patient

Parkinson's Patient

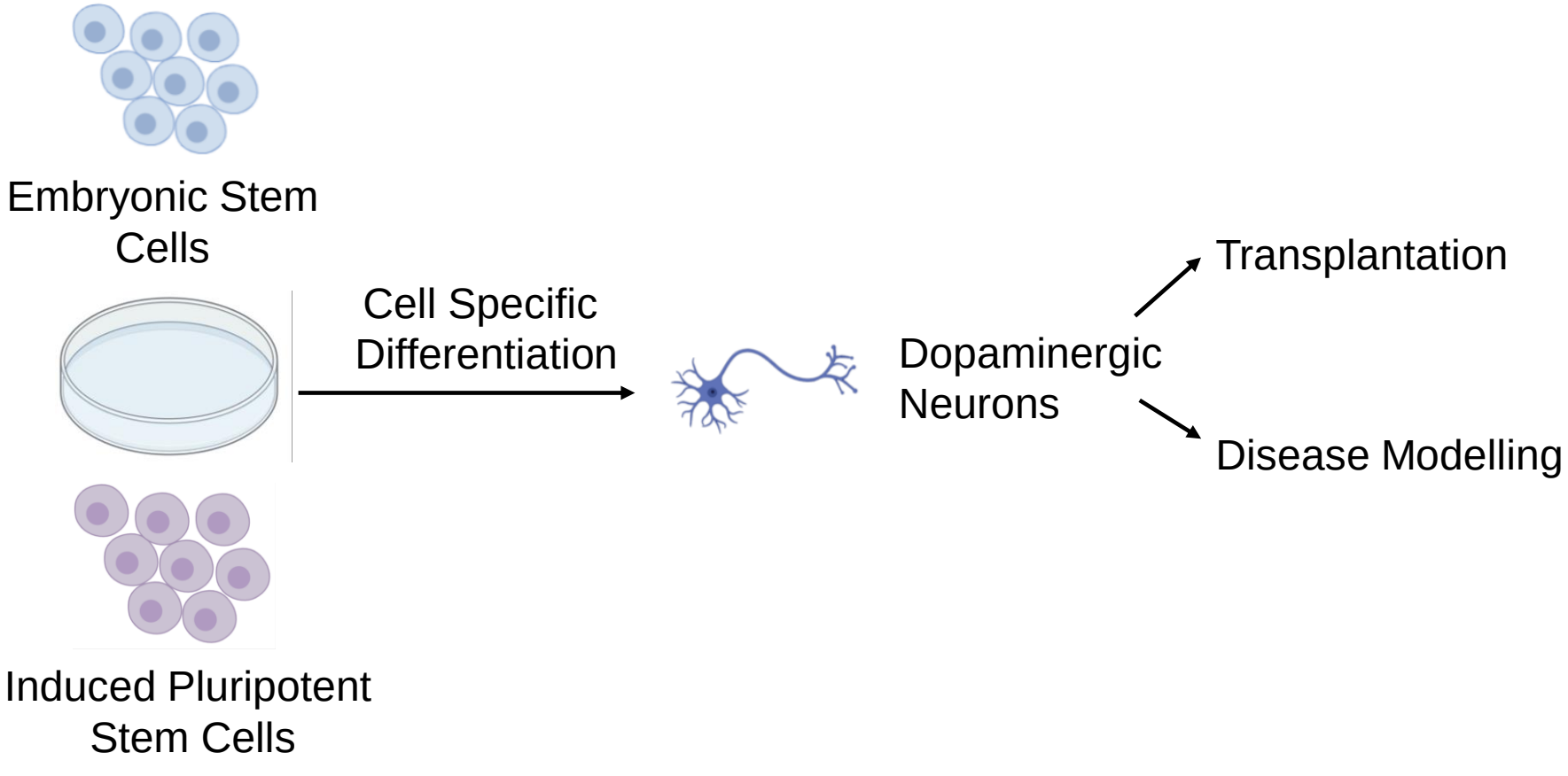


Dopaminergic Neurons

Loss of Dopaminergic Neurons

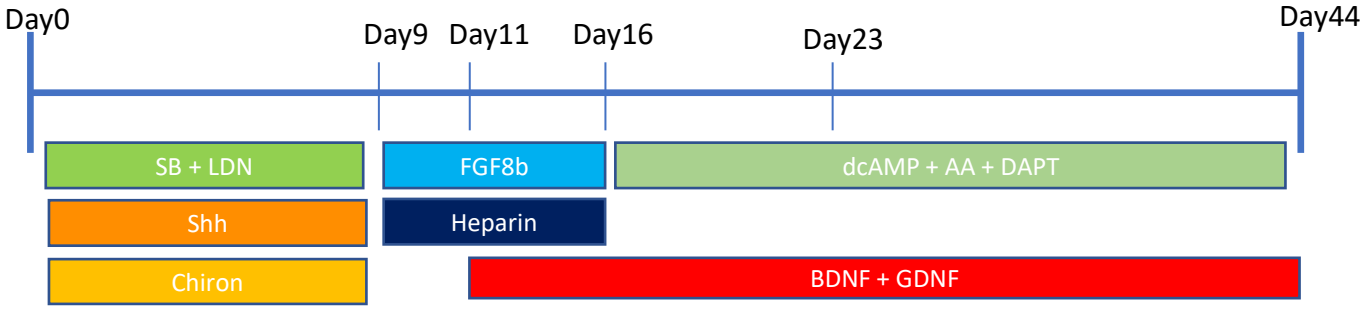
Human embryonic stem cells and induced pluripotent stem cells

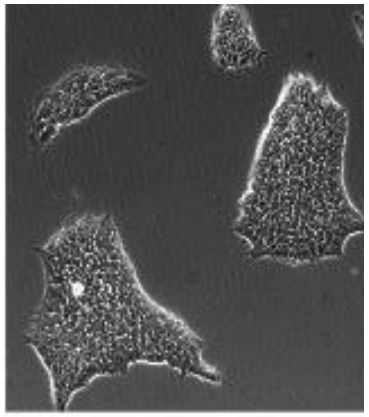
- A hallmark of Parkinson's is the progressive neurodegeneration of dopaminergic neurons within the Substantia Nigra (SNpc)
- Human embryonic stem cells and induced pluripotent stem cells can be differentiated into dopaminergic neurons
- Differentiated cells can be used for disease-modelling, drug screening and transplantation



Differentiation of dopaminergic neurons

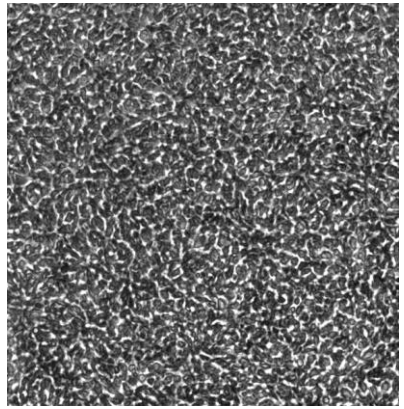
- Within the Kunath lab a robust protocol has been developed to produce a pure culture of dopaminergic neurons
- Microscopes are one technique of monitoring neuron development





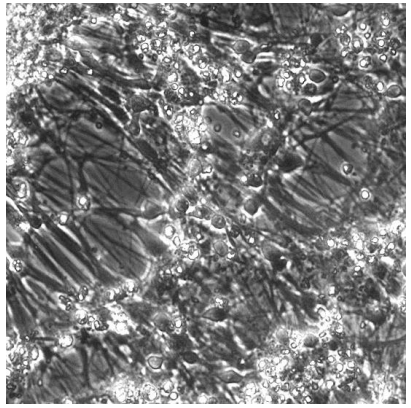
Embryonic Stem Cells

~16 days

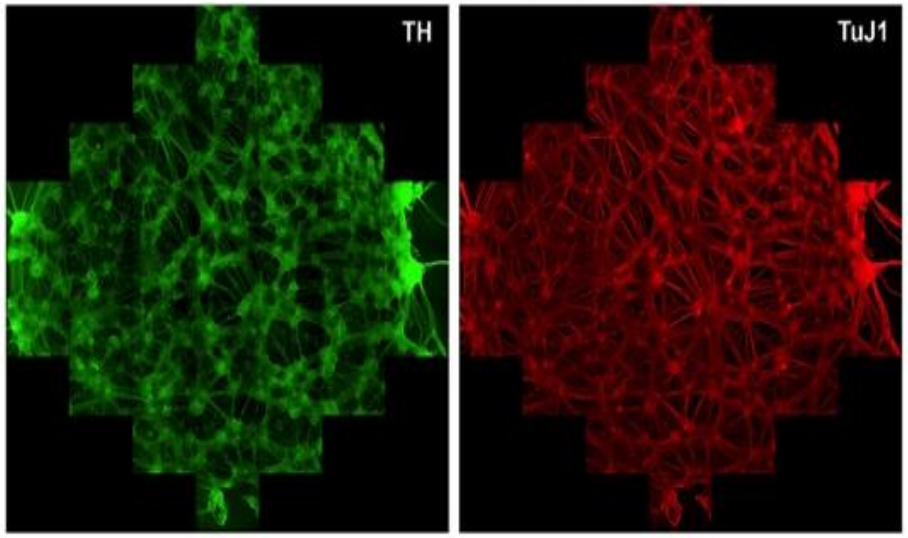


Dopaminergic precursors

>30 days



Dopaminergic neurons
(modeling)

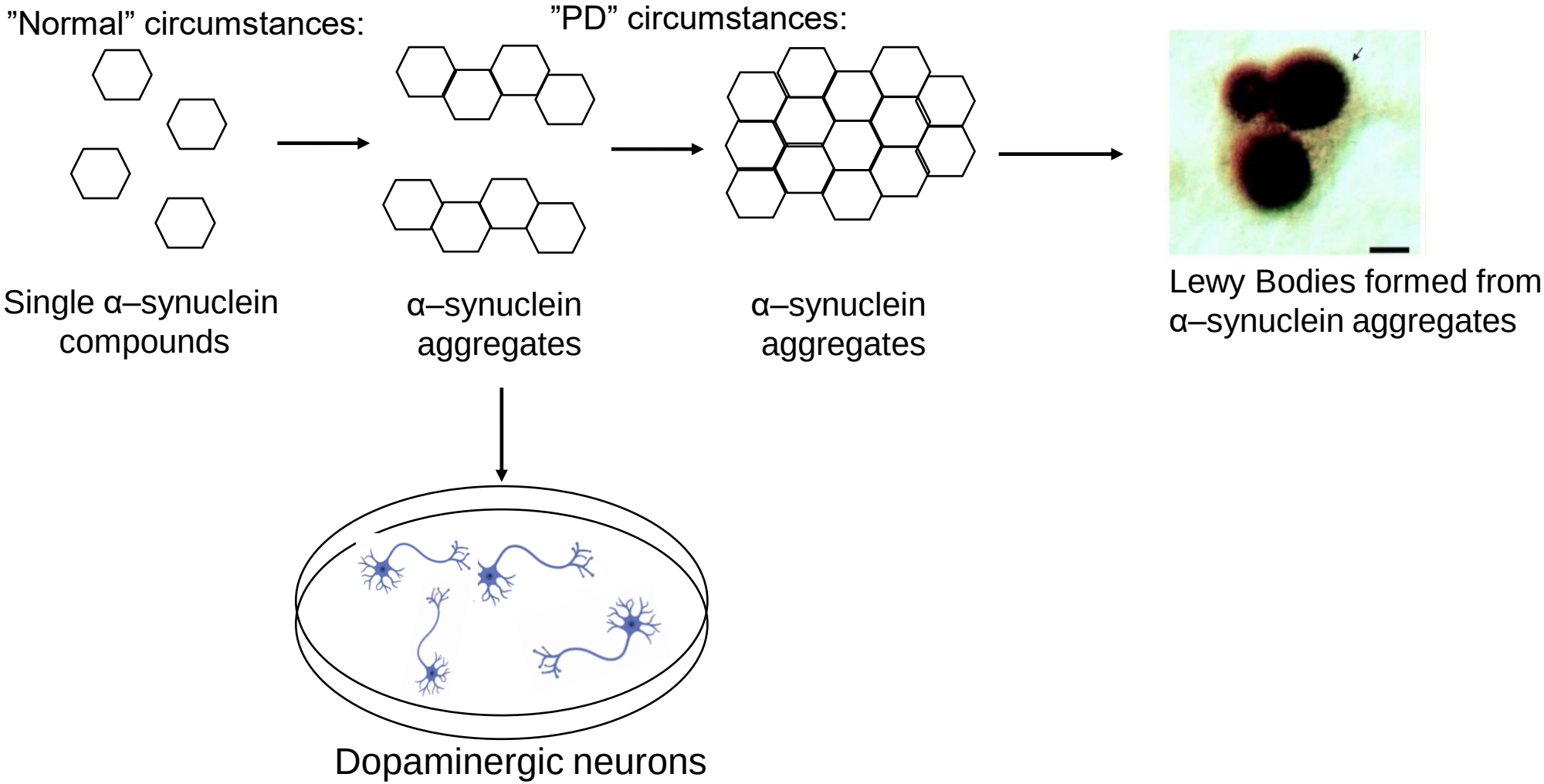


TH: dopaminergic neuron marker
TuJ1: general neuronal marker

Inducing Lewy Body-Like pathology within neurons

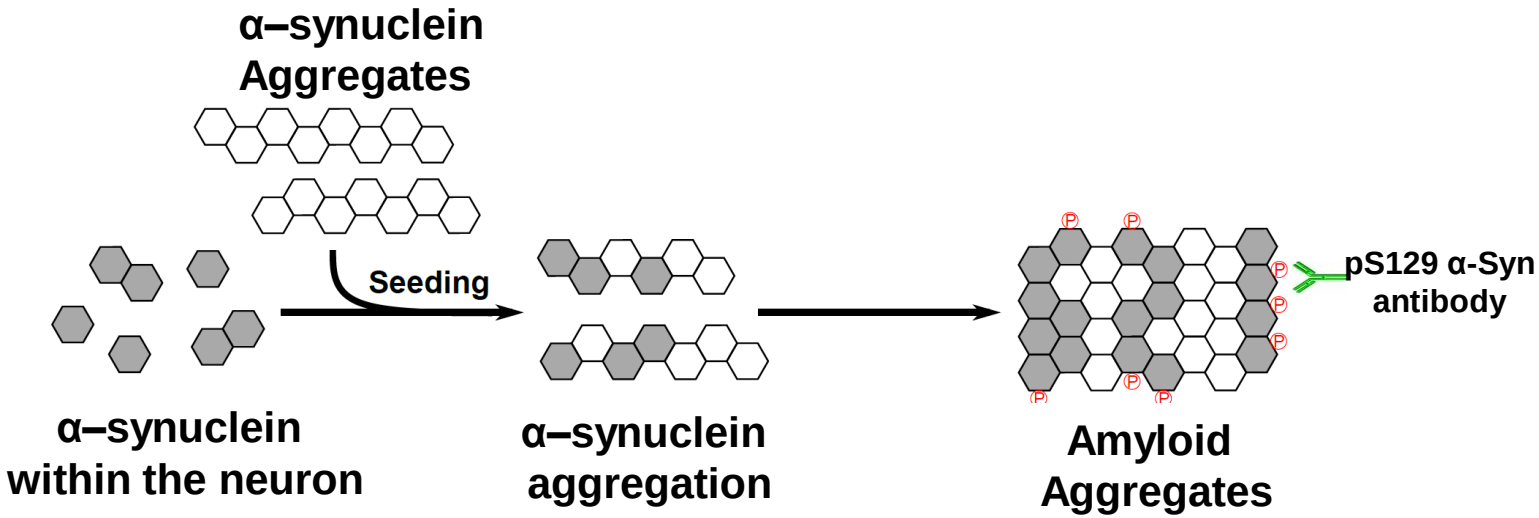
- A hallmark of Parkinson's is the formation of protein aggregates known as Lewy Bodies
- α–synuclein forms the main component of Lewy Bodies

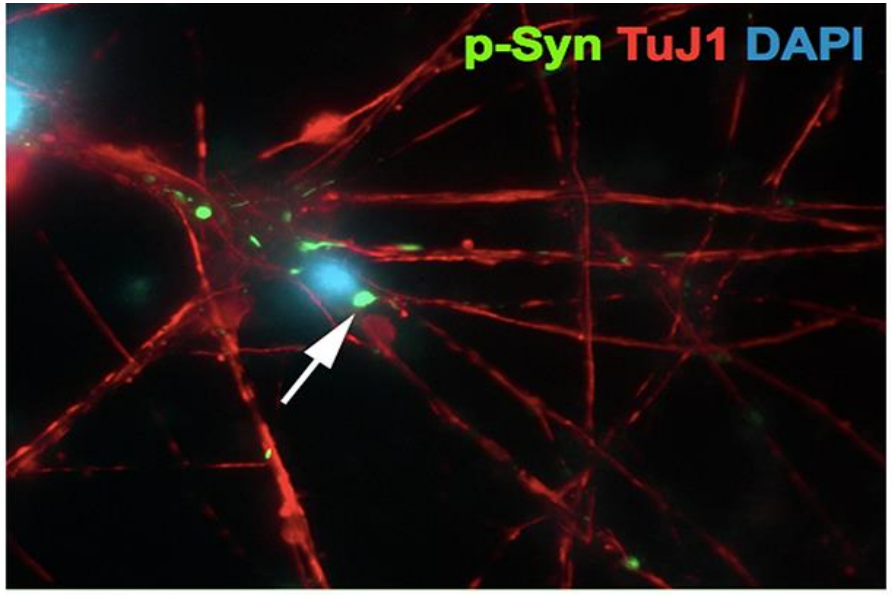
- α–synuclein aggregates produced within the lab can be used to induce pathology within a culture of neurons

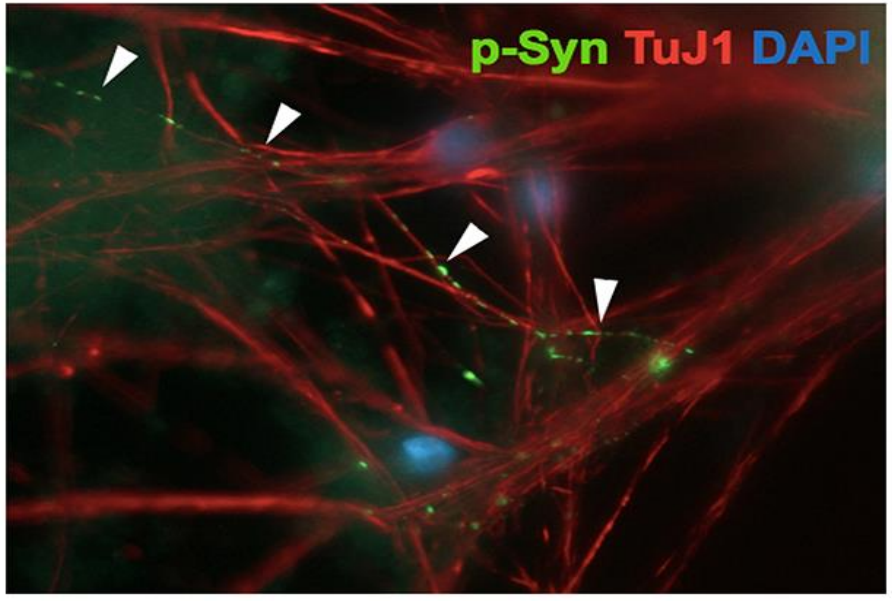


Inducing Lewy Body-Like pathology within neurons

- α–synuclein aggregates produced within the lab can be used to induce pathology within a culture of neurons
- The added α–synuclein aggregates with the α–synuclein already present within the neurons

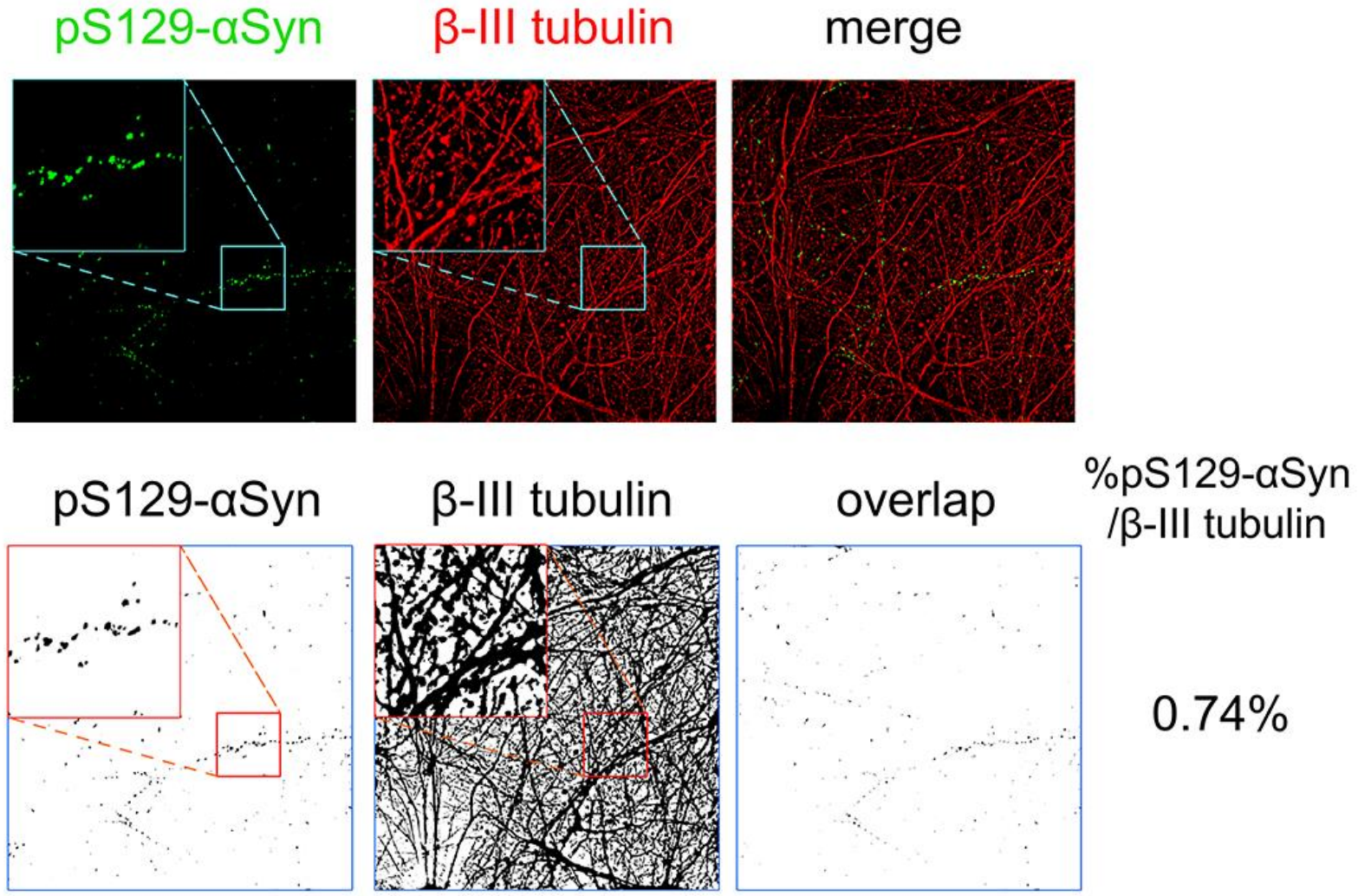






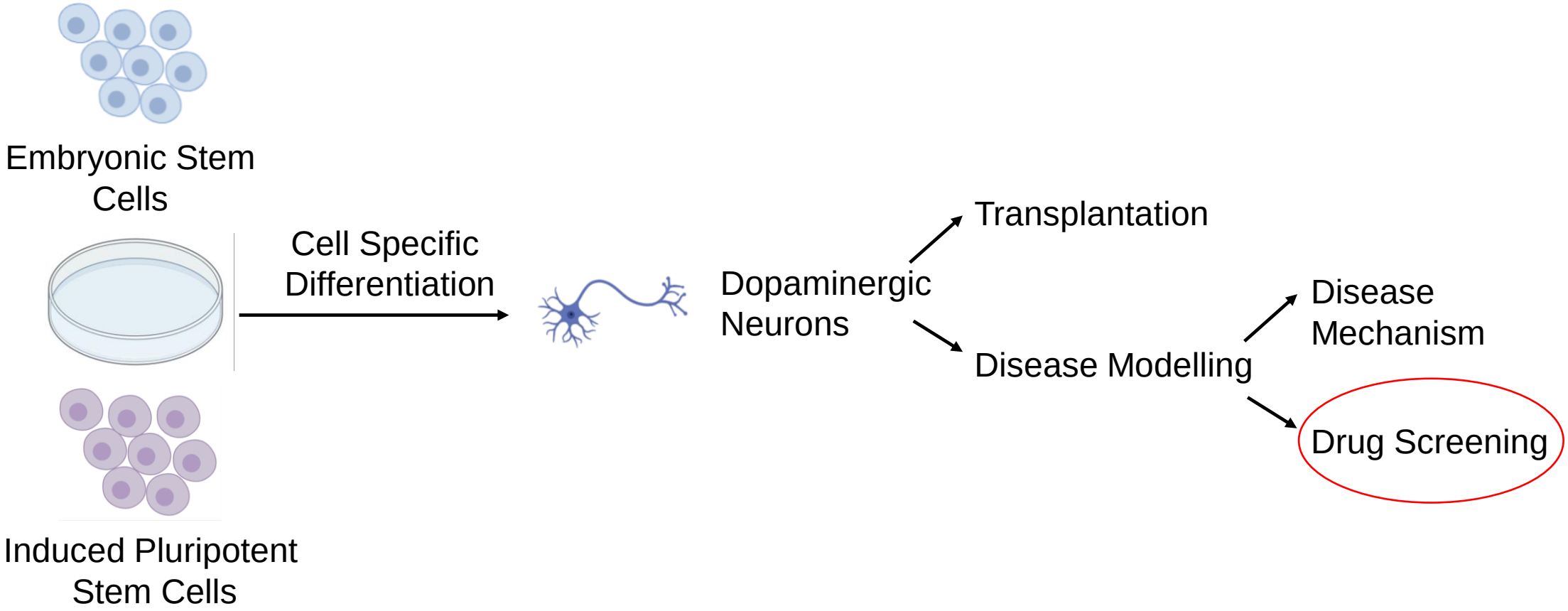
Inducing Lewy Body-Like pathology within neurons

- **By converting the images into binary, the relative amount of Lewy-like pathology can be <u>quantified</u>**



Human embryonic stem cells and induced pluripotent stem cells

- Using dopaminergic neurons as a disease model, drug screening can be performed



Drug Screening for compounds which inhibit Lewy like pathology

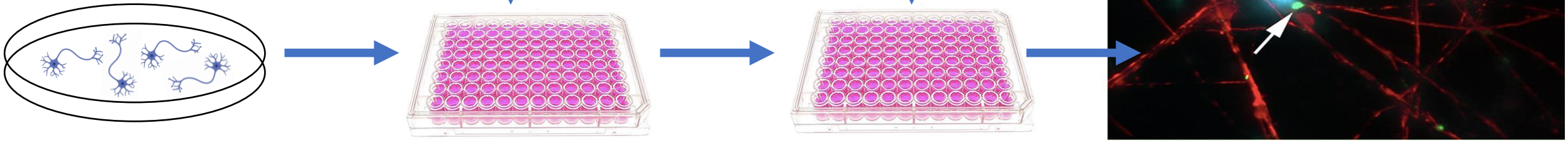
- Ongoing project to test the ability of 8 drugs to decrease the formation of α–synuclein aggregates
- Novel compounds will be tested along with repurposed drugs
- Quantifiable side by side comparison of drugs within a human system

+/–

α–synuclein Aggregates

+/–

Drugs



1. Culturing the neurons

2. Initiating α–synuclein pathology

3. Treatment of neurons with drugs

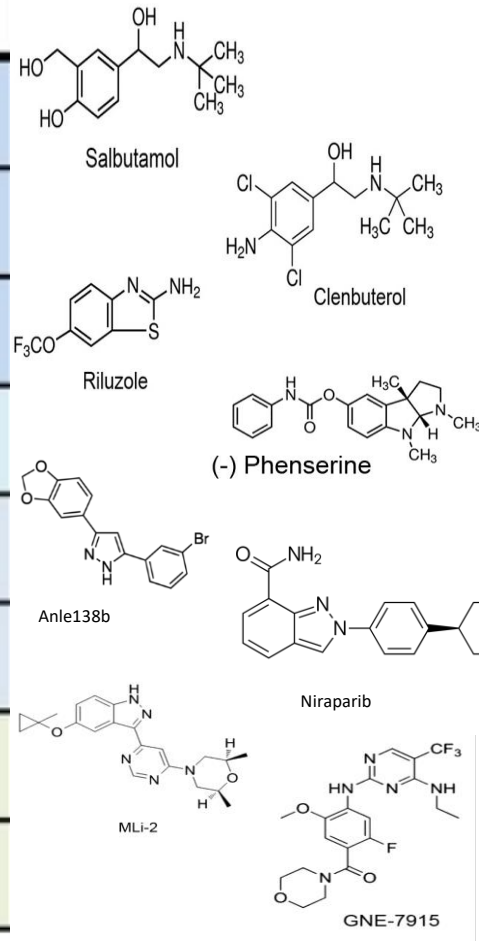
4. Quantify the effect of the drug

Drug Screening for compounds which inhibit Lewy like pathology

The Cure Parkinson's Trust

- Ongoing project to test the ability of 8 drugs to decrease the formation of α–synuclein aggregates
- Novel compounds will be tested along with repurposed drugs
- Quantifiable side by side comparison of drugs within a human system

Drug	Class	Reference	Reason
Salbutamol	Beta2 adrenoreceptor agonist	Mittal et al 2017	Reduces aSyn transcription
Clenbuterol	Beta2 adrenoreceptor agonist	Mittal et al 2017	Reduces aSyn transcription
Riluzole	Anti-glutametergic	Mittal et al 2017	Reduces aSyn transcription
(-) Phenserine	Anti-cholinesterase	Rogers et al 2011	Reduces aSyn translation
Anle138b	Novel	Wagner et al 2013	Inhibits aSyn aggregation
Niraparib	PARP inhibitor	Kam et al 2018	Reduces aSyn aggregation/toxicity
MLi-2	Novel	Scott et al 2017	LRRK2 inhibitor
GNE-7915	Novel	Fuji et al 2015	LRRK2 inhibitor

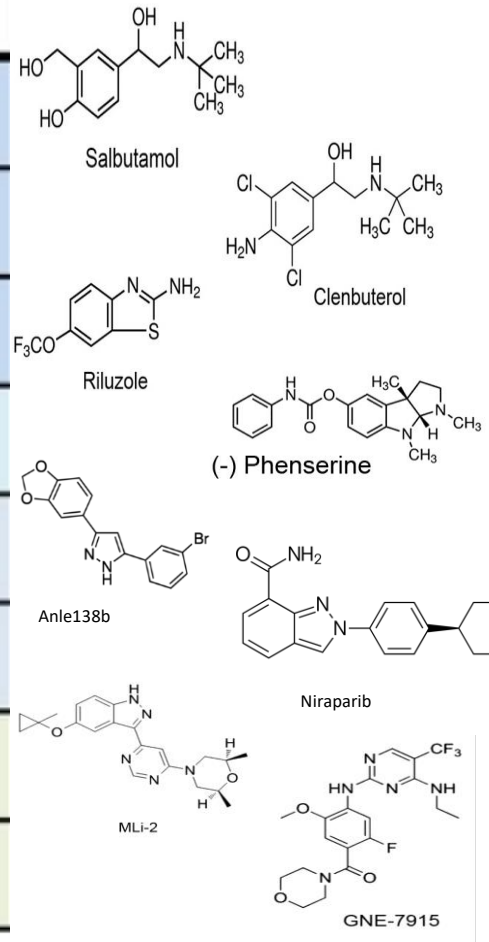


Drug Screening for compounds which inhibit Lewy like pathology

The Cure Parkinson's Trust

- Ongoing project to test the ability of 8 drugs to decrease the formation of α–synuclein aggregates
- Novel compounds will be tested along with repurposed drugs
- Quantifiable side by side comparison of drugs within a human system

	Drug	Class	Reference	Reason
α–synuclein treatment	Salbutamol	Beta2 adrenoreceptor agonist	Mittal et al 2017	Reduces aSyn transcription
	Clenbuterol	Beta2 adrenoreceptor agonist	Mittal et al 2017	Reduces aSyn transcription
	Riluzole	Anti-glutametergic	Mittal et al 2017	Reduces aSyn transcription
	(-) Phenserine	Anti-cholinesterase	Rogers et al 2011	Reduces aSyn translation
	Anle138b	Novel	Wagner et al 2013	Inhibits aSyn aggregation
	Niraparib	PARP inhibitor	Kam et al 2018	Reduces aSyn aggregation/toxicity
LRRK2 treatment	MLi-2	Novel	Scott et al 2017	LRRK2 inhibitor
	GNE-7915	Novel	Fuji et al 2015	LRRK2 inhibitor



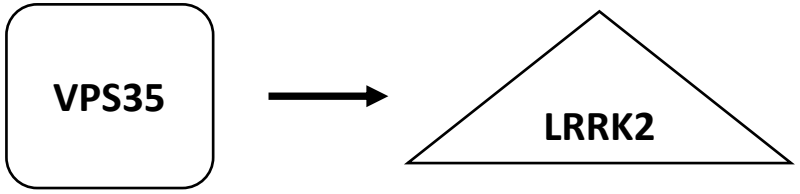
What is the role of LRRK2 and VPS35 in Parkinson's Disease?

- Around 10% of Parkinson's cases are familial

LRRK2:
- Mutations within LRRK2 are the most common cause of familial Parkinson's
- Mutations are known to increase the activity of LRRK2
- There is pharmaceutical interest in the development of LRRK2 inhibitors as a therapeutic strategy for Parkinson's

VPS35:
- The mutation D620N within VPS35 account for 0.115% of all PD cases
- VPS35 is known to act upstream of LRRK2
- D620N-VPS35 increases LRRK2 activity, to a greater extent than mutations within LRRK2 itself

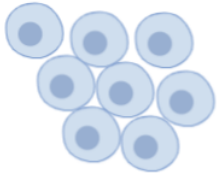
Understanding how these mutations are linked to Parkinson's will be critical to understanding how the disease develops and propagates



Drug	Class	Reference	Reason
MLi-2	Novel	Scott et al 2017	LRRK2 inhibitor
GNE-7915	Novel	Fuji et al 2015	LRRK2 inhibitor

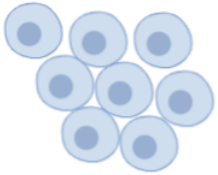
Embryonic Stem Cells: investigating disease mechanisms

- Embryonic stem cells can be genetically modified to introduce mutations associated with Parkinson's disease
- Induced pluripotent stem cells can be produced from patients carrying these genetic mutations
- Dopaminergic neurons can be used for biochemical experiments to determine the effect of these mutations



Embryonic Stem Cells

CRISPR/Cas9



Genetically modified Embryonic Stem Cells

Cell Specific Differentiation

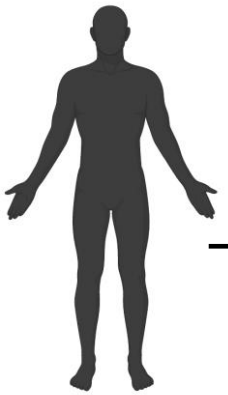
Dopaminergic Neurons

Transplantation

Disease Modelling

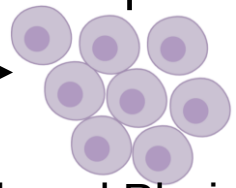
Disease Mechanism

Drug Screening



Patient with genetic mutation

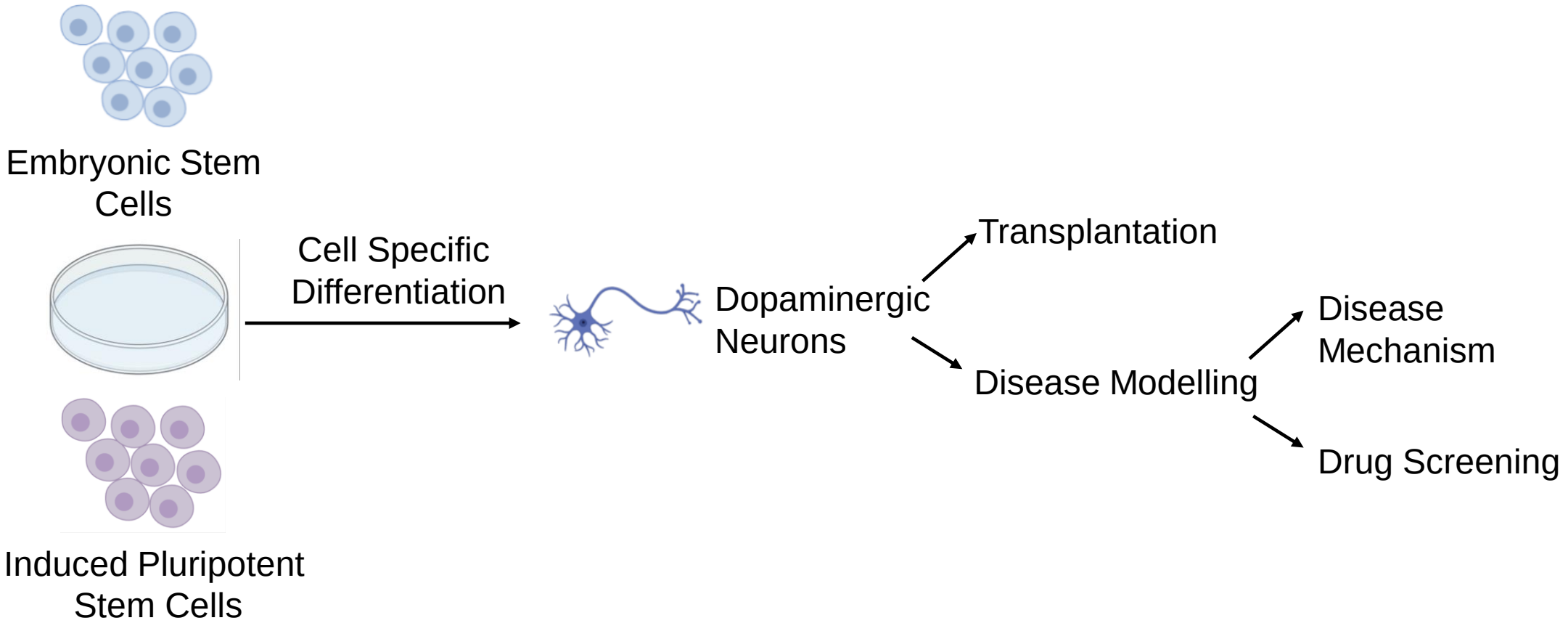
Skin Cells



Induced Pluripotent Stem Cells from patients carrying a genetic mutation

Summary

- Embryonic stem cells and induced pluripotent stem cells are an excellent tool for disease modelling
- α–synuclein pathology can be recapitulated within a culture of dopaminergic neurons
- Dopaminergic neurons can be used for drug screening of novel or repurposed compounds for their ability to inhibit/ slow down α–synuclein pathology
- Genetic modification of human embryonic stem cells allows us to investigate the effect of specific PD linked mutations
- Understanding how the disease initiates and propagates is critical to the development of novel therapies



Thank you

Centre for Regenerative Medicine

Kunath Lab
Tilo Kunath
Nicola Drummond
Stephen West
Yixi Chen
Ammar Natalwala
Craig Leighton
David McNay

Collaborators:
MRC PPU, Dundee:
Dario Alessi
Pawel Lis
Matt Taylor
Kerryn Berndsen

Forrester Lab:
Lesley Forrester
Alisha May
Telma Venturra

Burdon Lab
Tom Burdon

SCRM facilities:
Fiona Rossi (FACs)
Matthieu Vermeren (Imaging)

The Cure Parkinson's Trust

MRC Medical Research Council
CRM at 10
MRC Medical Research Council
THE UNIVERSITY of EDINBURGH
Anne Rowling Regenerative Neurology Clinic
MRC Protein Phosphorylation and Ubiquitylation Unit
University of Dundee