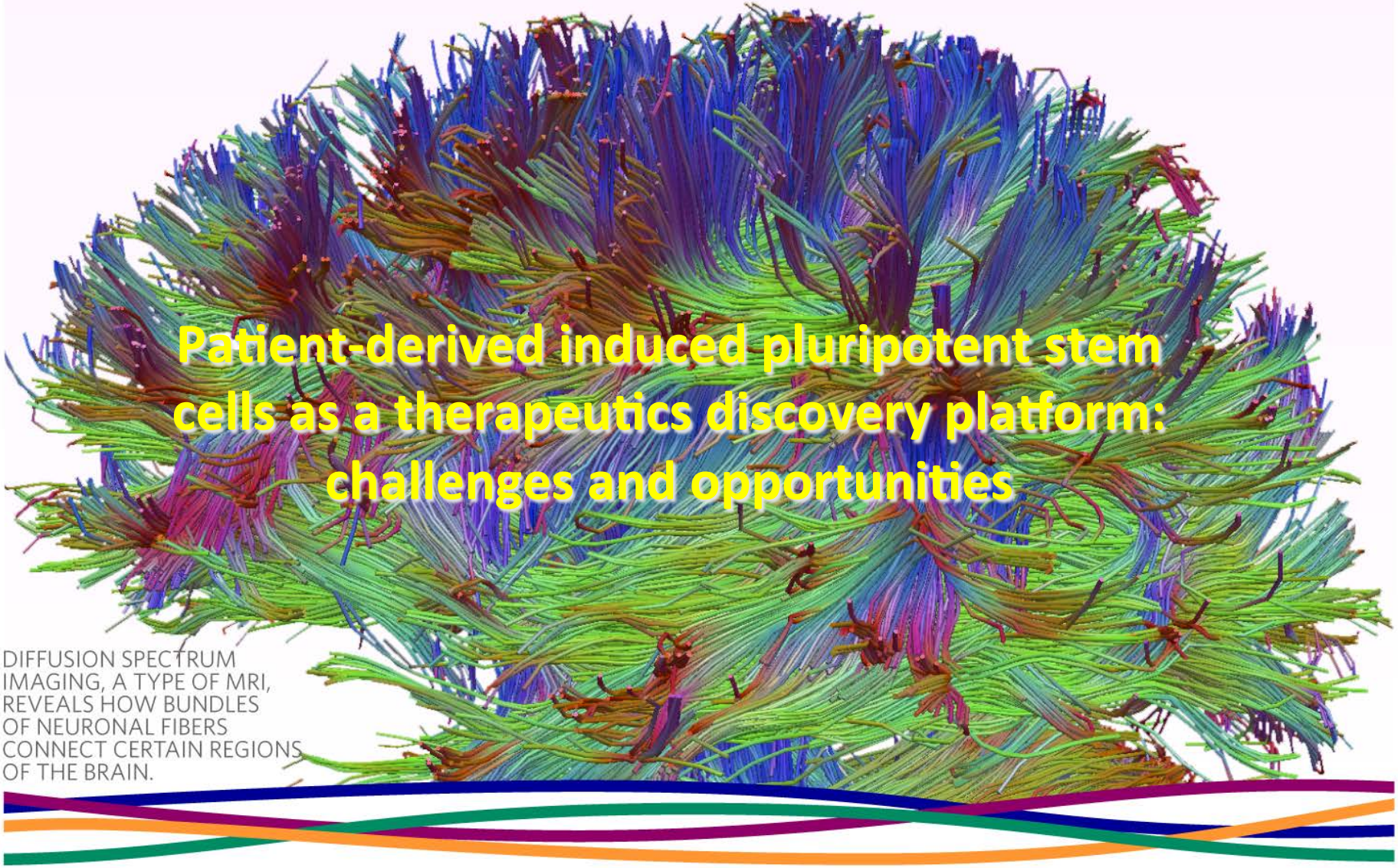


Patient-derived induced pluripotent stem cells as a therapeutics discovery platform: challenges and opportunities

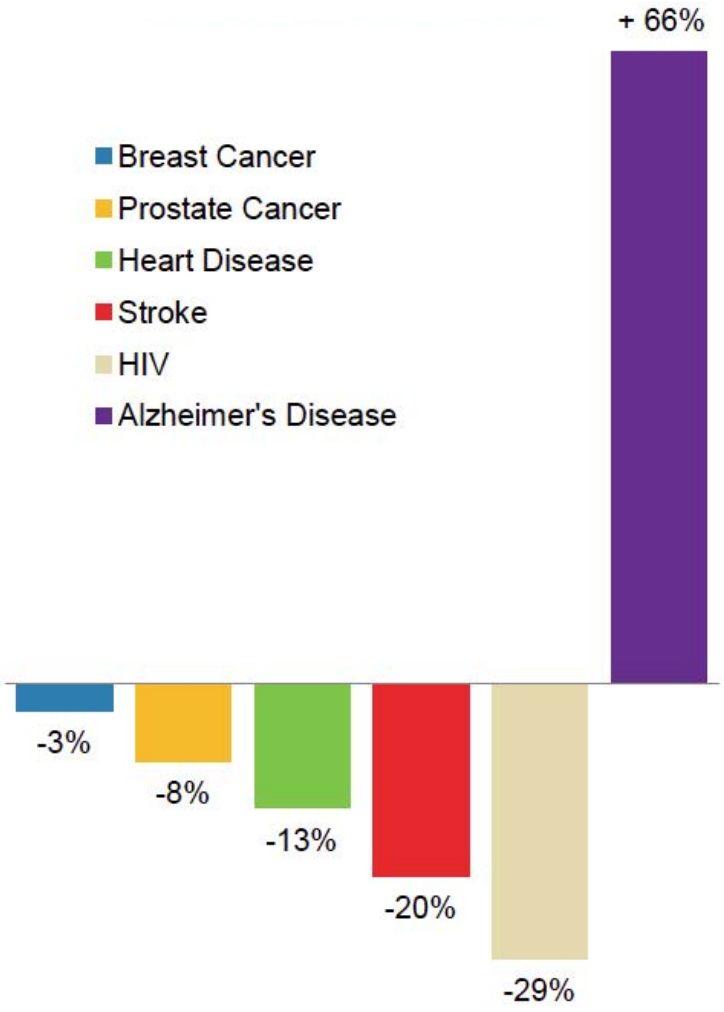
DIFFUSION SPECTRUM IMAGING, A TYPE OF MRI, REVEALS HOW BUNDLES OF NEURONAL FIBERS CONNECT CERTAIN REGIONS OF THE BRAIN.

Steven Finkbeiner, MD, PhD
Professor, UCSF & Gladstone
Director Taube/Koret Center

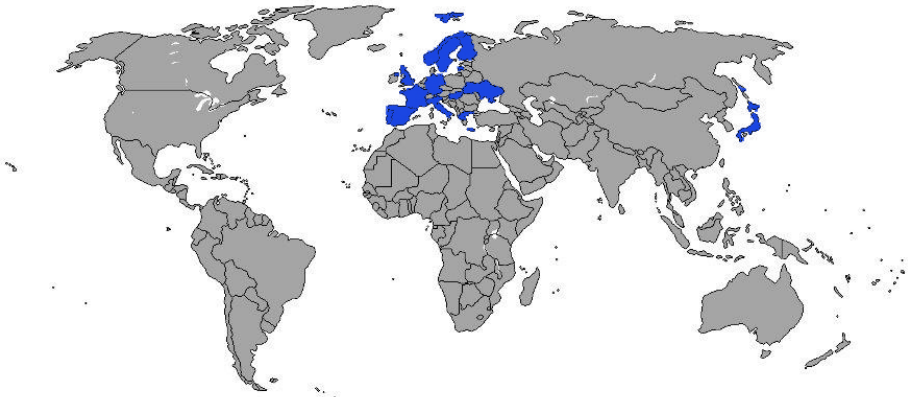
CIRM Webinar

The Enormous Therapeutic Challenge of Neurodegenerative Disease

Change in Number of Deaths 2000–2008

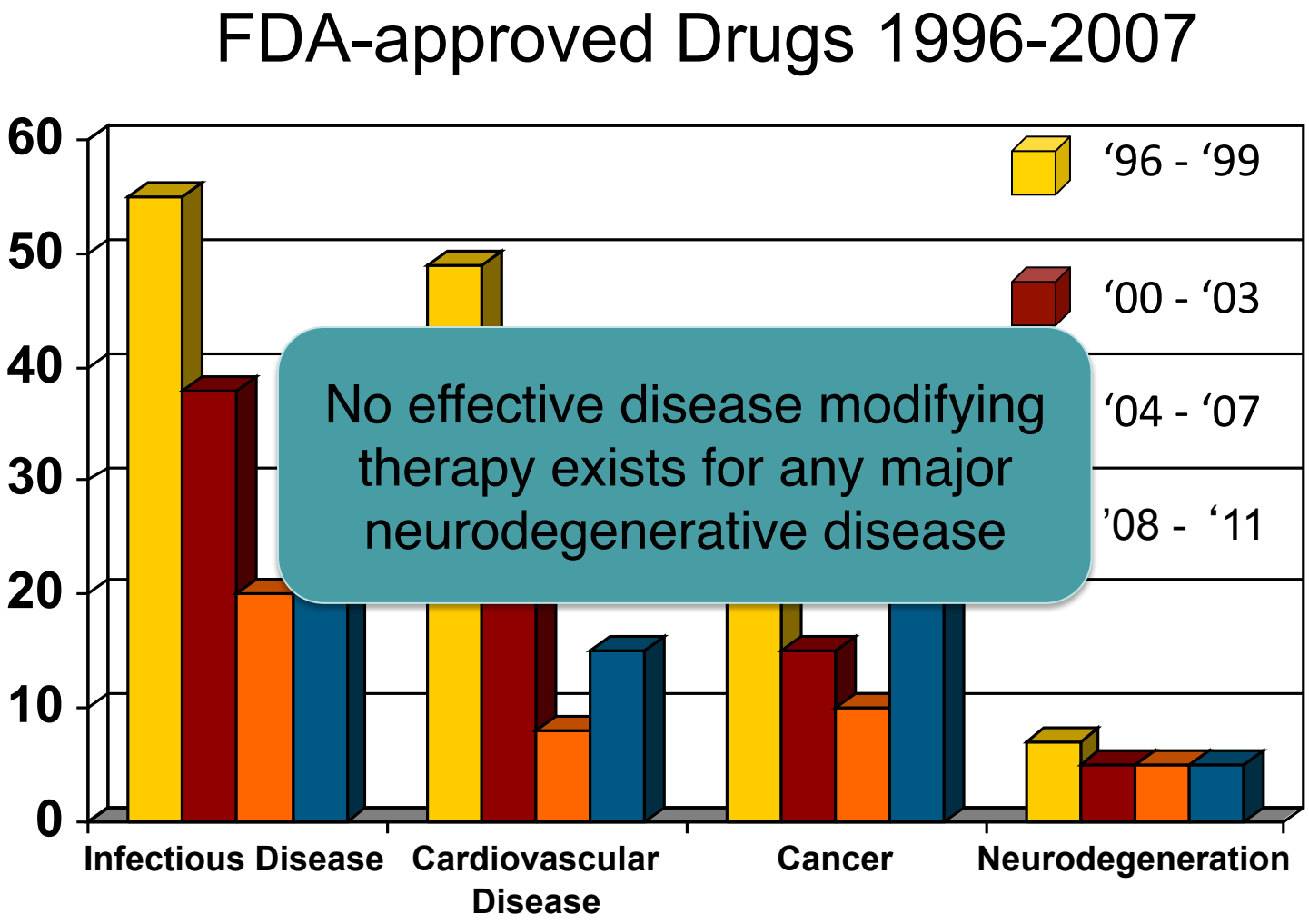


TODAY



> 20% over age 65

The Challenge of Drug Development for Neurological Disease



© 2011 Harvard NeuroDiscovery Center. Courtesy Adrian Ivinson. Data from Thompson Centerwatch

Murine Models of Human Disease

Genetic similarities are striking…

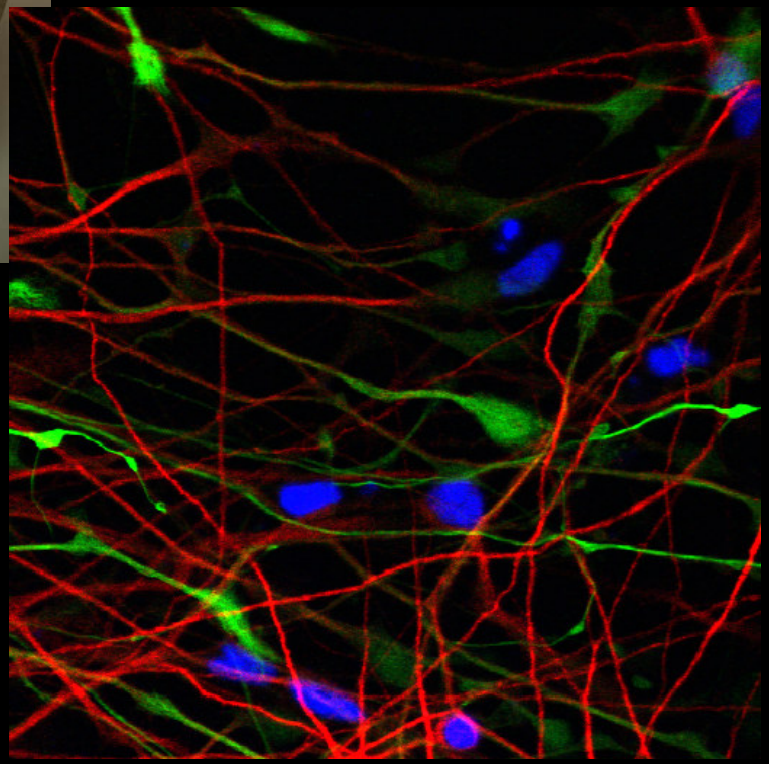
…but to a drug, mice and people may be very different

Developing Disease Models Based on Patient-Derived Induced Pluripotent Stem Cells

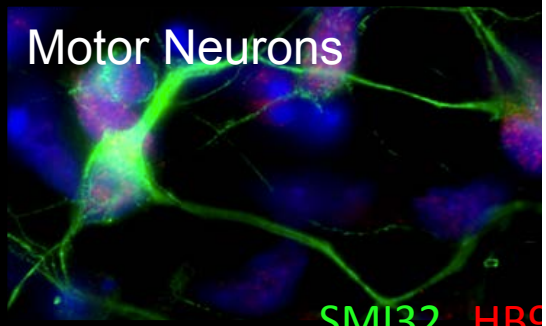
Patient fibroblasts

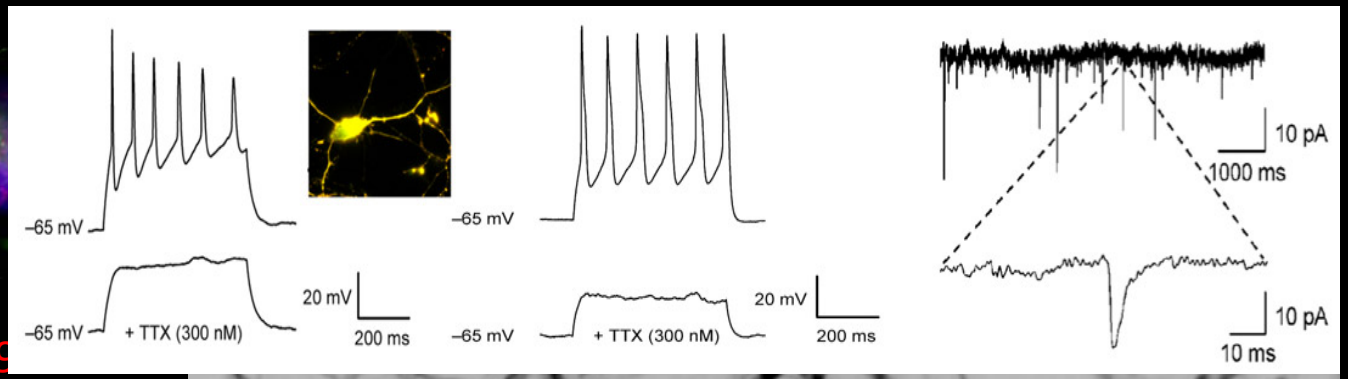
Induced pluripotent stem cells

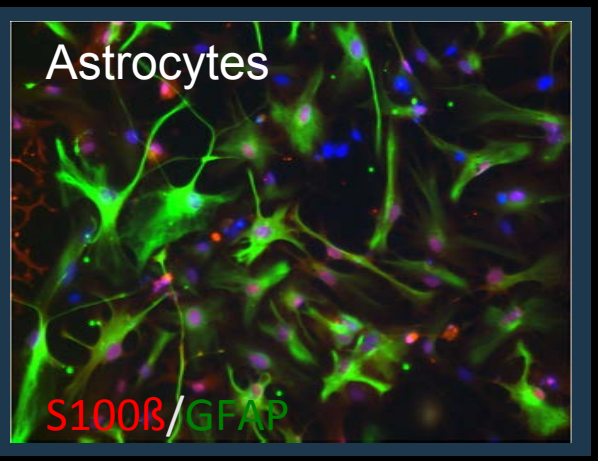
Human neurons

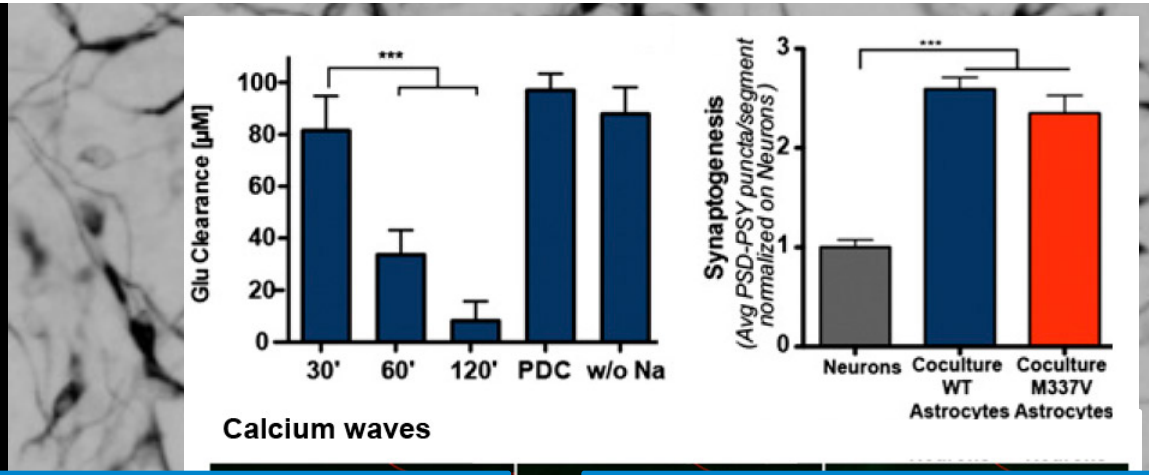


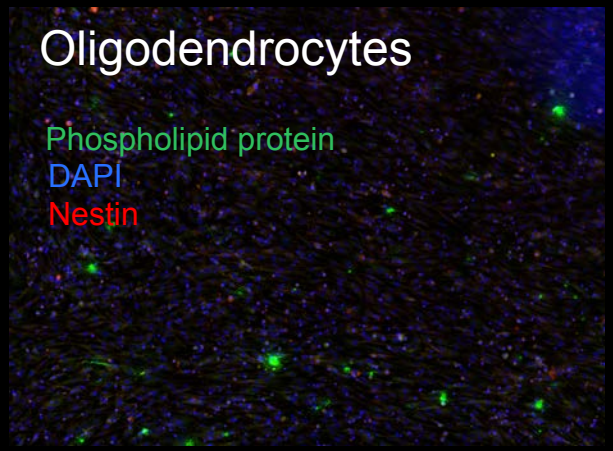
Characterizing Human iPSCs Differentiated Toward Brain Cells

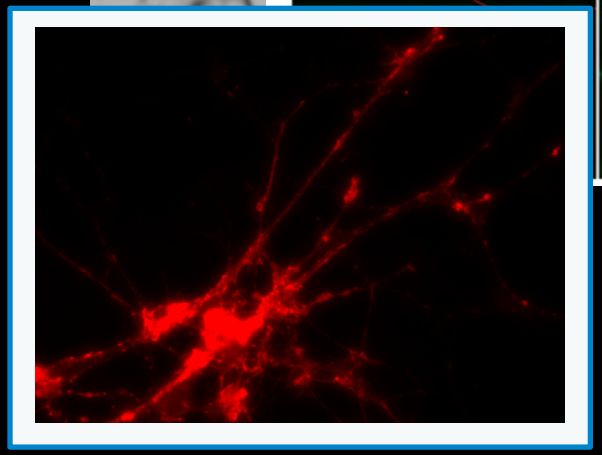


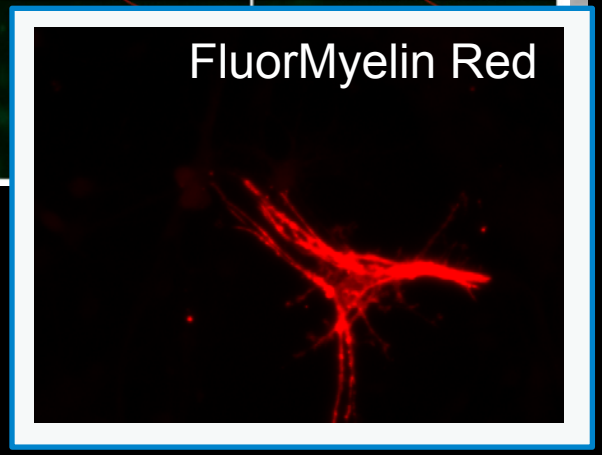












Challenges to iPSCs as a Discovery/Analytical Platform

Cell Type	Differentiation	Efficiency
Forebrain neurons	3 weeks	~90%
Striatal neurons	8 weeks	~1-5%
Dopaminergic neurons	4-8 weeks	~50-70%
Motor neurons	6 weeks	~40-50%
Astrocytes	12-24 weeks	~25%
Oligodendrocytes	4-18 weeks	~1-10%
Microglia-like	6 weeks	~10-20%

Heterogeneity remains a challenge

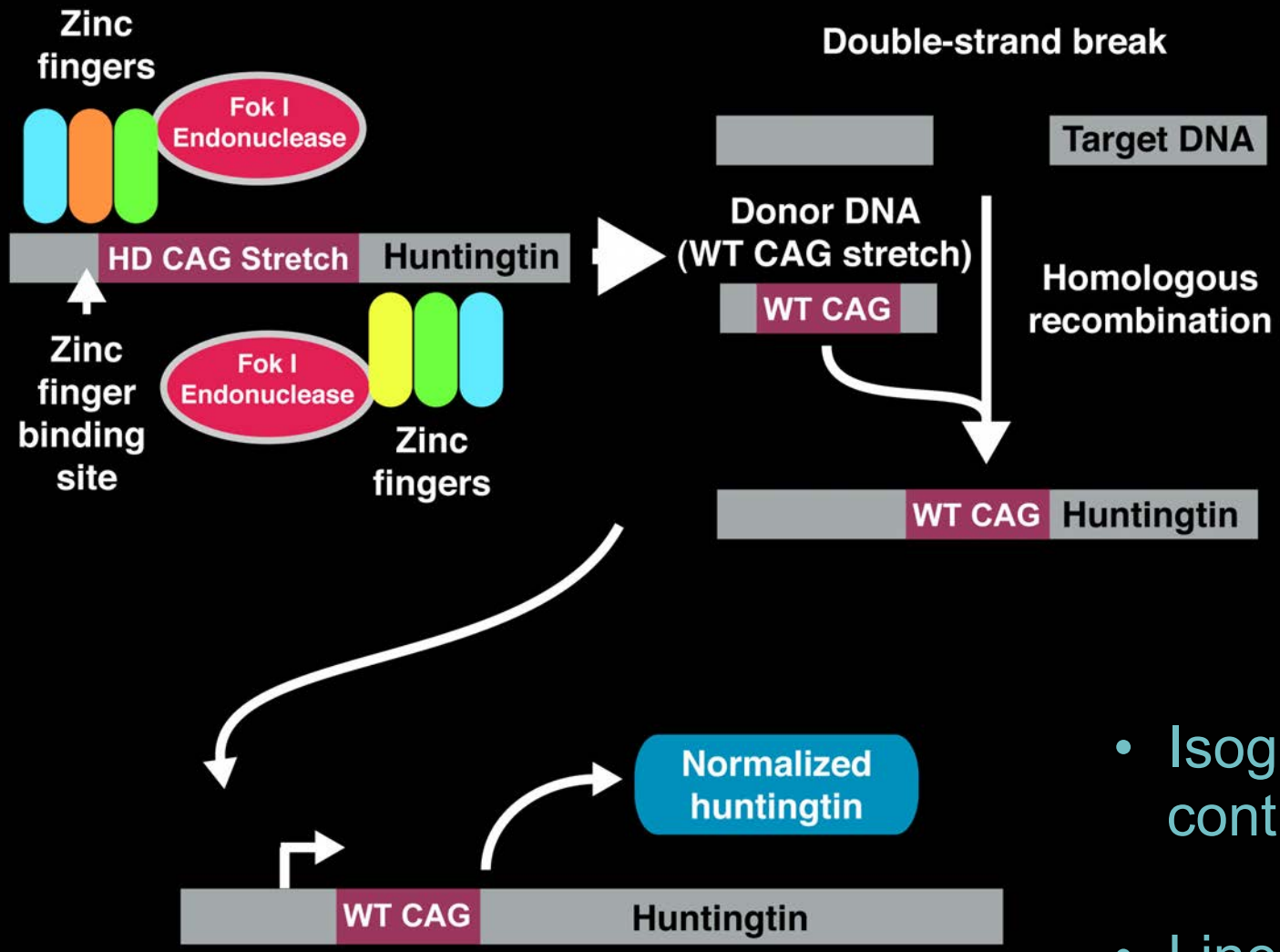
- Line-specific variation (possible importance of reprogramming techniques; donor heterogeneity)
- Prep variation (differentiation)
- Maturity (persistence of dividing cells)
- Cell type

Addressing Heterogeneity: Making Many iPSC Lines

Disease	Mutation	Number Of Patients	Number Of iPSC Clones
Huntington's Disease	26-70 CAG expansion	10	28
	>70 CAG expansion	4	13
Parkinson's Disease	LRRK2 G2019S	2	2
	LRRK2 G2019S (at risk)	2	2
	LRRK2 R1441C (at risk)	2	2
	α-SYNUCLEIN triplication	4	4
	PARK2	1	1
	Sporadic PD	1	1
ALS	TDP43	3	7
	ANGIOGENIN	1	1
	SOD1 (Fast & Slow Progressing)	7	7
	FUS	3	3
	c9ORF72 expansion	7	7
	Sporadic ALS (Fast & Slow Progressing)	6	6
FTD	PROGRANULIN	1	3
Healthy Controls (relatives and unrelated)	--	24	37
Total		78	124

Confidential

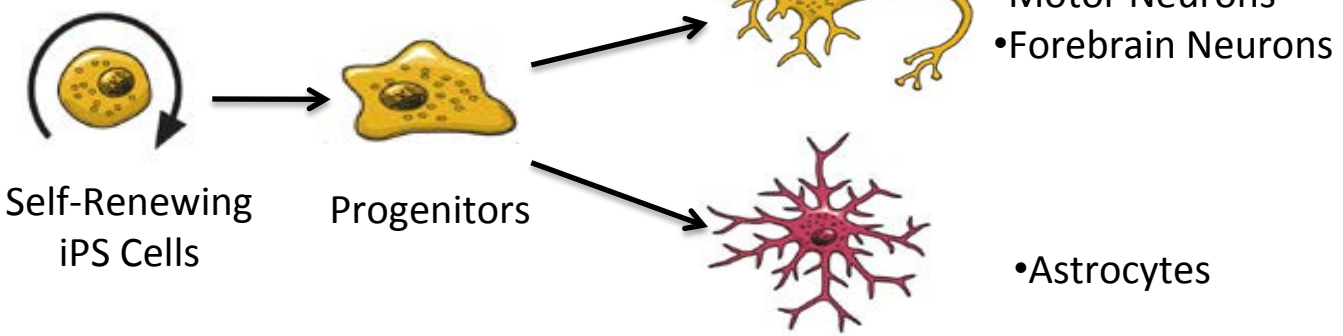
Addressing Heterogeneity: Genome Editing

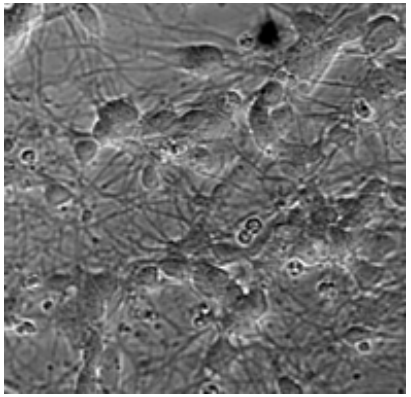


- Isogenic controls
- Lineage reporter lines

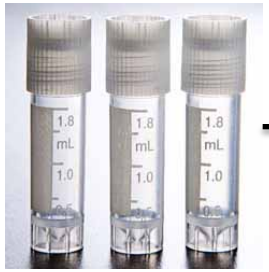
Julia Kaye & Kelly Haston

Addressing Heterogeneity: Scaling Differentiation

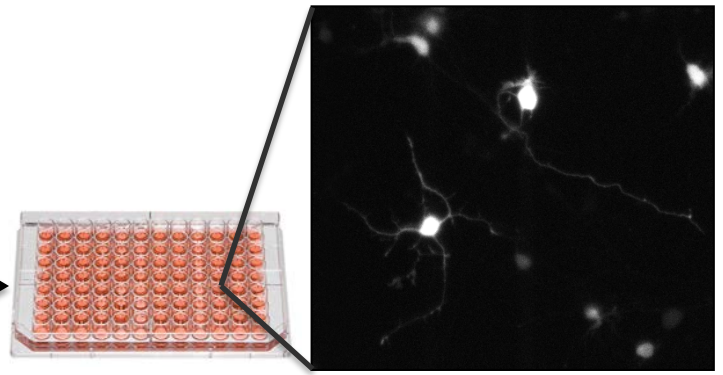




iPSC-Neurons Grown
In Monolayer



Characterized and Banked iPSC-Neurons



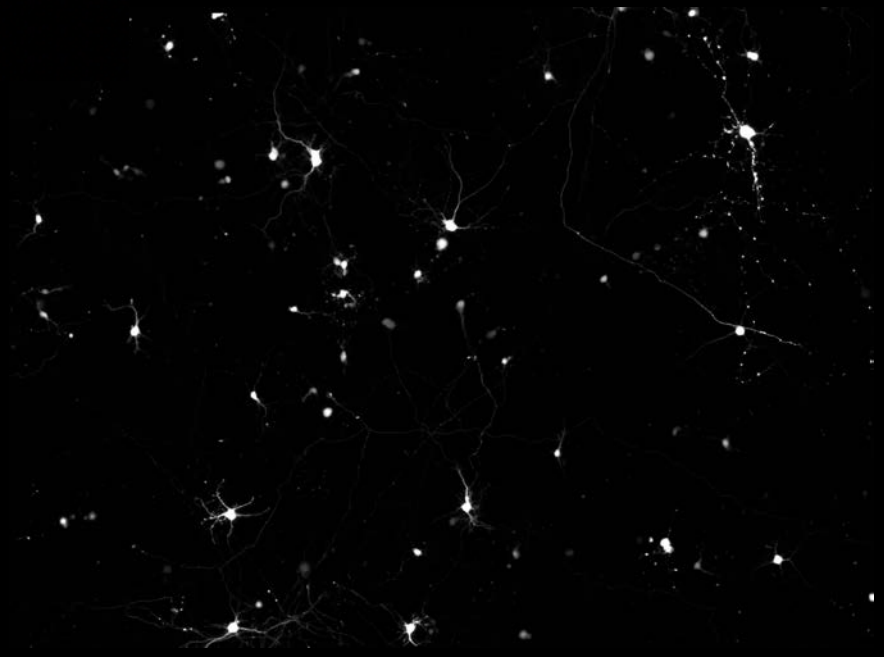
Transfected iPSC-Neurons
Imaged by
Automated microscopy

Ashkan Javaherian

High Throughput Longitudinal Single Cell Analysis

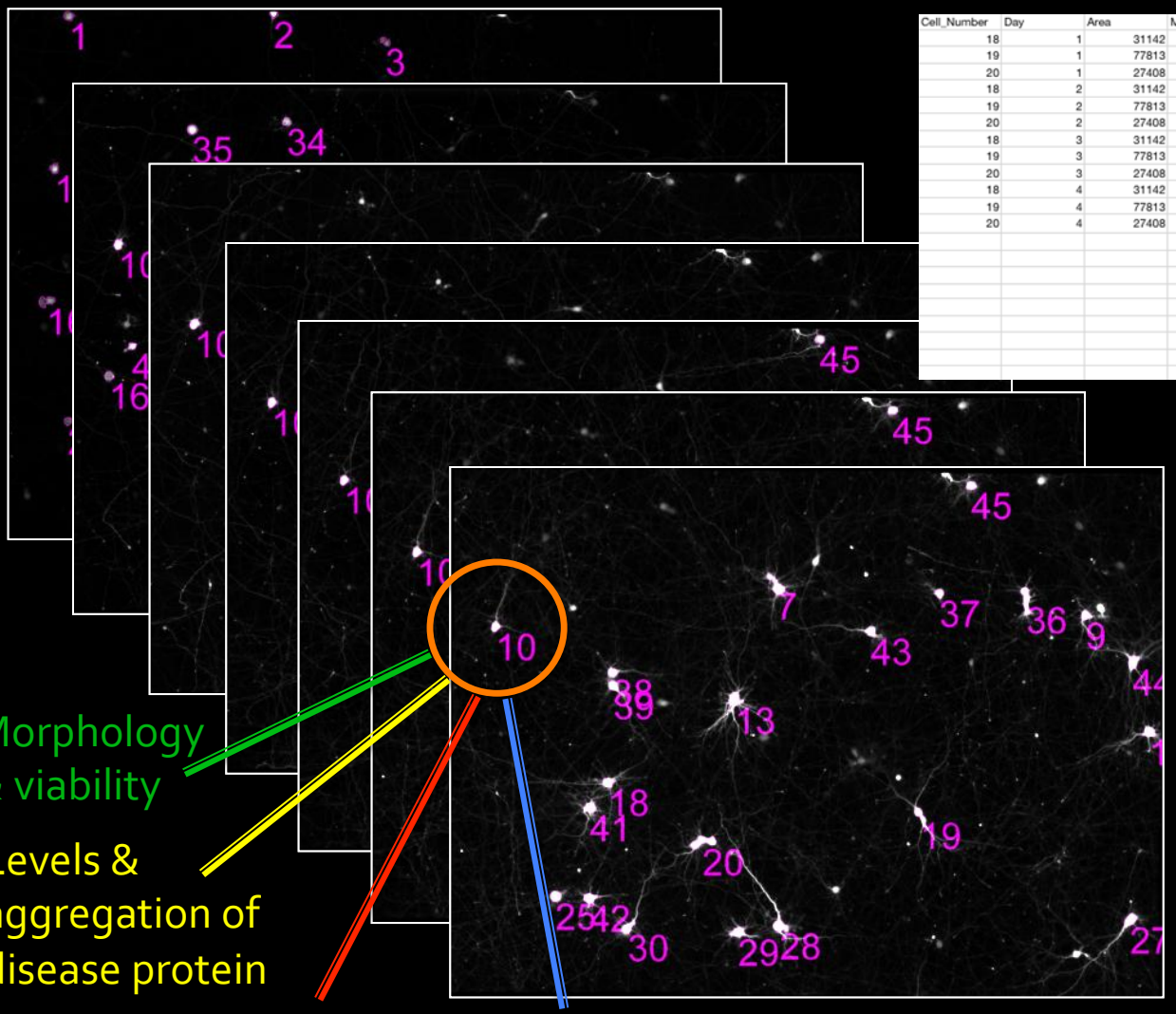
3rd Generation
Automated
Microscope
(Robo III)

7 day movie



#thebrainbot

Longitudinal Tracking



Day 4

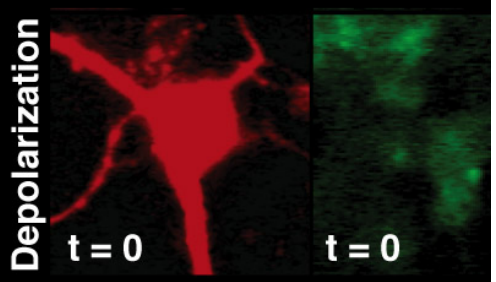
Cell_Number	Day	Area	Mean	StdDev	Mode	Min	Max	Perim.
18	1	31142	597.957	180.342	536	514	1638	704.024
19	1	77813	567.874	105.085	537	513	1494	1024.611
20	1	27408	553.433	54.340	534	513	927	760.918
18	2	31142	998.997	1260.216	546	520	7996	704.024
19	2	77813	754.168	714.393	544	522	6564	1024.611
20	2	27408	591.102	149.516	538	518	1567	760.918
18	3	31142	1287.203	2099.407	550	525	12478	704.024
19	3	77813	801.182	833.485	547	524	7768	1024.611
20	3	27408	616.433	206.510	542	517	1906	760.918
18	4	31142	1243.333	1964.293	555	526	13445	704.024
19	4	77813	915.302	1190.803	552	526	10324	1024.611
20	4	27408	658.185	299.814	542	518	2512	760.918

Well suited to deal with cell-specific stochastic phenomena

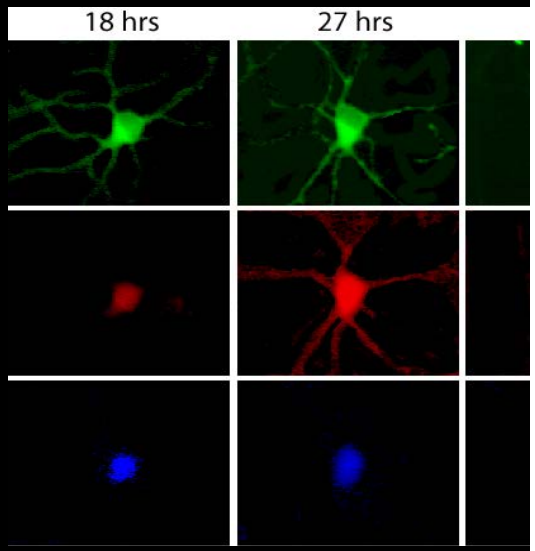
- Reduced user bias
- Reduced observer bias
- Powerfully exploits cell-to-cell variability

Assays for Ph … c Phenotypes

Cellular Phenotypic Assays
Neuronal survival
Protein aggregation/ inclusion body formation
Protein levels and clearance
Protein localization
Protein half-life by optical pulse labeling*
Neurite morphology
Synapse number
Ubiquitin proteasome function
Autophagy function
Mitochondrial morphology
Mitochondrial trafficking
Mitophagy**
Axonal trafficking
Signal transduction/ gene expression pathways
Calcium signaling

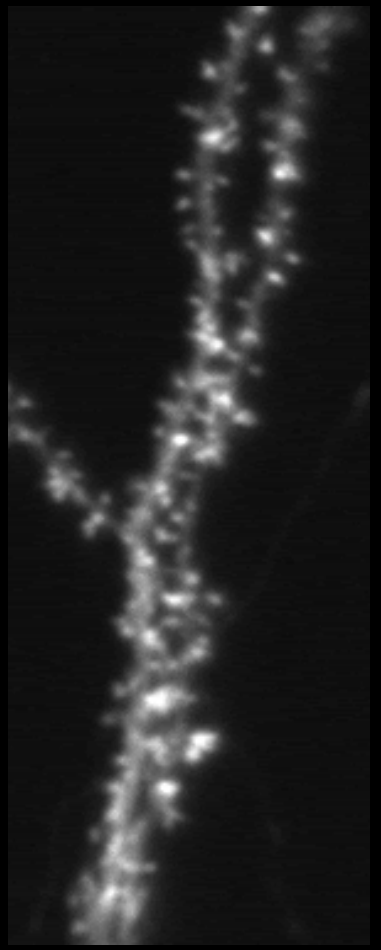


Gene Transc…



Proteasome Func…

Spines

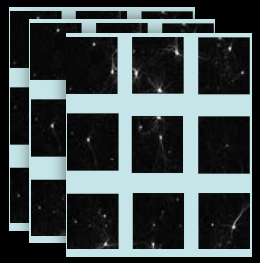


Mitochondria

Google Collaboration II: Overview

Goal: Substantially More Powerful Image Analysis

Raw Images



Neuro-Analysis Pipeline

OpenCV

Measurements of Tracked Brain Cells

Cell_Number	Day	Area	Mean	StdDev
18	1	31142	597.957	180.342
19	1	77813	567.874	105.085
20	1	27408	553.433	54.340
18	2	31142	998.997	1260.216
19	2	77813	754.168	714.393
20	2	27408	591.102	149.516
18	3	31142	1287.203	2099.407
19	3	77813	801.182	833.485
20	3	27408	616.433	206.510
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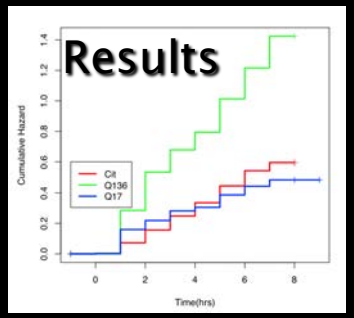


Construct	Risk of Death	P Value
Q17	0.8623	0.269
Q136	2.3972	1.83e-12

Machine Learning

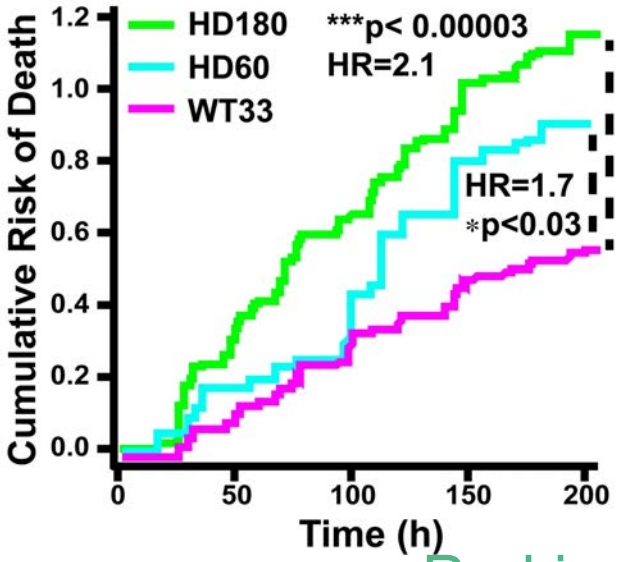
Web-based Image Annotation

- "Systems"cell biology
- Quantitative predictive model of fate
- Blueprint for rational intervention

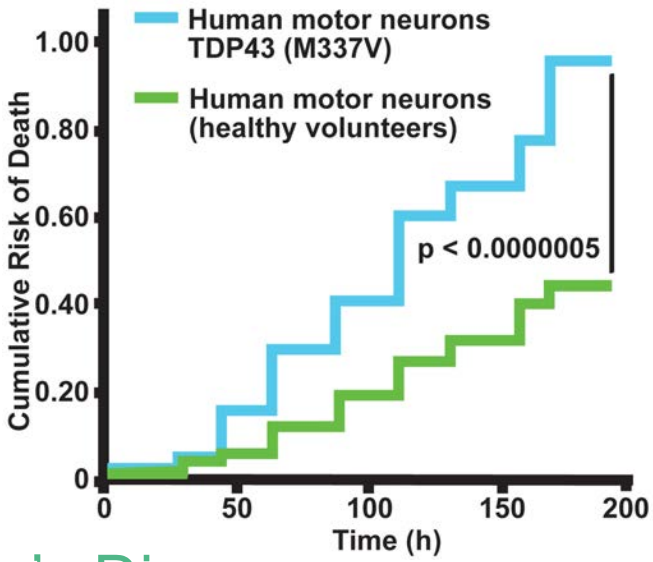
Confidential – not in the public domain

Human iPSC-based Models of Neurodegenerative Disease

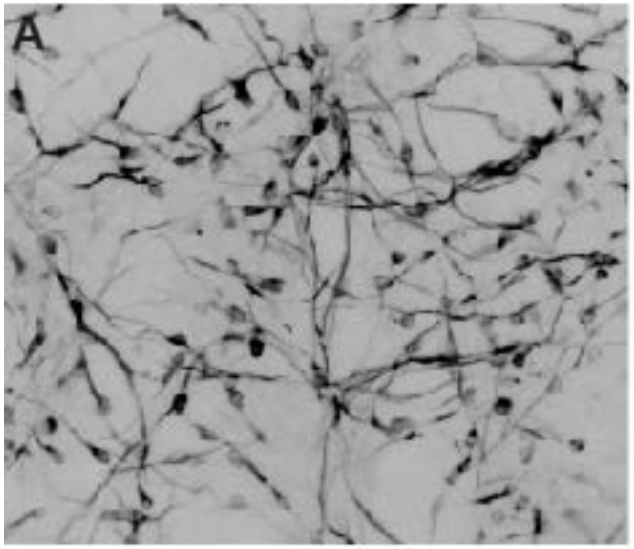
Huntington's Disease

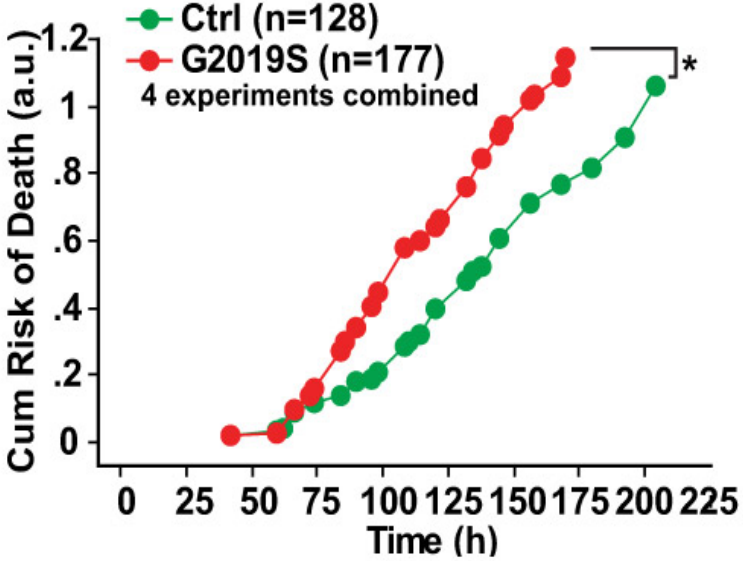


ALS/ FTD



Parkinson's Disease





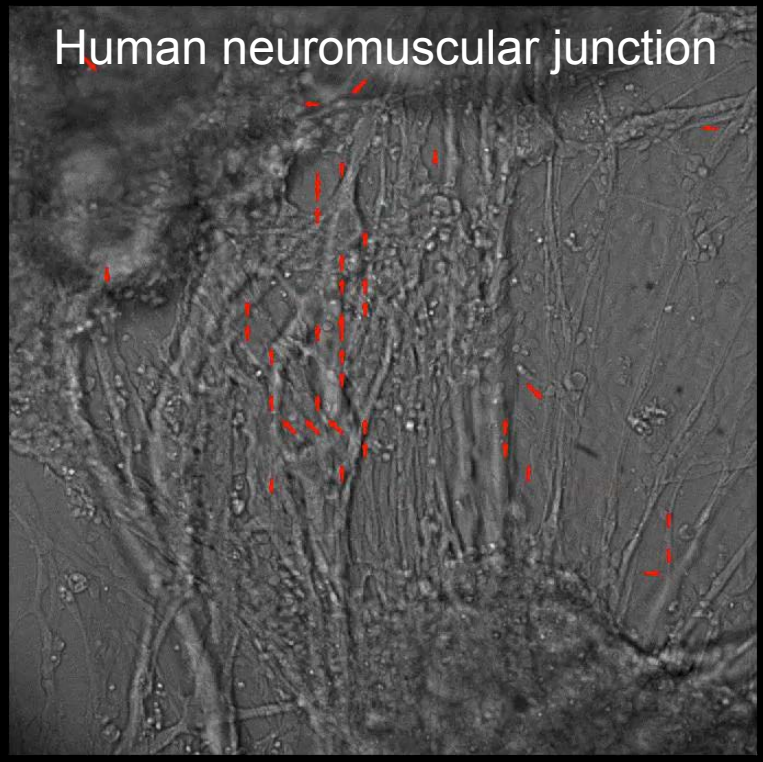
Disease-relevant phenotypes:

- Survival
- Neurite length
- Calcium signaling
- Glial phenotypes
- Electrophysiology changes
- Gene profiling changes
- Response to stress

PNAS, 2012
Cell Stem Cell, 2012; PNAS, 2013; J. Neurosci, 2014; Nat. Chem. Biol., 2014, in press

Current Applications & Future Directions

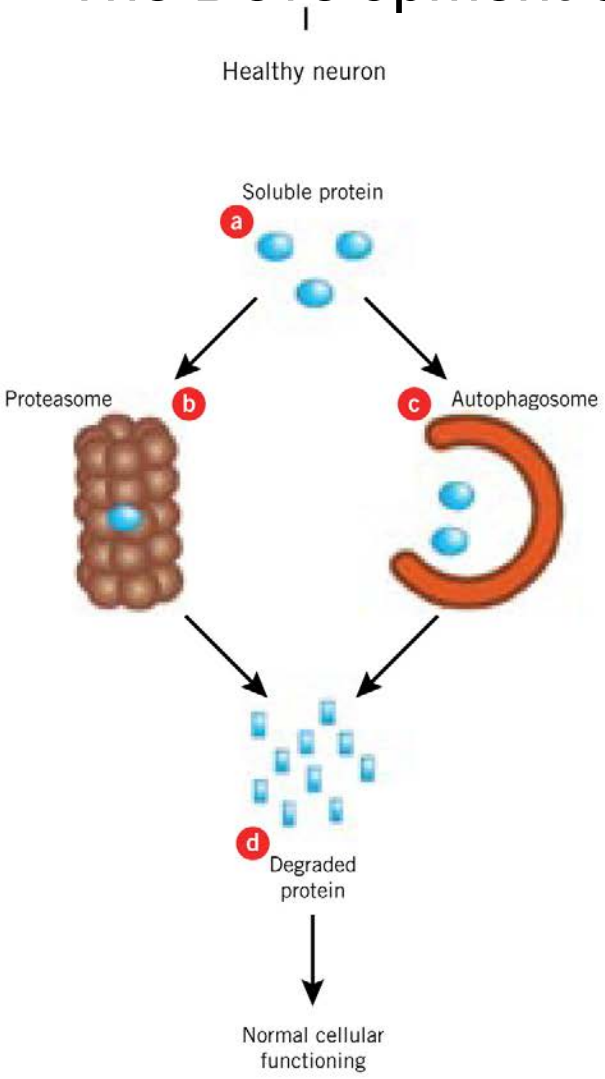
- Mechanisms of disease (NIH, CIRM, Foundations)
- Genetics (NIH, Michael J Fox Foundation, Pharma)
  - Bottom up: RNAi screens
  - Top down: functional validation of human genetics results
- Small molecule discovery (ALS-TDI, Pharma)
- Lead optimization (NIH, Pharma)
- Long-term toxicology, especially when mouse models won't do (Pharma)



- More relevant/complex models
- Integrate AI / deep learning into phenotypic analysis (Google) to predict clinical results

nature biotechnology

iPSCs & Small Molecule Discovery and Lead Optimization: The Development of Small Molecule Autophagy Inducers



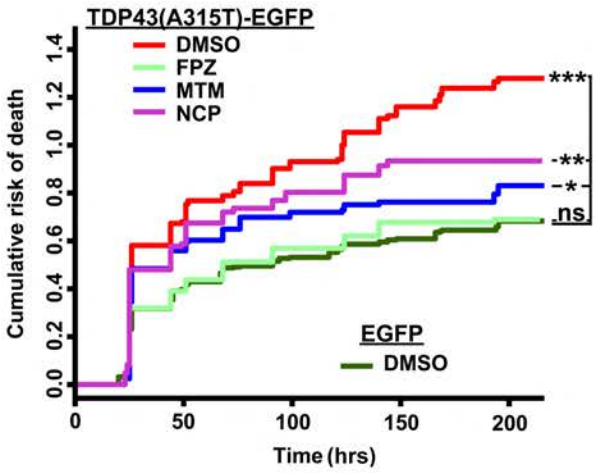
SciBX 3:5-8, 2010

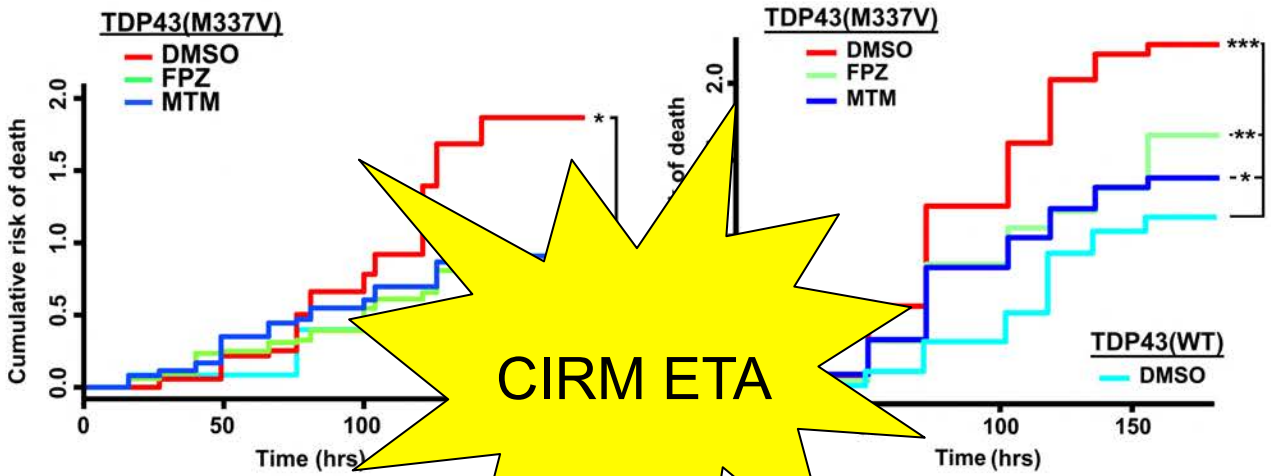
Application of Small Molecule Autophagy Inducers Mitigates TDP43 (A315T) Toxicity

TDP43 (A315T) in Rodent Neurons

Patient-derived human motor neurons (TDP43 M337V)

Patient-derived human astrocytes (TDP43 M337V)





CIRM ETA

New 3rd Generation inducers: Novel structures with potencies ≤ 5 nM, better side effect profile, and good predicted BBB penetration

Barmada et al., Nat. Chemical Biology, 2014; Javaherian et al. unpublished

Acknowledgments

Finkbeiner Lab

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Julia Kaye
Ian Kratter
Eva LaDow
Julia Margulis
Amanda Mason
Jason Miller
Siddhartha Mitra
Gaia Skibinski
Tina Tran
Andrey Tsvetkov
Hengameh Zayed
Adam Ziemann

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Joan Steffan
Leslie Thompson

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Chris Ross
Clive Svendsen
Leslie (again!)
Nick Allen
Paul Kemp

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David Galas
Lee Hood

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California Institute for Regenerative Medicine
National Institute of Neurological Disorders and Stroke
ALS Association — Fighting Lou Gehrig's Disease
Taube-Koret Center for Huntington's Disease Research