HARVARD STEM CELL INSTITUTE

HSCRB
harvard department of stem cell & regenerative biology

Using Stem Cells to Study Neurodegenerative Disease

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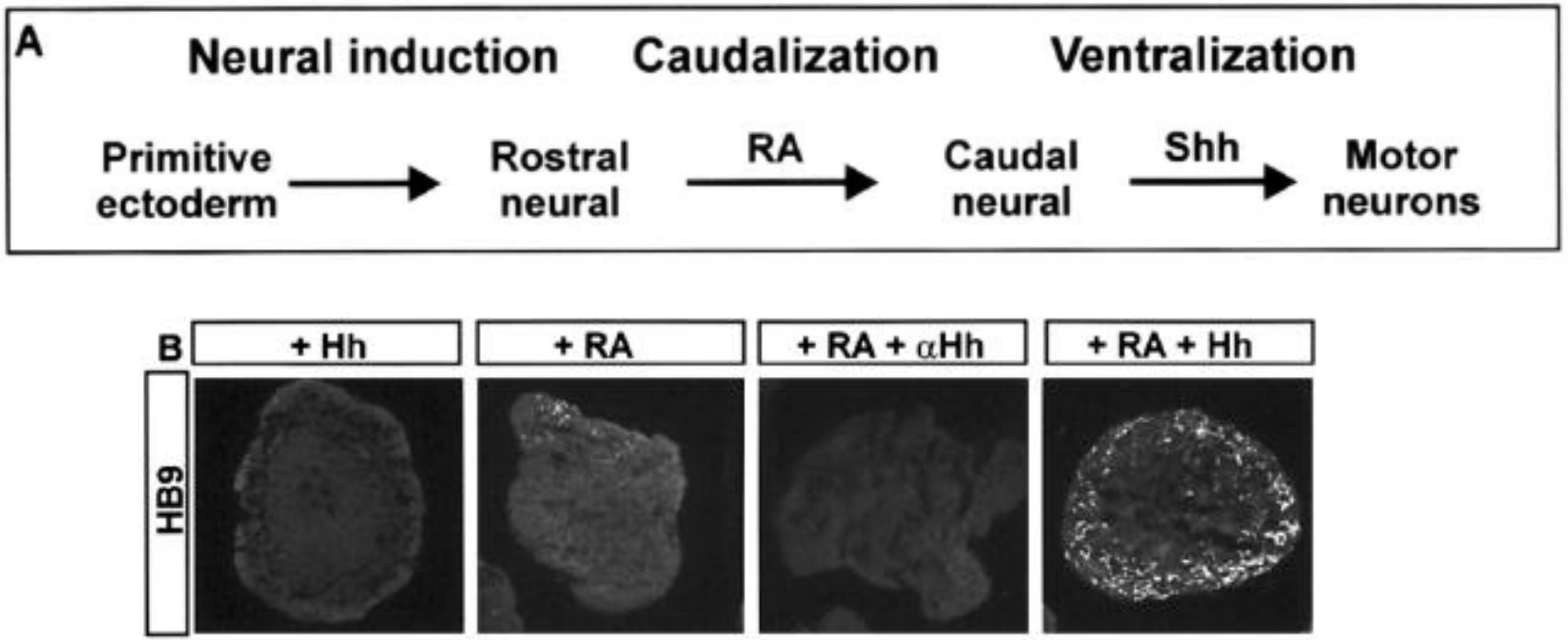
Introduction

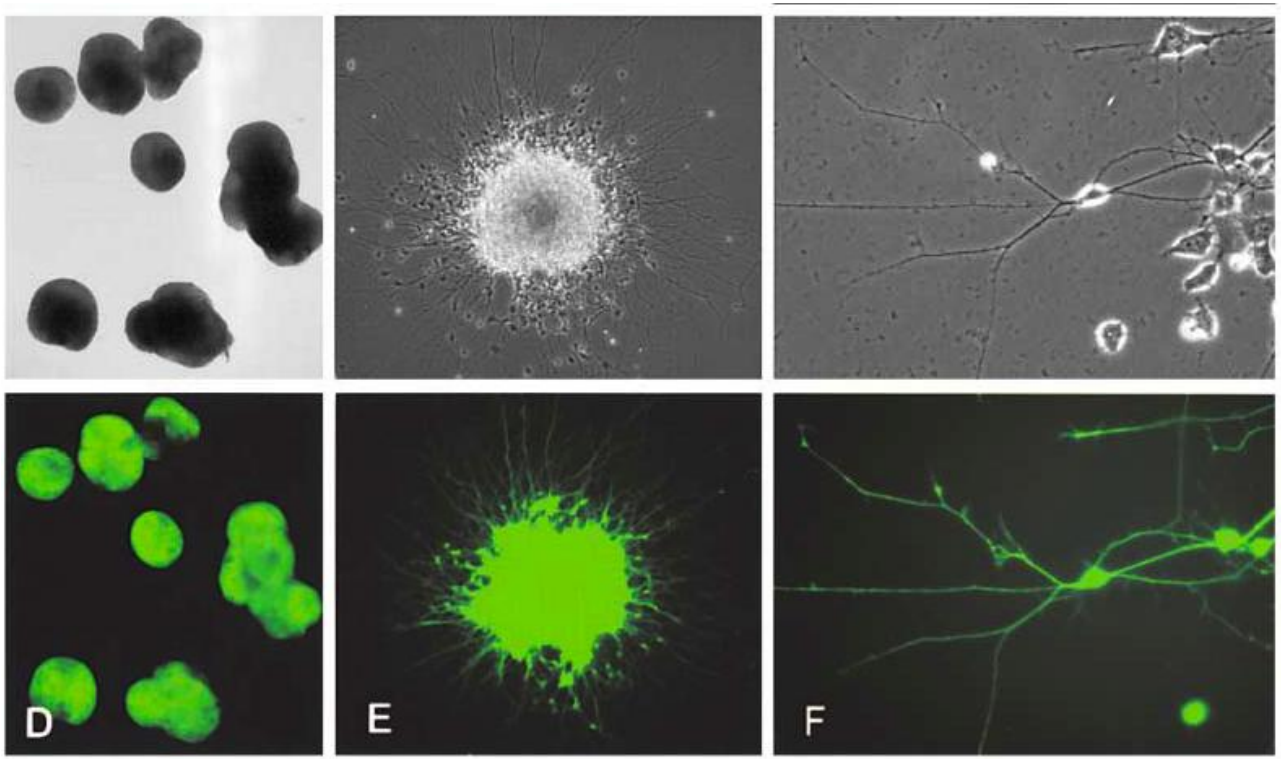
- Major advances in the last decade have led to a potential new use for stem cells – as the foundation of a new drug discovery system.
  - Adult cells (from patients or controls) can be **reprogrammed** into iPS cells which resemble, but are not identical to, ES cells.
    - Theoretically, this can be done at industrial scale (from many different patients).
  - A variety of methods for producing **differentiated** cells, either directly from patient fibroblasts, or indirectly from iPS cells, have been described.

Specific Morphogenetic Pathways Regulate Embryonic Development



Wichterle and Jessell, Cell 2002

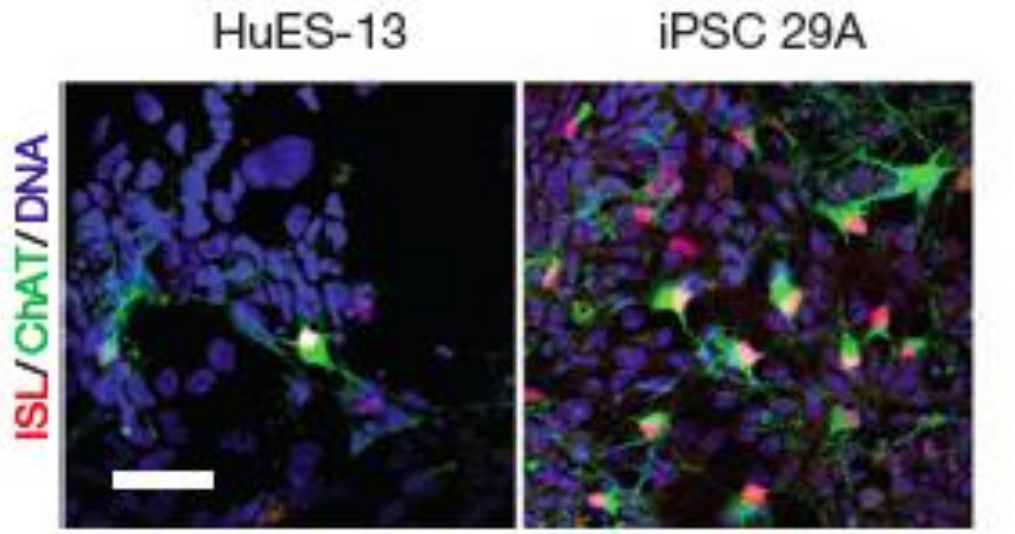
Small molecule Hh agonists induce motor neuron differentiation from mouse (and human) ES cells.



This means that **large numbers** of motor neurons can be produced from various patient populations, including those with motor neuron disease.

Can this method for making motor neurons give us some guidance about how to make other types of neurons and other types of cells?

Putting these two methods together: iPS Cells (from healthy donors or from those with motor neuron disease) can be differentiated equally well into motor neurons.

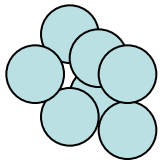


Boulting et al., Nature Biotech, 2011

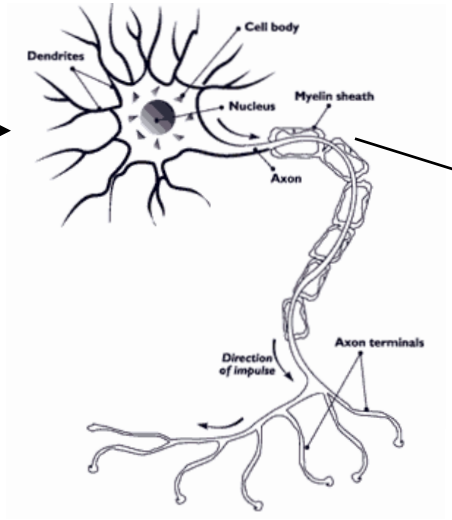
A New Way to Study Human Disease

- Identify cohorts of patients with diseases of interest.
- Derive fibroblasts or other cells from each patient.
- Reprogram and differentiate into desired cell types (or just transdifferentiate without reprogramming).

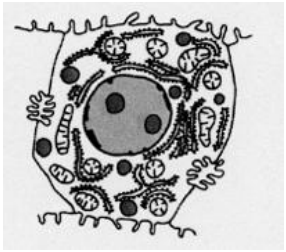
How Can Stem Cells Help Us to Understand Human Disease and Find Better Drugs?



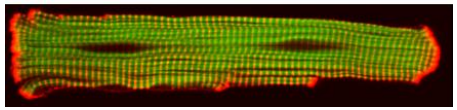
Produce iPS cells from normal people or those with disease and **differentiate** them



Screen for therapeutics



Hepatocyte



Cardiac myocyte

Specific types of normal or diseased neurons

Disease mechanism studies

In vitro clinical trial

More efficacious drugs?

Safer drugs?

Issues that need to be resolved

- What is the reliability of the reprogramming process?
- How similar are differentiated cells produced from individual iPS lines all derived from a single patient?
- How similar are differentiated cells produced from iPS lines from multiple patients with the same (genetic) disorder?
- How mature are the differentiated cells?

Issues that need to be resolved

- Can late onset disease pathology be reproduced when starting from iPS cells?
  - In other words, can diseases that take decades to occur in patients be modeled in vitro in a reasonable amount of time?
- Most neurodegenerative disorders are seemingly sporadic, rather than familial (including AD, PD and ALS).
  - How can iPS cell-based studies be used to model these diseases?
- Big question: Is iPS-based drug discovery predictive and how generally useful will it be?

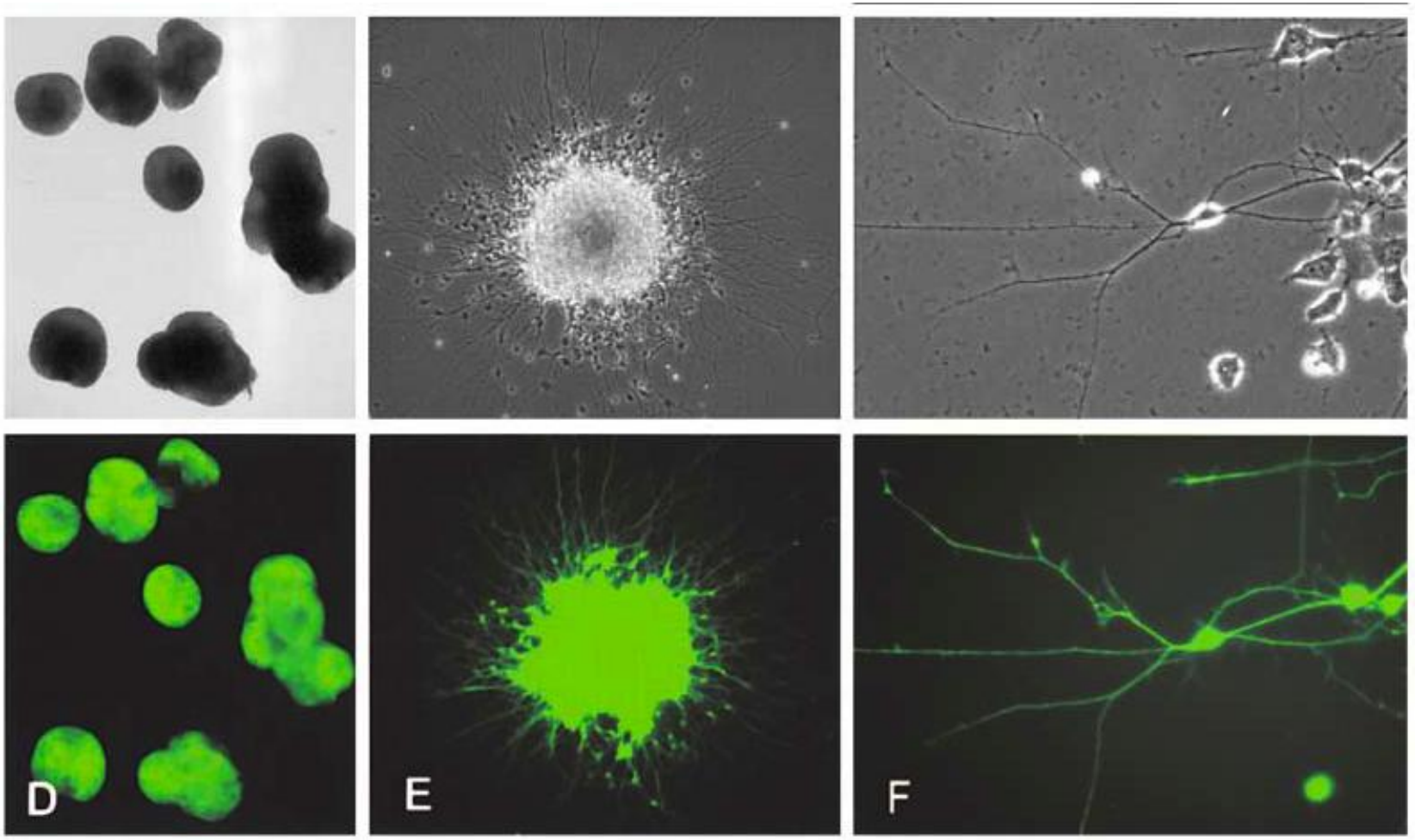
Other practical issues that need to be resolved

- When are disease-relevant cells produced from patients absolutely required in the drug discovery process?
  - Primary screening stage?
  - Secondary assays?
  - In vitro “clinical trial”?

Examples of neurons we've made from ES cells

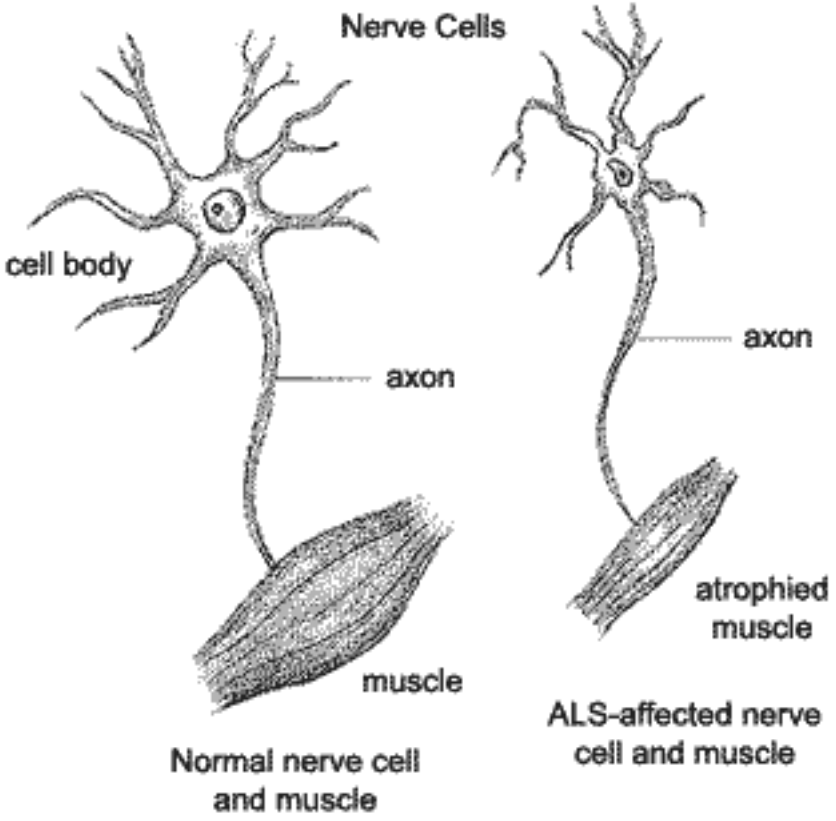
- Nociceptors (pain)
- Dopaminergic neurons (Parkinson's disease)
- Motor neurons (Spinal Muscular Atrophy, ALS)

Small Molecule Hh Agonists Induce Motor Neuron Differentiation from Mouse ES Cells



Wichterle et al., Cell, 2002

ALS (Lou Gehrig's Disease) is a mostly sporadic, adult-onset, fatal degenerative disease of motor neurons



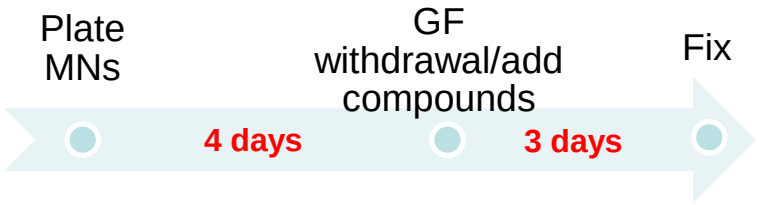
Characteristics of ALS

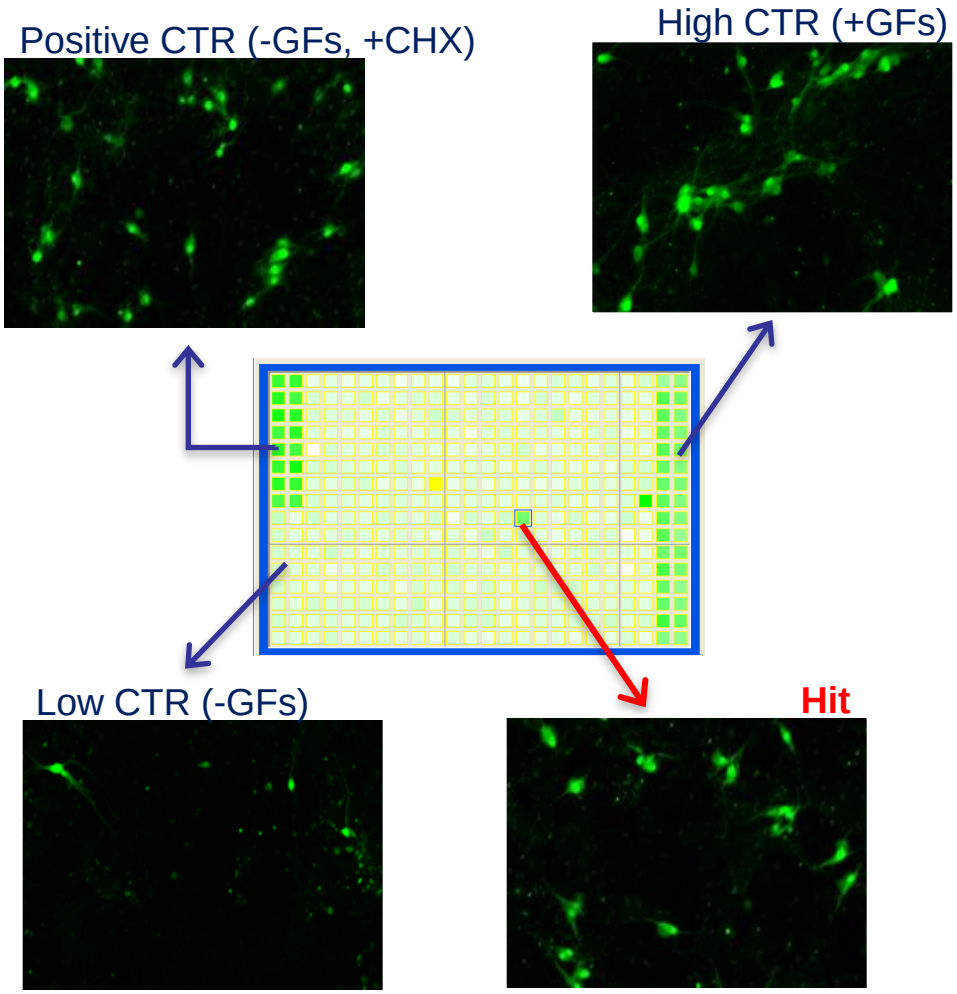
- About 90% of cases of ALS have no clear genetic association.
  - The most frequent genetic cause is a gain of function mutation in superoxide dismutase.
- The late onset and sporadic nature of the disease make it difficult to model.
  - Is it even possible to recapitulate in culture a disease that takes decades to become apparent in humans and months in the most severe mouse model?
  - Which environmental factors contribute to the onset of this disease? Can the disease be modeled without knowing their identities?

A motor neuron screen for ALS therapeutics

- We produced large numbers of motor neurons from wildtype mouse ES cells and from ES cells carrying a mutation (G93A) in superoxide dismutase found in some patients with ALS (ES cells kindly provided by Kevin Eggan).
- We carried out survival screens using a focused, annotated, library containing thousands of small molecules.

Growth factor-withdrawal screen





Summary

- We identified many small molecules that extended the life of wildtype MNs, G93A (ALS) motor neurons or both.
  - These studies could not have been done using other approaches.
- Some act directly on the motor neurons; others on glial cells in the culture.
- They are now being tested on human ALS motor neurons produced with iPS cells (in collaboration with Kevin Eggan at Harvard).
  - Genetic and sporadic forms of the disease

Spinal Muscular Atrophy: an often fatal, childhood onset, genetic disease caused by low expression of the *Survival of Motor Neuron (SMN)* gene

Infant with typical bell-shaped thorax, frog-leg posture, and "jug-handle" position of upper limbs

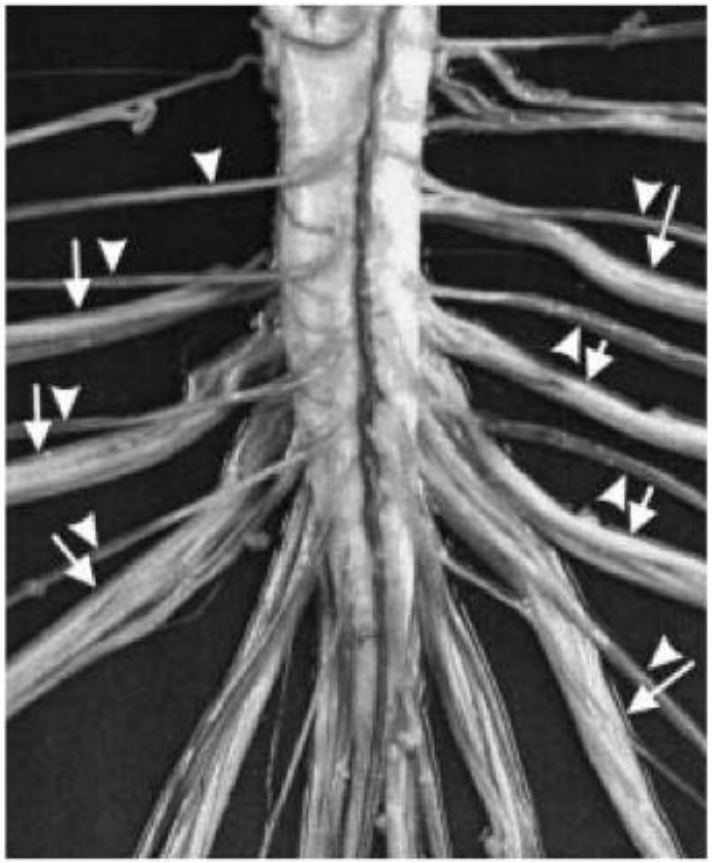


FIGURE 8–3

Gross appearance of caudal spinal cord in a case of spinal muscular atrophy type II. Note the very thin ventral roots (arrowheads), *in contrast to the dorsal roots at the same level* (arrows).

From Neuromuscular Disorders of Infancy, Childhood, and Adolescence: A Clinician's Approach, Jones, De Vivo, and Darras, "Spinal Muscular Atrophies,"145-166, 2003.

Spinal Muscular Atrophy

- Since this is a purely genetic disease, it should be simpler to study than ALS.
- **Also it is already known that there is a direct and strong correlation between the amount of SMN and the length of survival in children and in mouse models**.
- A drug that elevates SMN levels should be therapeutic.

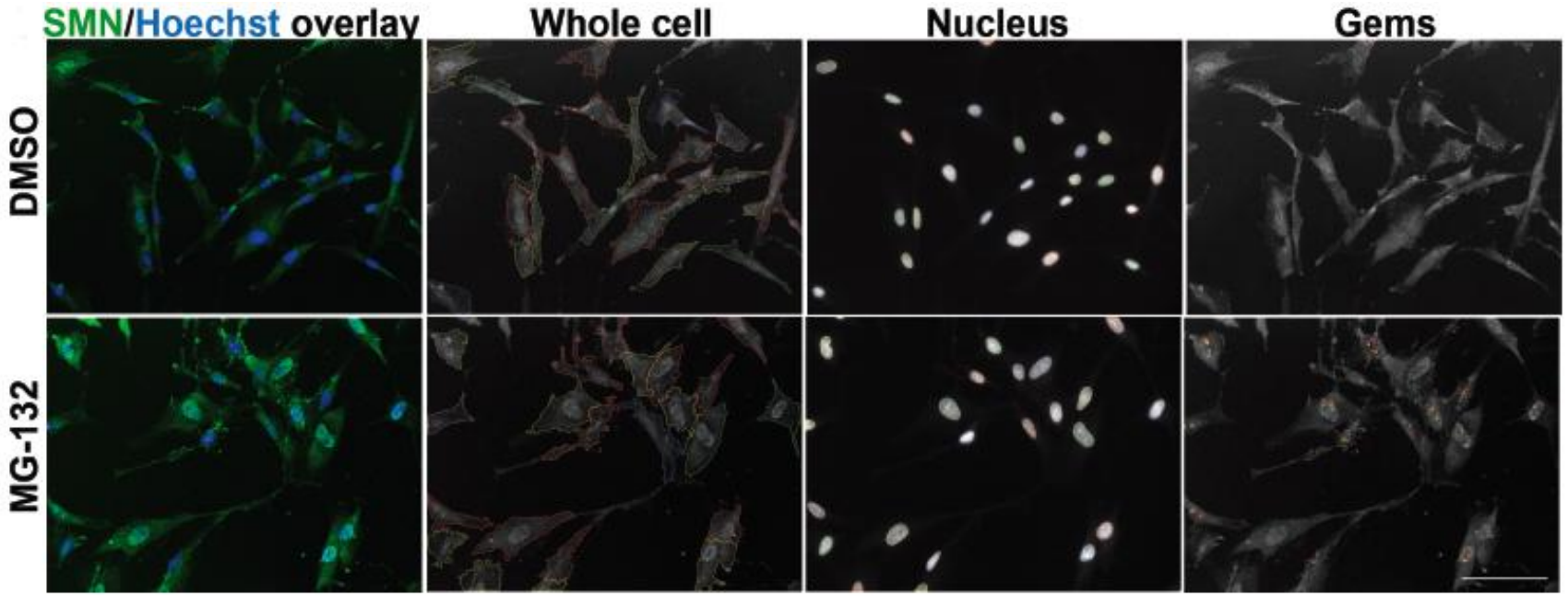
Spinal Muscular Atrophy (SMA)

- How to find effective therapeutics?
  - Previous screens used patient-derived fibroblasts.
  - Can more effective or more selective therapeutics be discovered by conducting motor neuron screens?
- Would a drug that only elevates SMN in motor neurons be therapeutic?
  - Probably not.

Therapeutic Screens for SMA

- We have carried out a series of screens – using chemical diversity libraries and sets of annotated collections -- on both human patient fibroblasts and on mouse motor neurons.
- There are very few hits shared between the two different types of cells.
  - Does this mean that you can only find compounds that work in motor neurons by screening in motor neurons?

An image-based screen in fibroblasts



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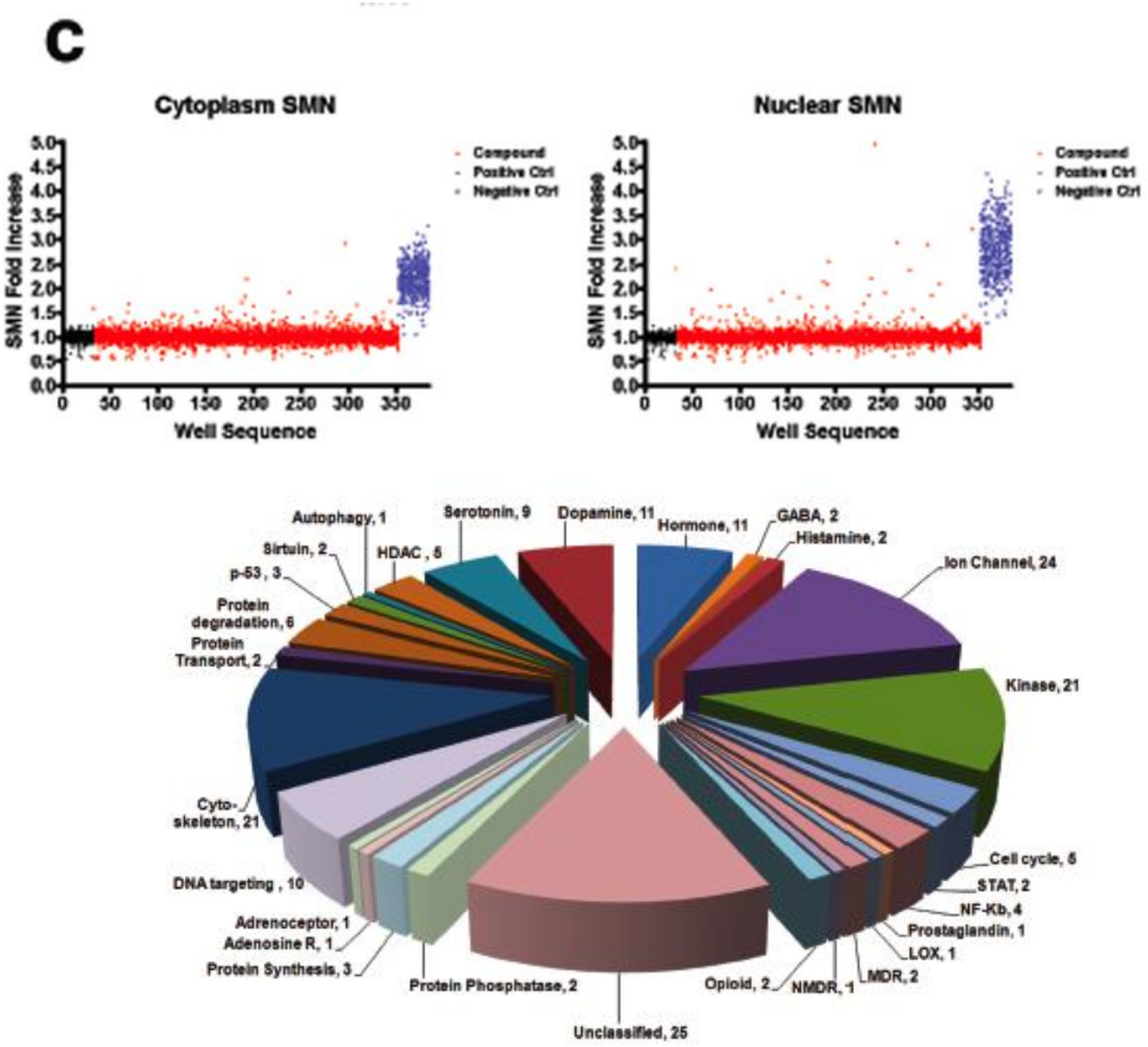
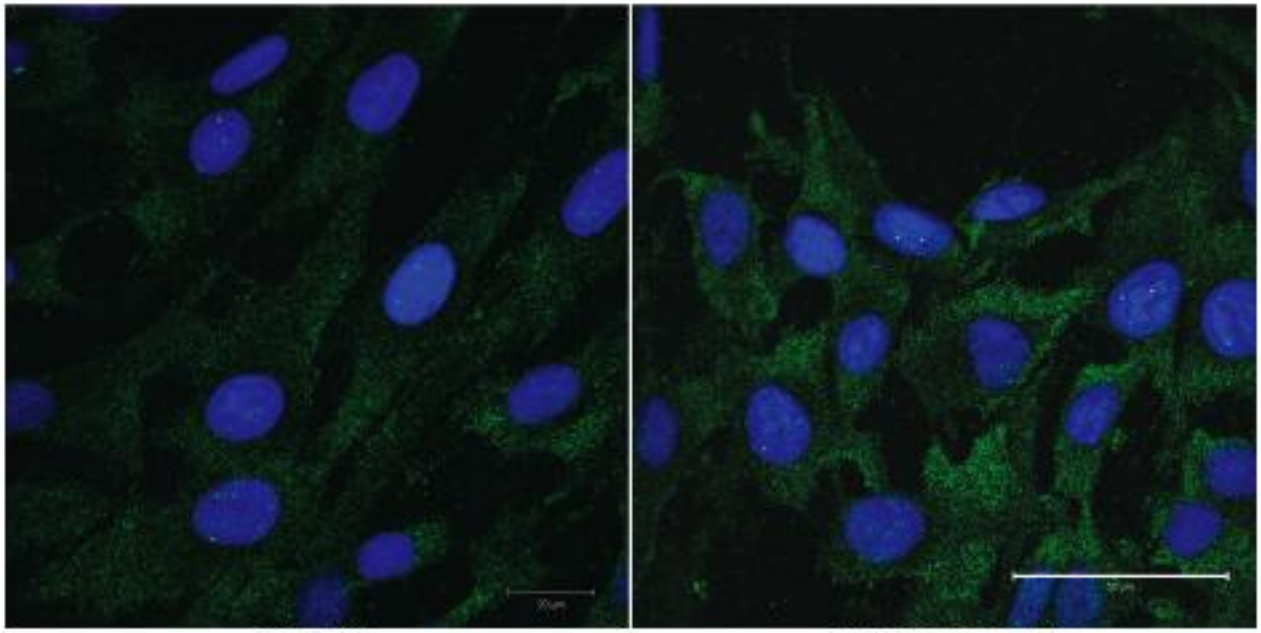
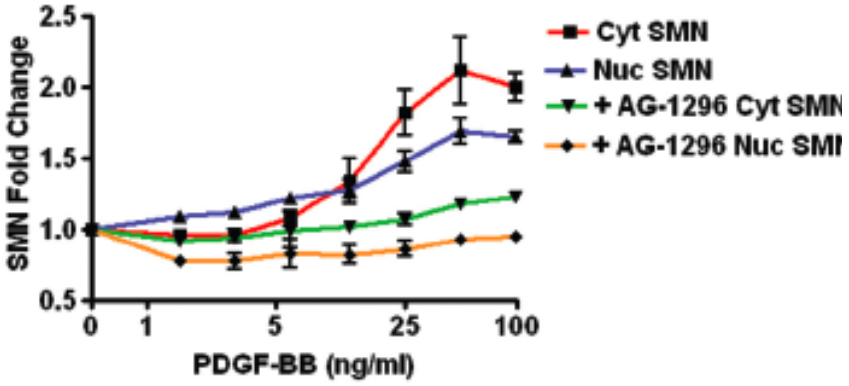
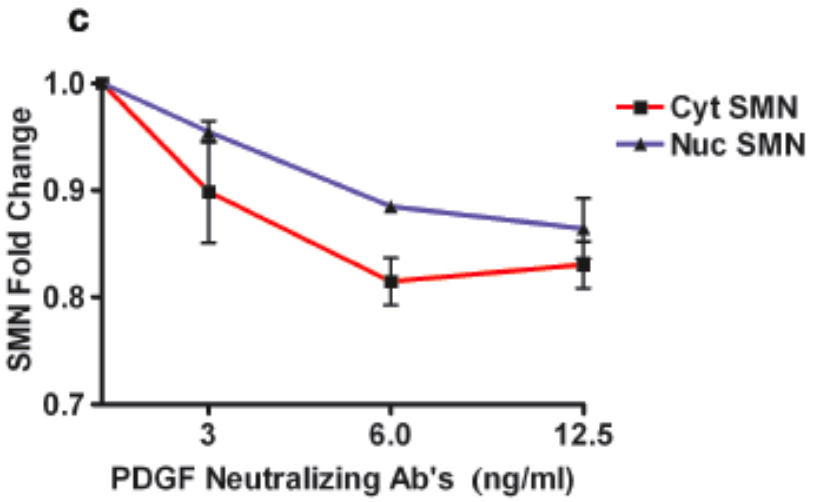


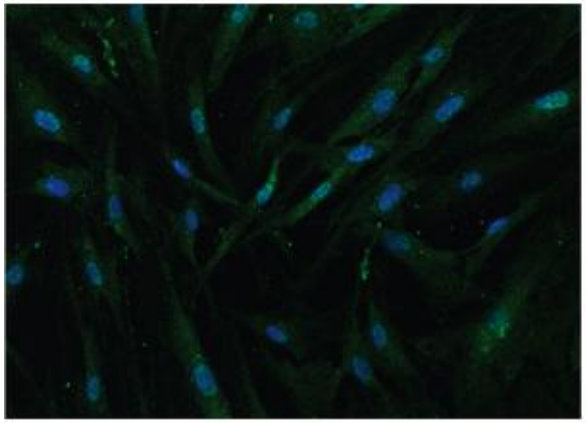
Hit Class: RTK Ligands



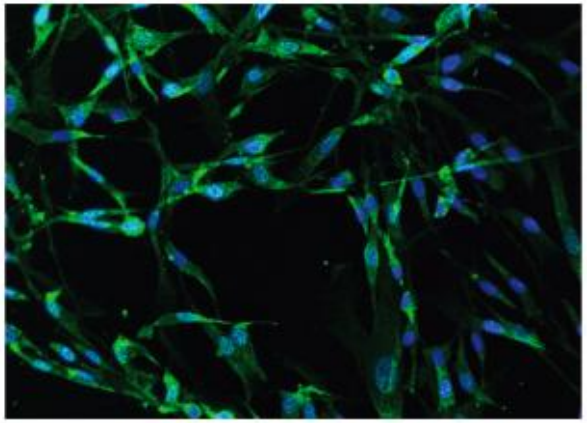




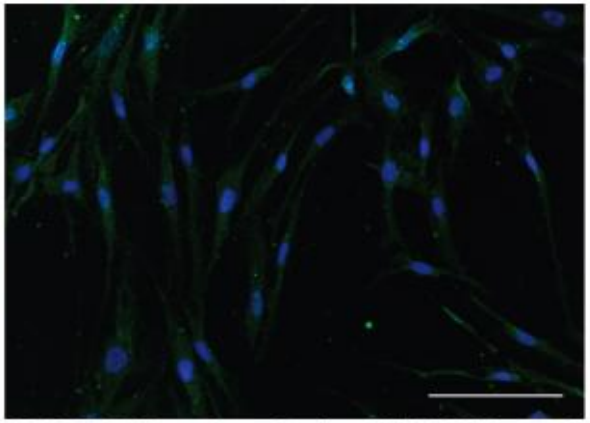
RTK Ligands Regulate SMN via activation of PI3-K/AKTa and by inhibition of the downstream kinase GSK-3β



CTRL, 72h

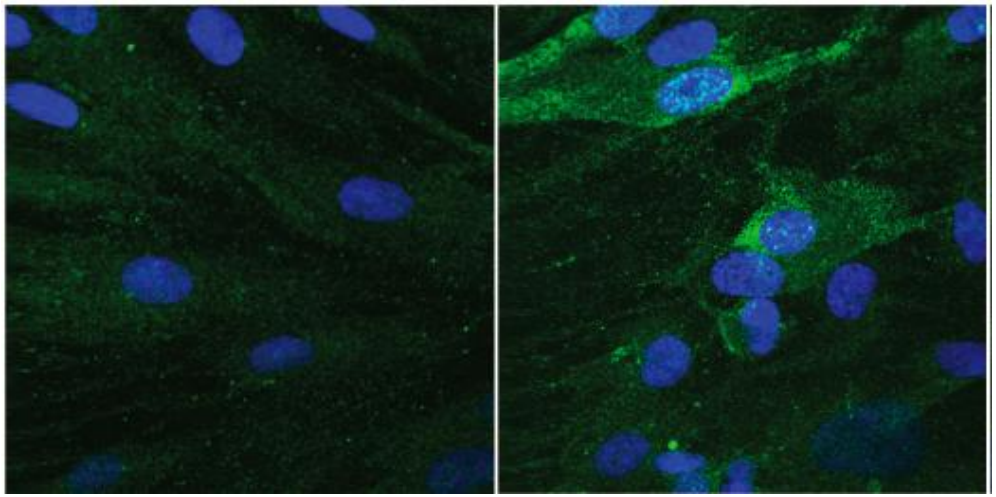


PDGF-BB, 50 ng/ml



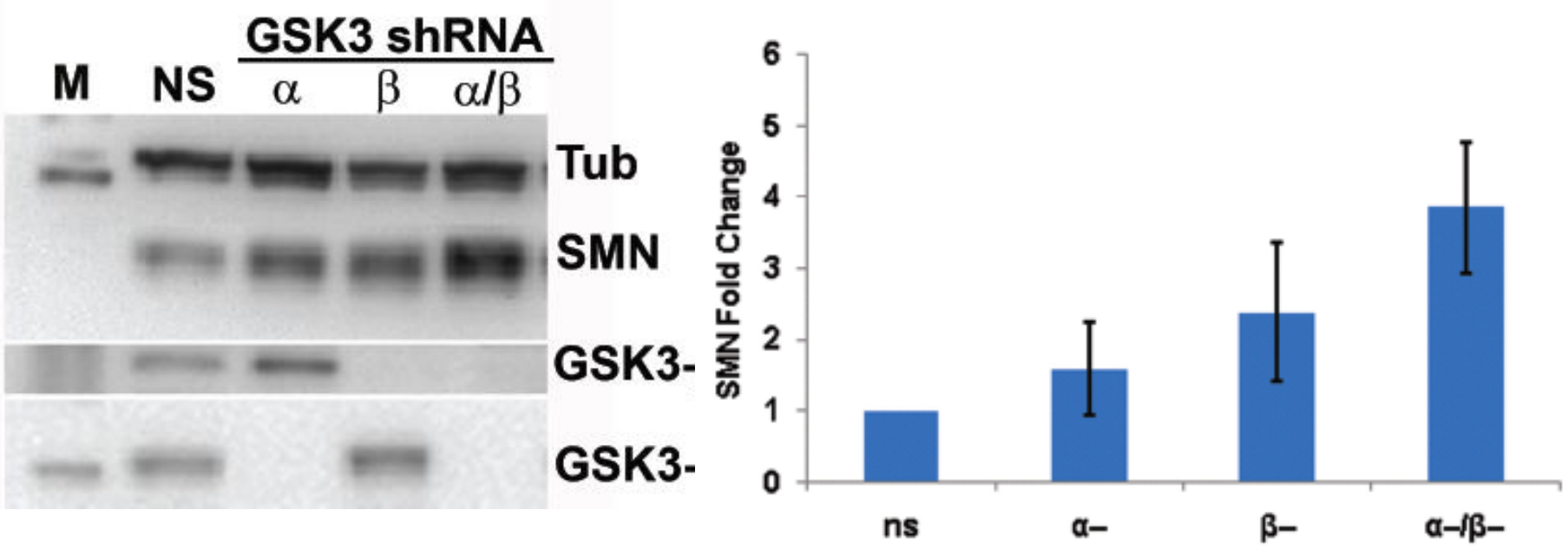
PDGF-BB, 50 ng/ml + LY 29004, 50uM

Control



GSK-3 Inh

GSK-3 knockdowns cause a large increase in SMN levels in fibroblasts.



How does this work: GSK-3 phosphorylates SMN and targets it for degradation.

Can GSK-3 inhibition increase SMN in motor neurons?

Can this produce any functional improvement in motor neuron properties?

ES Cells to Motor Neurons

- ES cells were isolated from a mouse model of type 1 Spinal Muscular Atrophy (severe disease).
  - Mice die at about 4 days of age.
- These ES cells have low levels of SMN and proliferate and differentiate relatively poorly.
- The motor neurons tend to die rapidly (within 24 hrs of plating).

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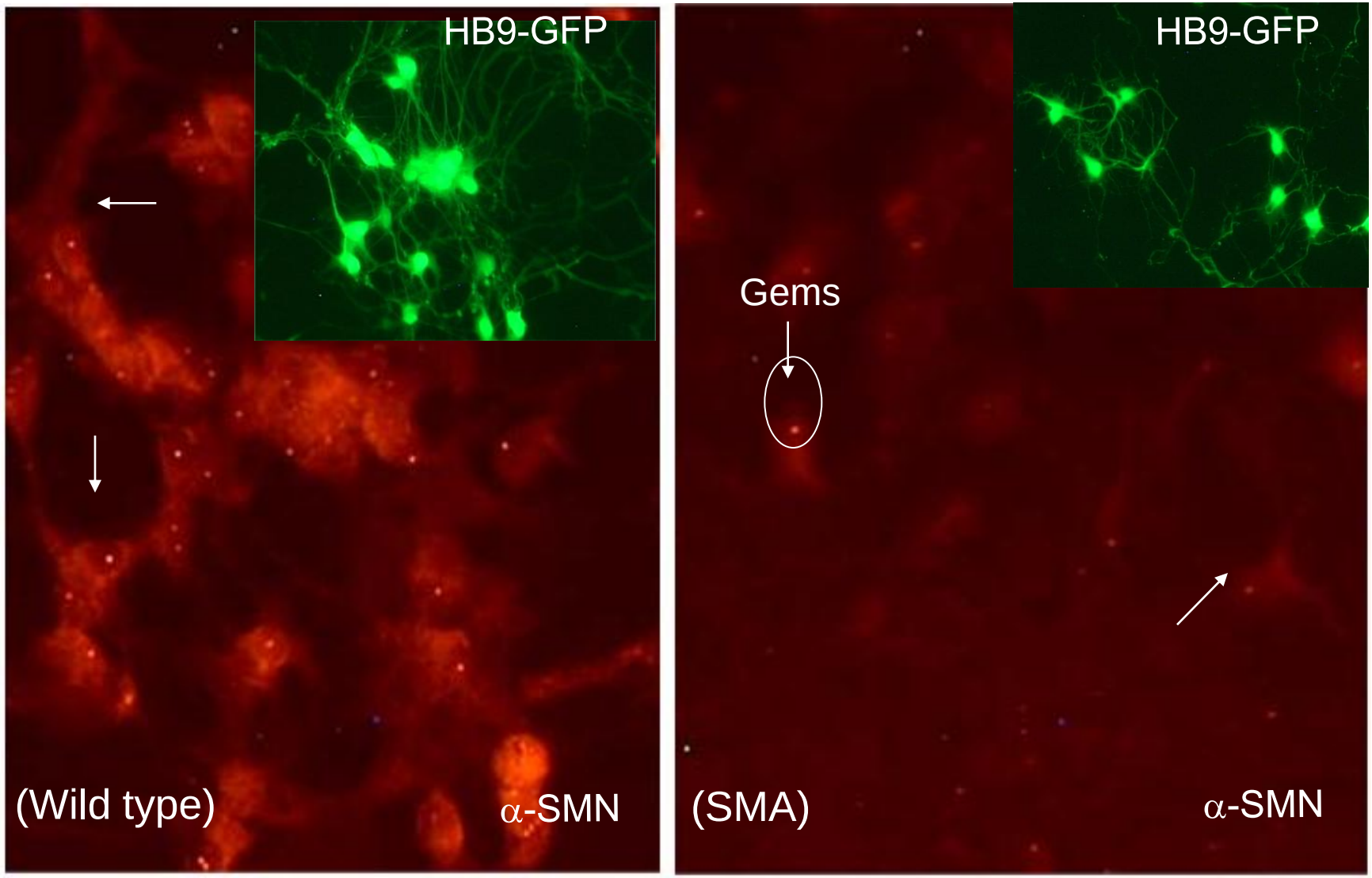
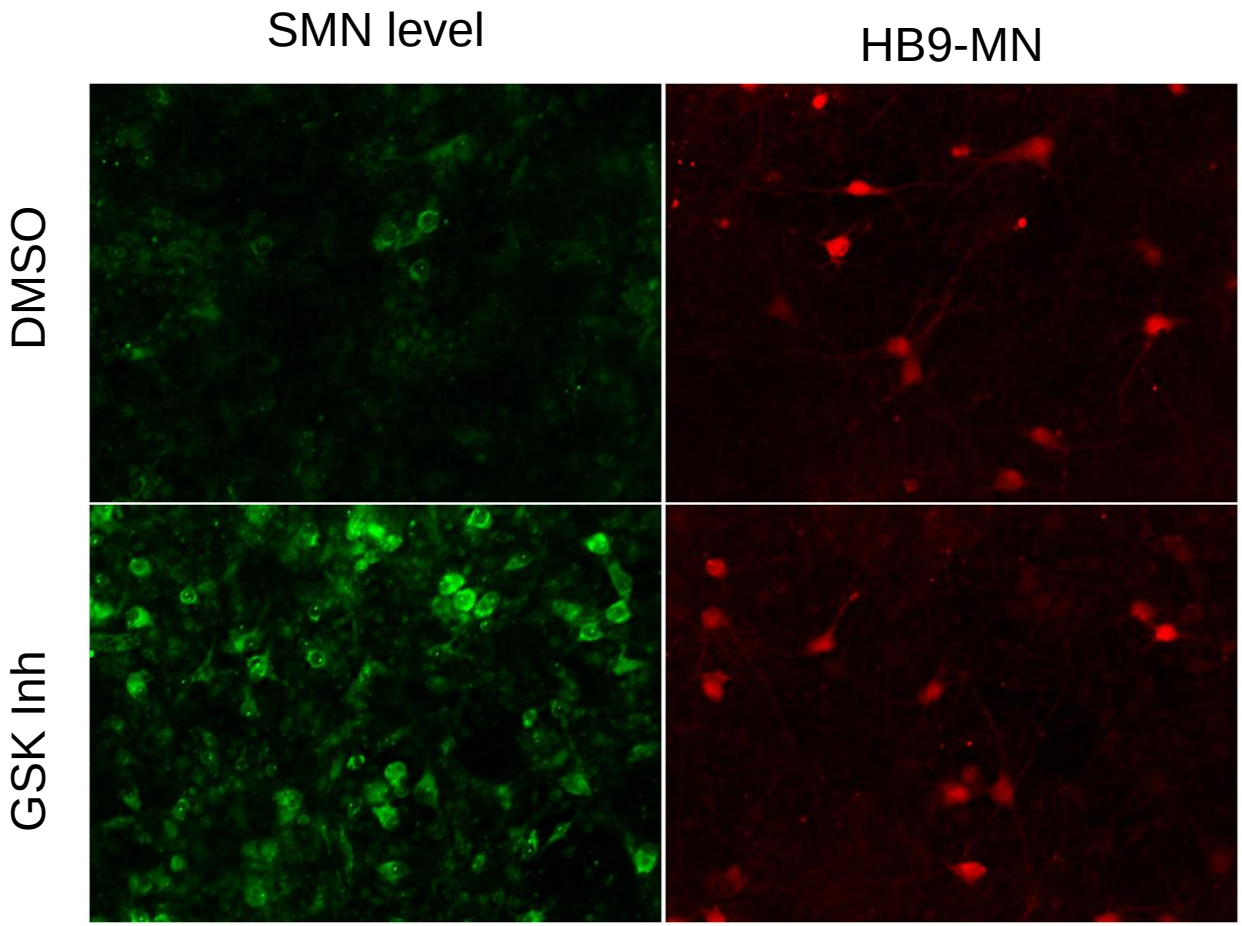
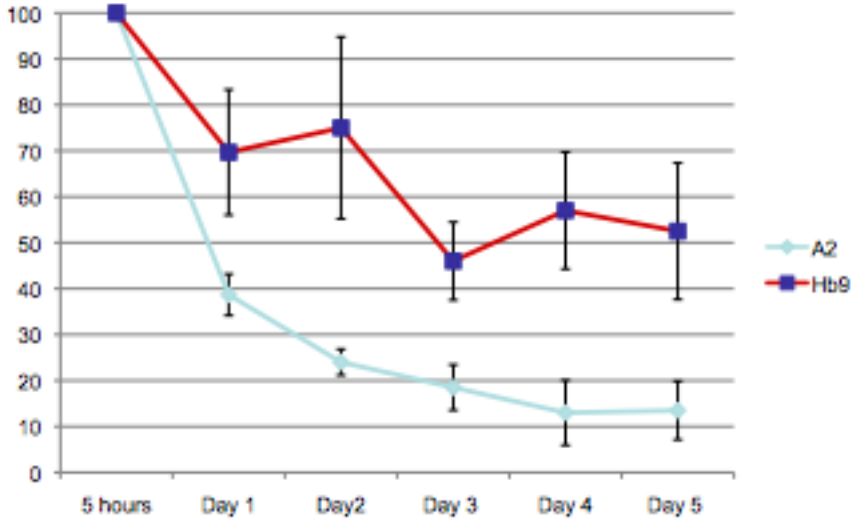
SMA Motor Neuron Screen

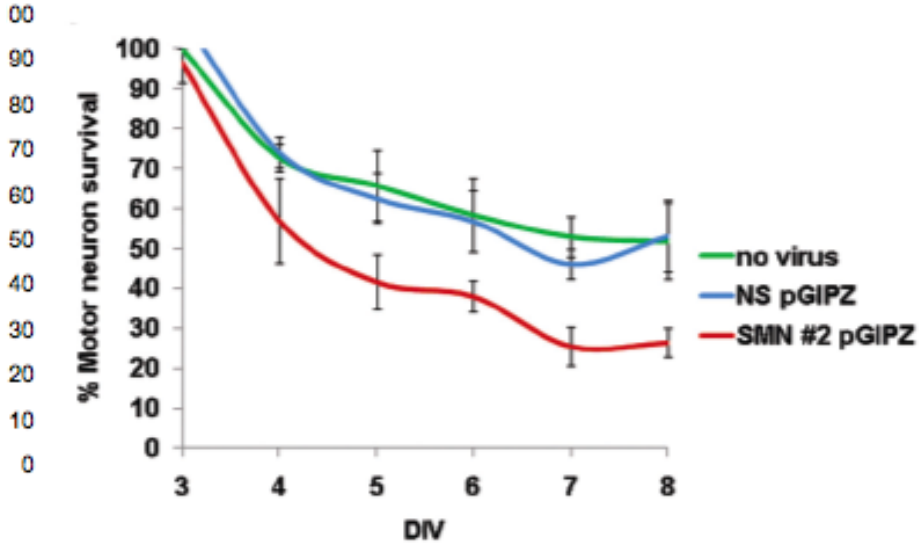


GSK-3 inhibitors elevate Smn Levels in motor neurons.

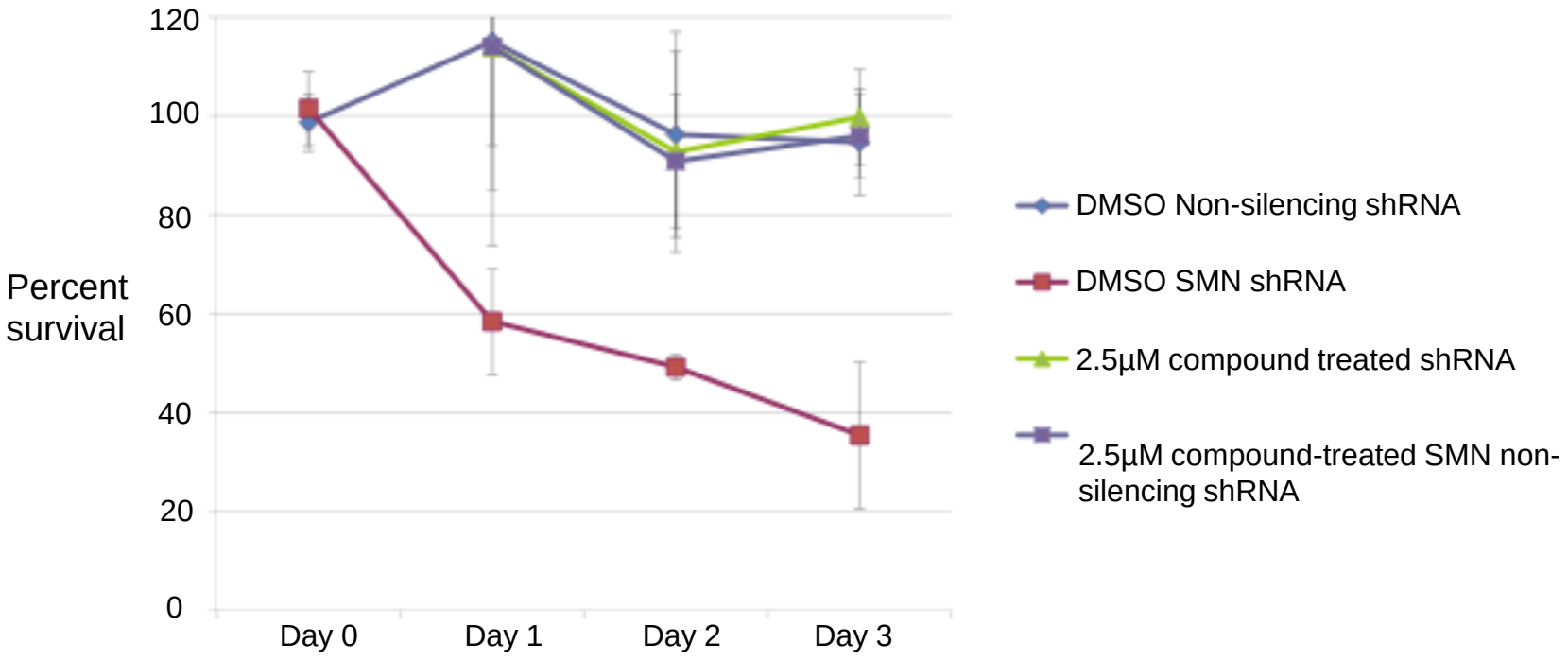


Can these inhibitors exert any positive effects on motor neurons?

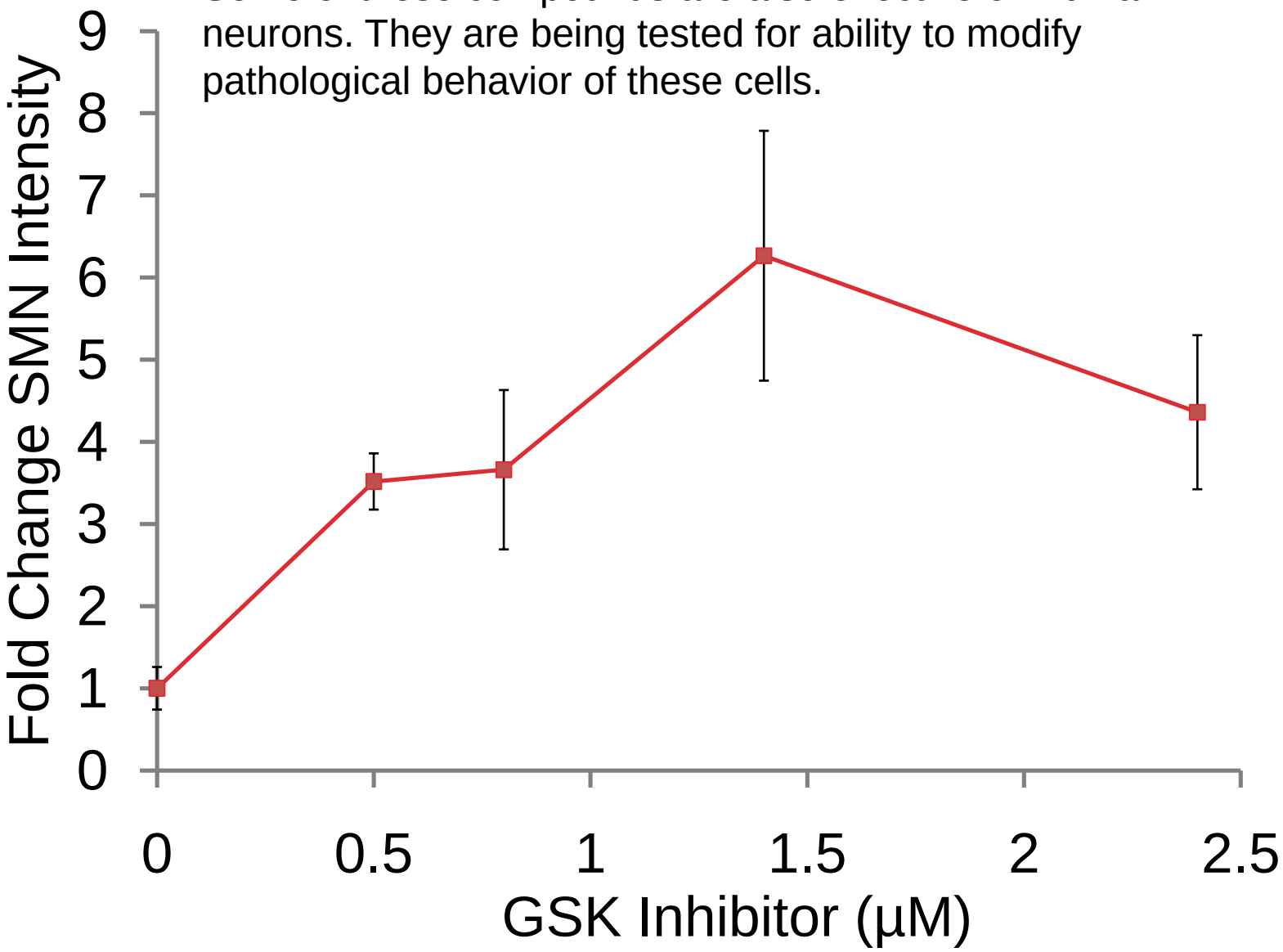




Activating RTK signaling rescues motor neuron survival after SMN knockdown



Some of these compounds are also effective on human neurons. They are being tested for ability to modify pathological behavior of these cells.

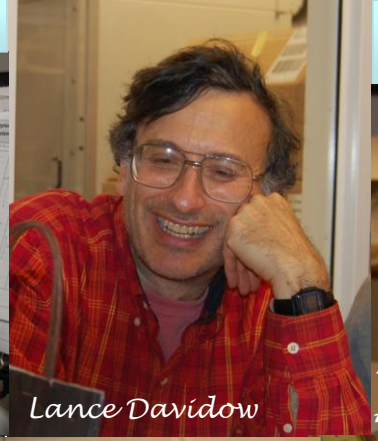


Summary

- Stem cell based approaches may help us understand more about the pathological basis of disease and help us set up new kinds of assays.
- Key features of this approach may include:
  - Reproducing human disease processes and testing potential therapeutics on human disease-relevant cells.
  - Pretesting drugs on patient cells for safety and efficacy prior to testing them in the clinic.

The Rubin Lab


Katie Krumholz, Amanda Wagner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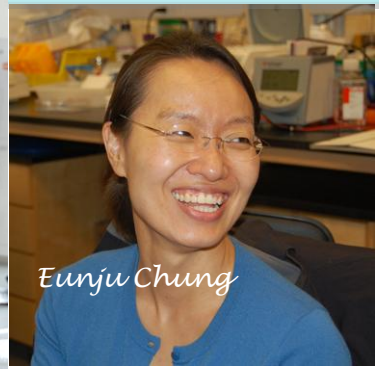
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