RNA-targeted Therapeutics for ALS

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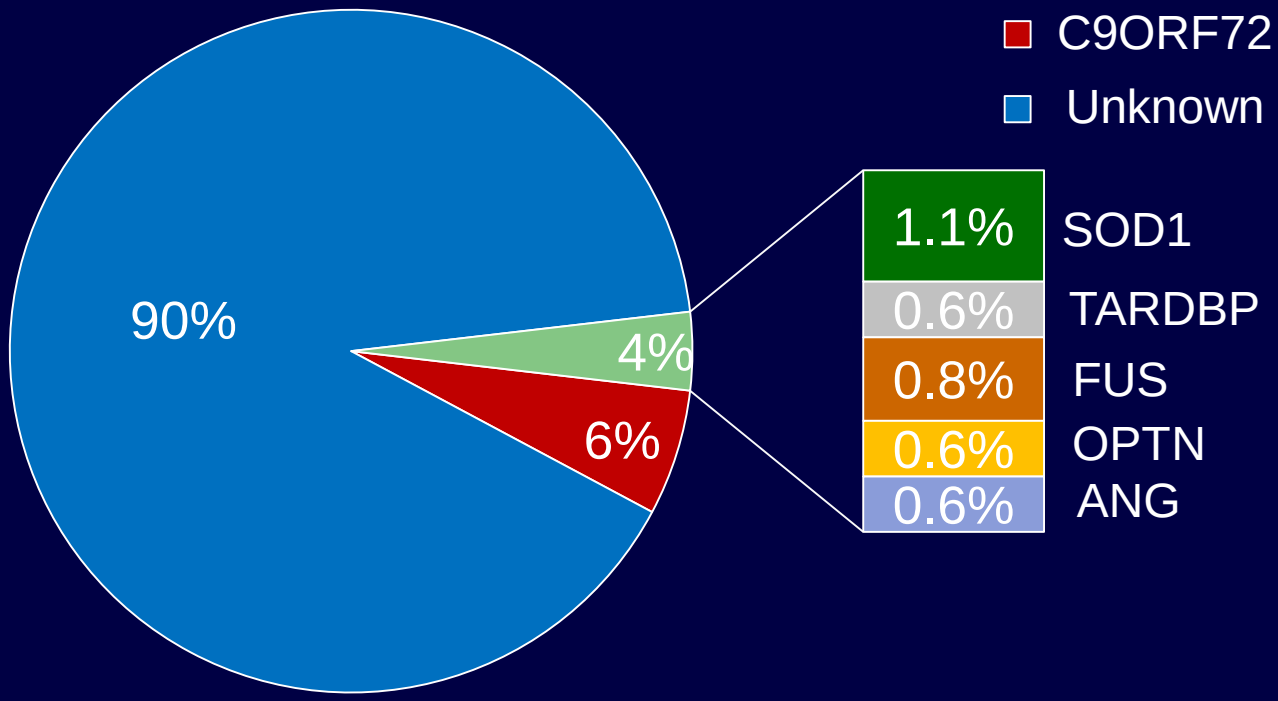
Disclosures

- **Ionis Pharmaceuticals**
  - –Licensing agreement, material support
- **Biogen Idec**
  - –Advisory board, support for clinical studies
- **Cytokinetics**
  - –Consultant
- **C2N**
  - –Licensing agreement
- **Regulus Therapeutics**
  - – antisense oligonucleotides
- **Current industry clinical trials**
  - –Biogen, Orion, Amylyx

Amyotrophic Lateral Sclerosis

- Motor neuron disease
- Causes progressive weakness, death
- No current adequate therapies

Genetic landscape of ALS



Singleton ("Sporadic")

Rationale for Lowering SOD1 as a Therapy for ALS

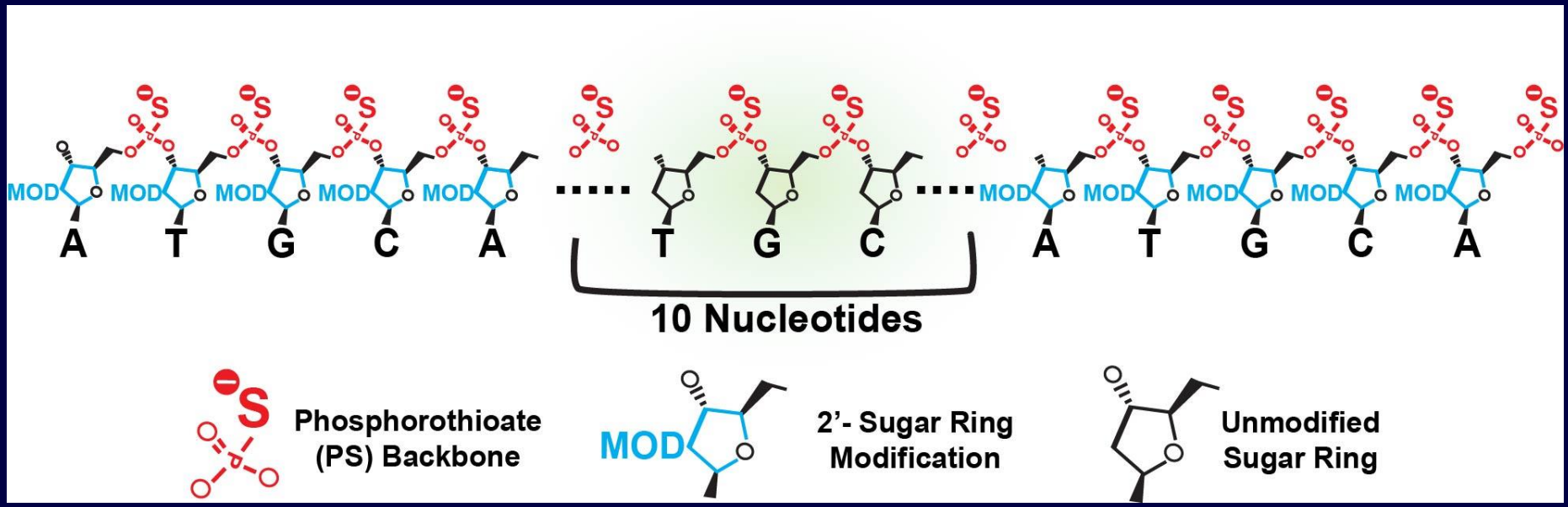
- Mutations in superoxide dismutase 1 (SOD1) cause 20% of familial ALS
- Mutant SOD1 acquires new toxic property
- Decreasing SOD1 likely beneficial
- Decreasing SOD1 likely safe

Methods to Clear/Improve Toxic Proteins

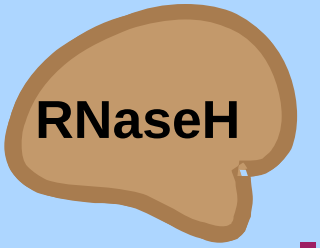
- Small molecules
- Use the immune system (vaccination or passive immunization)
- Gene editing
- RNA interference
- Antisense oligonucleotides

Incredible tool box for therapeutic development

Antisense Oligonucleotides (ASO)



DeVos and Miller, 2013

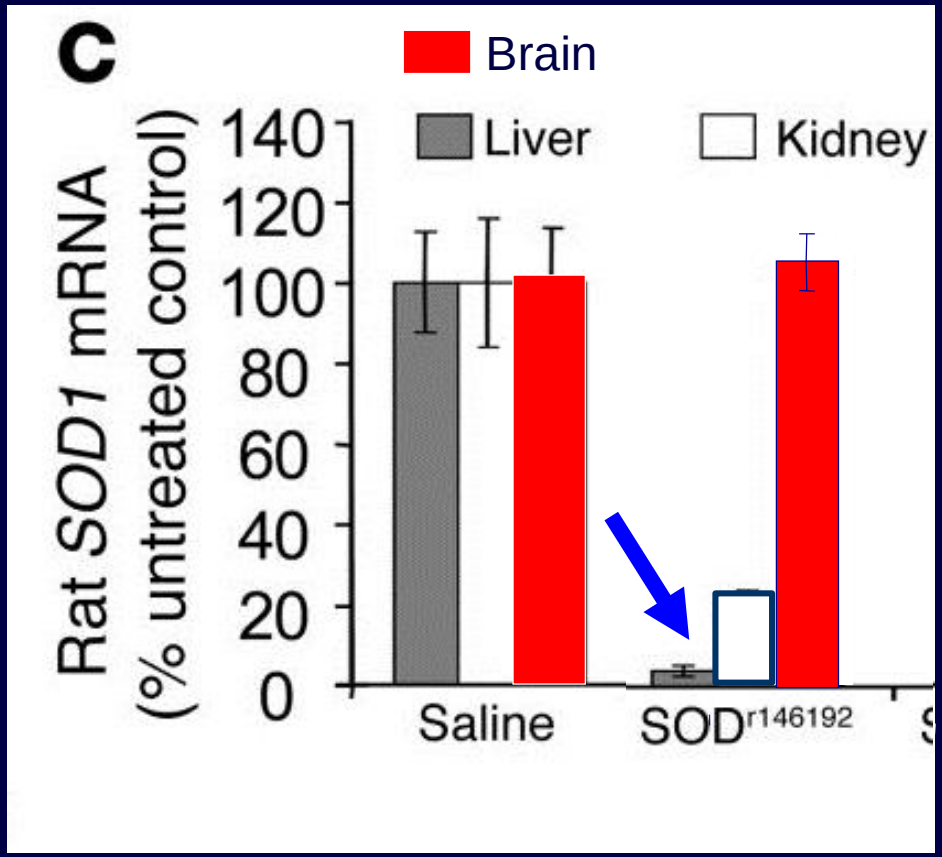


Target mRNA

RNaseH recruitment

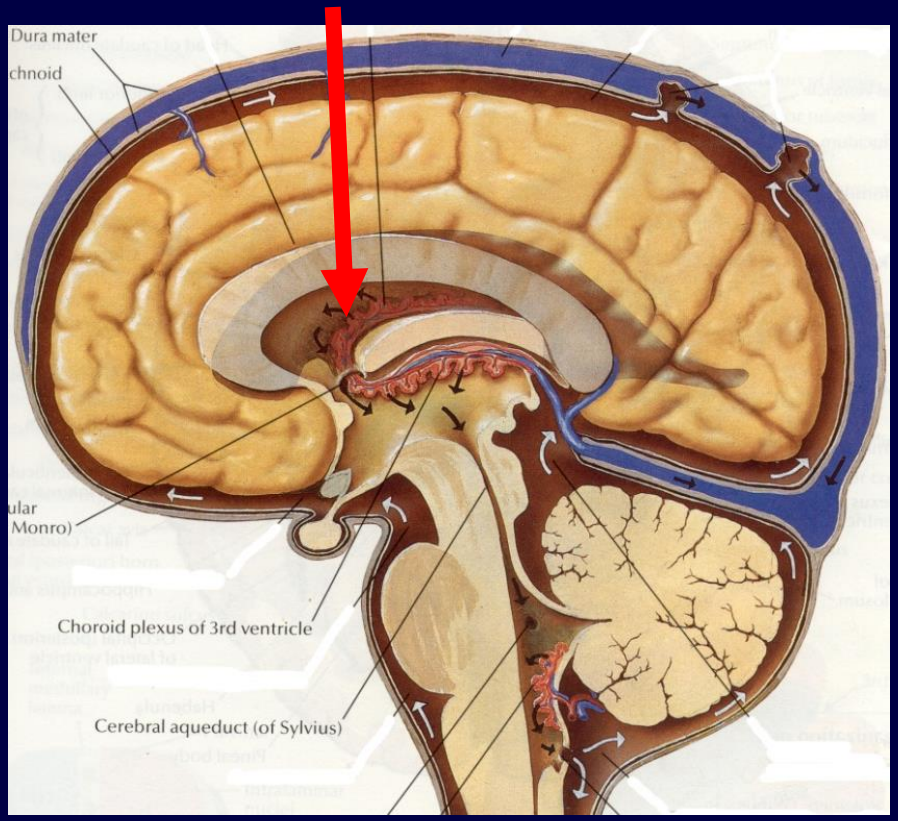
Schoch and Miller, Neuron 2017

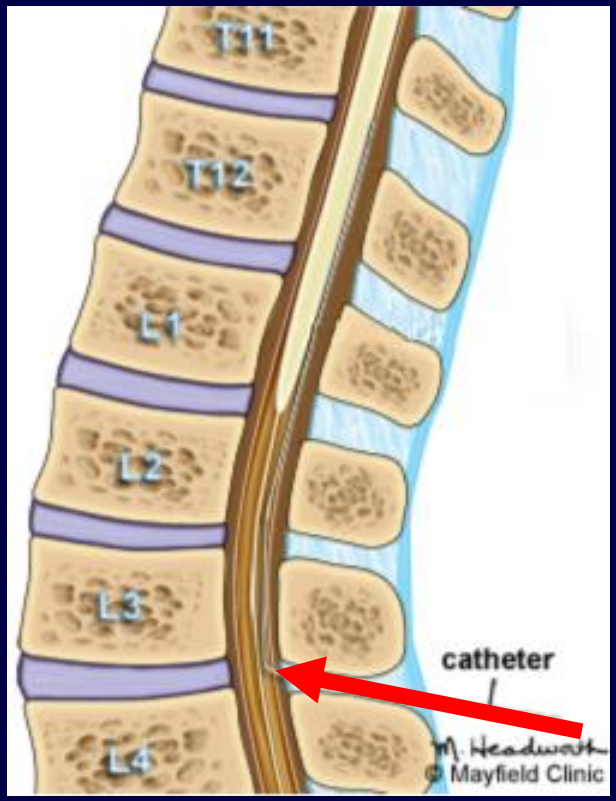
Intraperitoneal Administration Decreases SOD1 mRNA in Liver and Kidney, Not in Brain



Smith, Miller et al. JCI 2006

CSF Delivery of Antisense Oligos

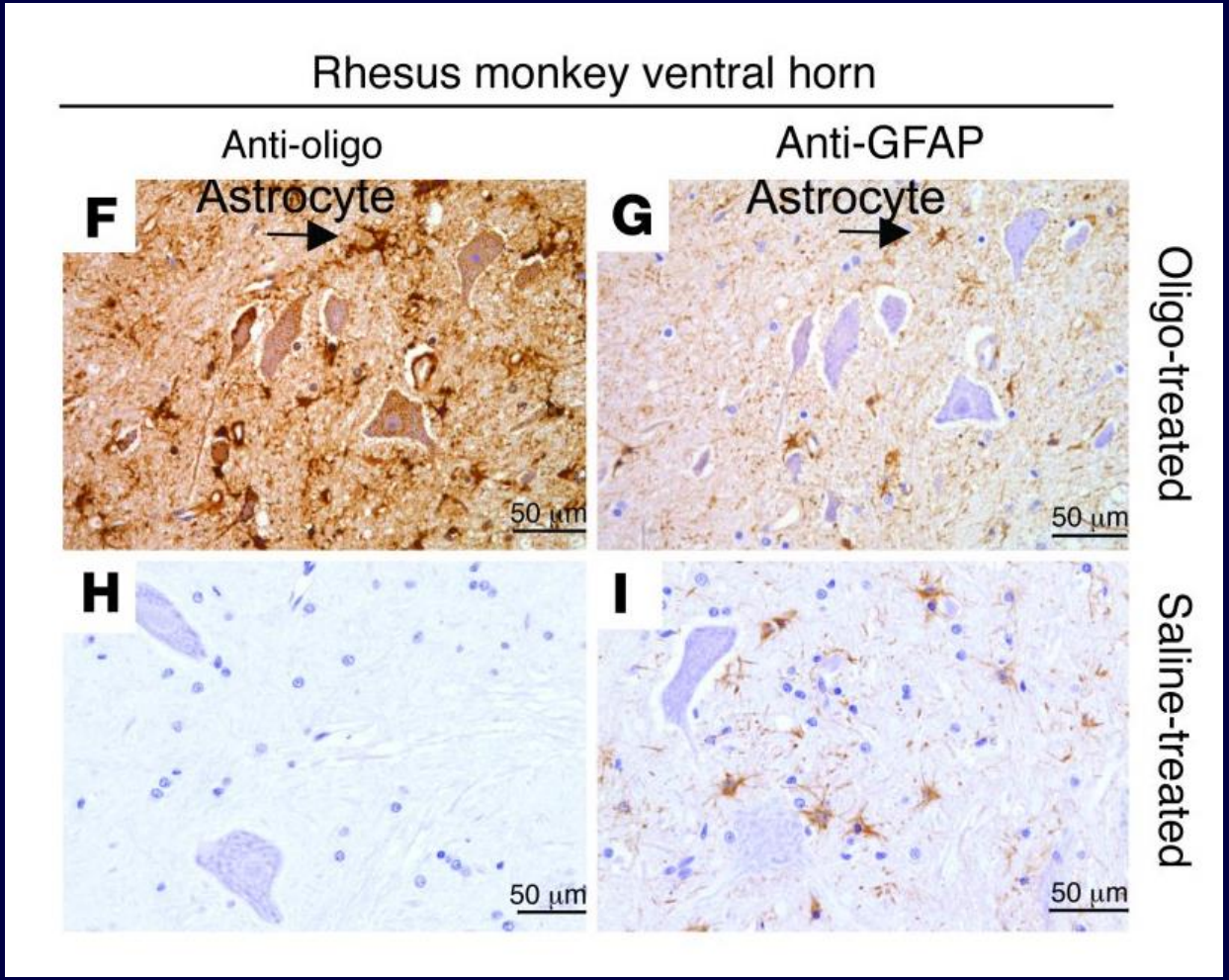




Atlas of Human Anatomy
Frank Netter
1989

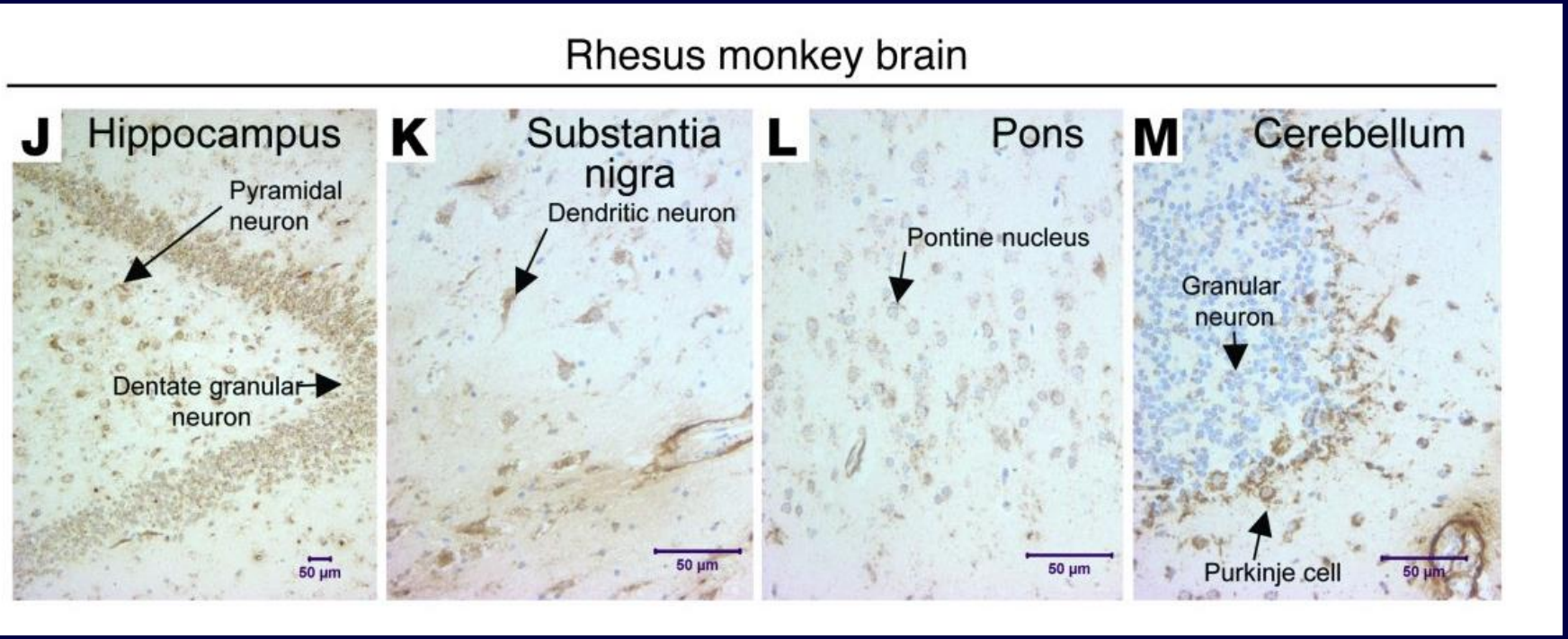
Mayfield Clinic
Cincinnati, Ohio

Intraventricular Administration Delivers Antisense Oligonucleotides Widely



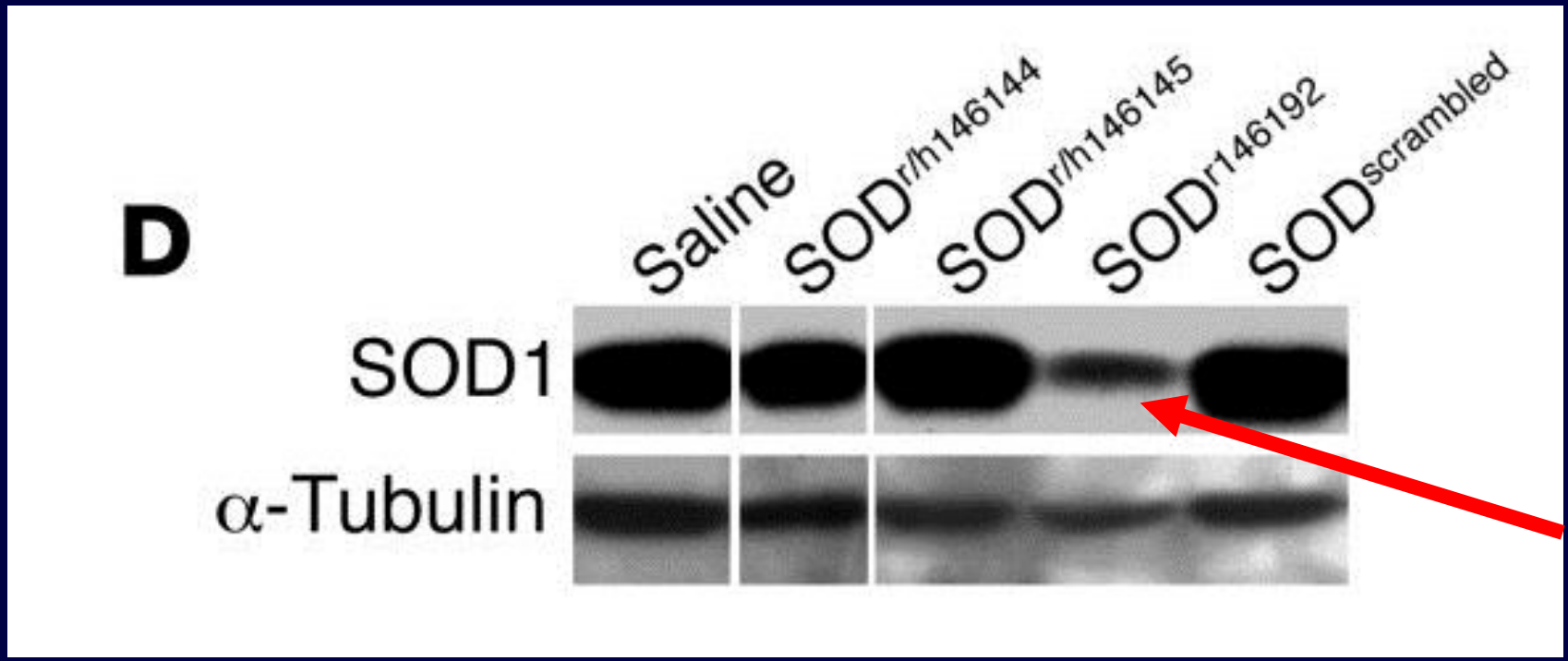
Smith, Miller et al. JCI 2006

Intraventricular Administration Delivers Antisense Oligonucleotides Widely



Smith, Miller et al. JCI 2006

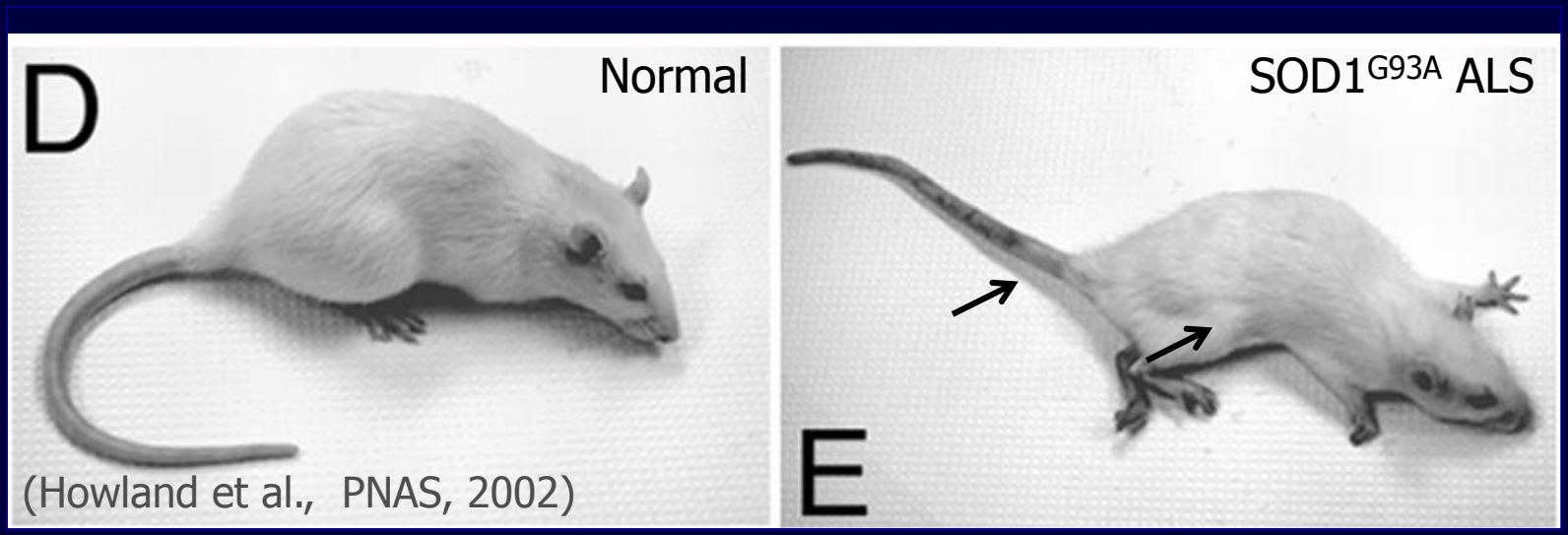
SOD1 ASO Lowers SOD1 Protein in Spinal Cord



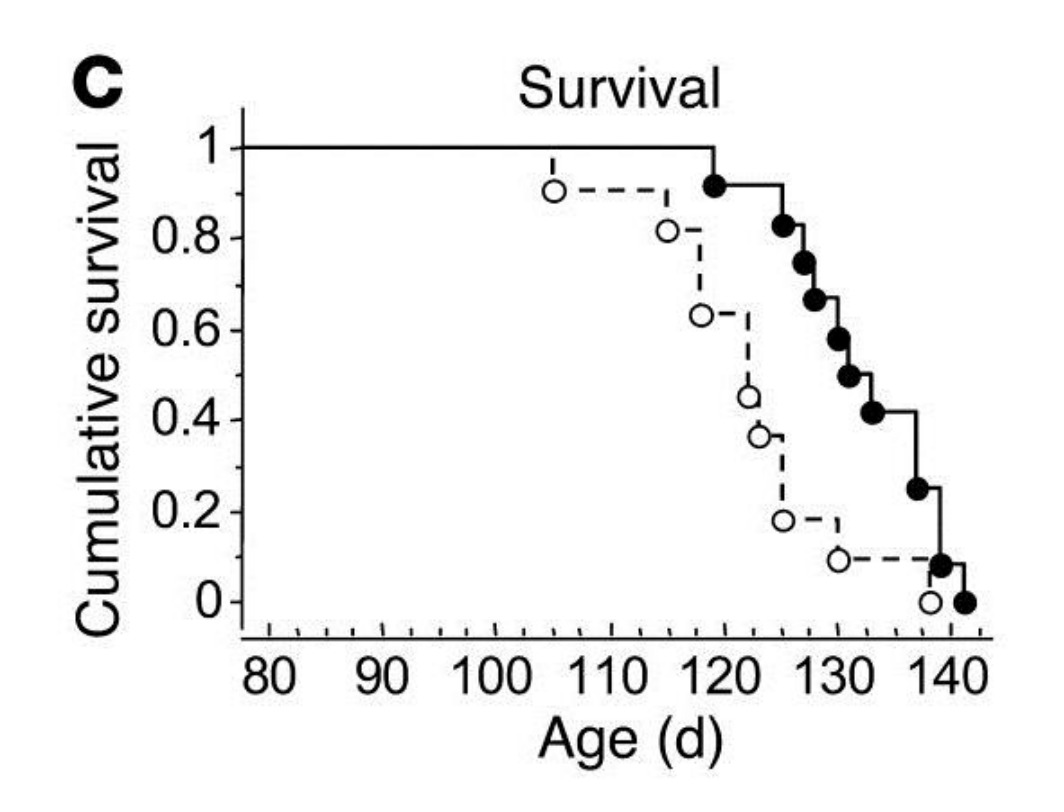
Smith, Miller et al. JCI 2006

Mutant SOD1 Causes ALS-like phenotype in Rodents

- Mice, rats develop weakness and atrophy
- $SOD1^{G93A}$ Rat



SOD1 Antisense Oligo Extends Survival



Doubling of survival *after* onset

Smith, Miller et al. JCI 2006

Conclusions

- CSF Delivery Distributes throughout brain and spinal cord
- Target mRNA and Protein reduced
- Beneficial effects in disease model

Smith, Miller et al. JCI 2006

First-in-man SOD1 ASO Study

An antisense oligonucleotide against *SOD1* delivered intrathecally for patients with *SOD1* familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study

Timothy M Miller, Alan Pestronk, William David, Jeffrey Rothstein, Ericka Simpson, Stanley H Appel, Patricia L Andres, Katy Mahoney, Peggy Allred, Katie Alexander, Lyle W Ostrow, David Schoenfeld, Eric A Macklin, Daniel A Norris, Georgios Manousakis, Matthew Crisp, Richard Smith, C Frank Bennett, Kathie M Bishop, Merit E Cudkowicz

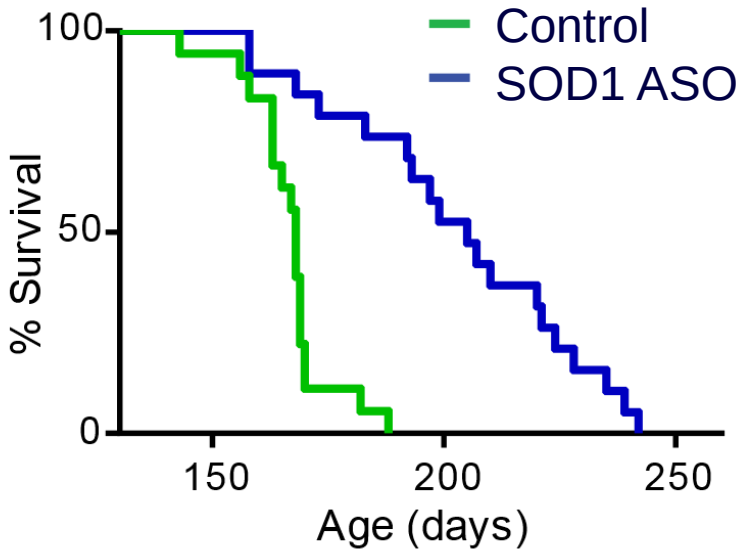
Timothy Miller, Alan Pestronk, Bill David, Jeff Rothstein, Erika Simpson, Stan Appel, Pat Andres, Katy Mahoney, Peggy Allred, Katie Alexander, Lyle Ostrow, David Schoenfeld, Eric Macklin, Dan Norris, George Manousakis, Matt Crisp, Richard Smith, Frank Bennett, Kathie Bishop, Merit Cudkowicz

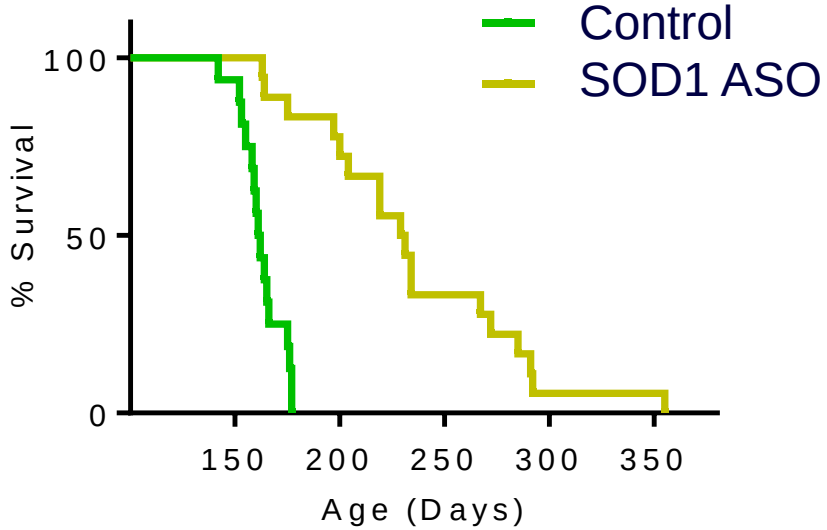
Lancet Neurology, 2013

Conclusions First SOD1 Trial

- Outstanding safety and important pharmacokinetics
- Good result, but decided to develop better SOD1 ASO
- New ASO, more potent in vitro and in vivo (data not shown)

SOD1 ASO Markedly Extends Survival





SOD1 G93A Mice Survival
Control: 168
SOD1 ASO: 205

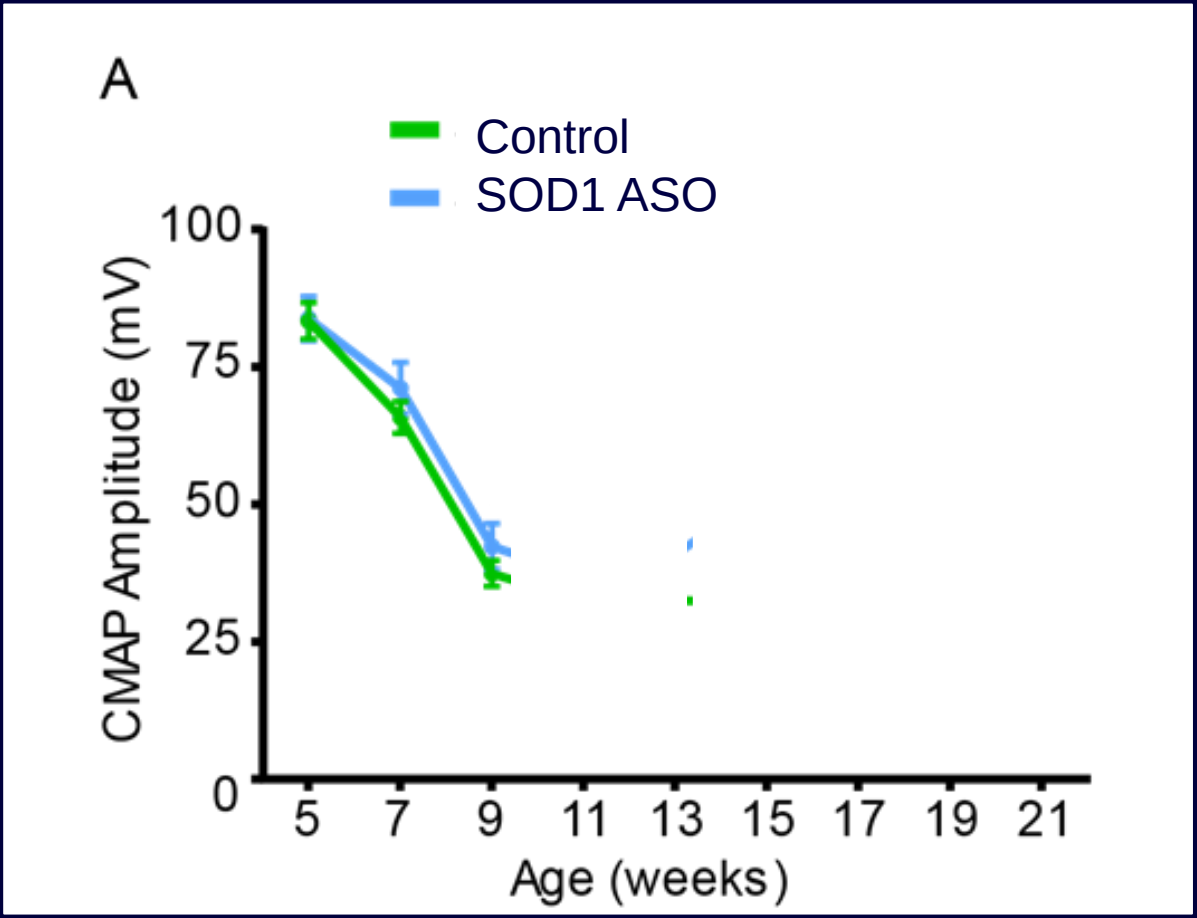
37 Days!

SOD1 G93A Rats Survival
Control: 166
SOD1 ASO: 230

64 Days!

Modified from McCampbell et al. JCI 2018

SOD1 ASO Reverses Decline in CMAP



ICV = intracerebroventricular

Modified from McCampbell et al. JCI 2018

Conclusions

SOD1 ASOs

- Distribute throughout brain & spinal cord
- Lower SOD1 mRNA and protein in rodents
- Markedly extends survival in rodent models
- Reverse decline in CMAP
- Lower SOD1 mRNA and protein in non-human primate
- Currently being tested in human clinical trial
- Similar strategy to be used for C9ORF72
- Similar strategy for multiple targets

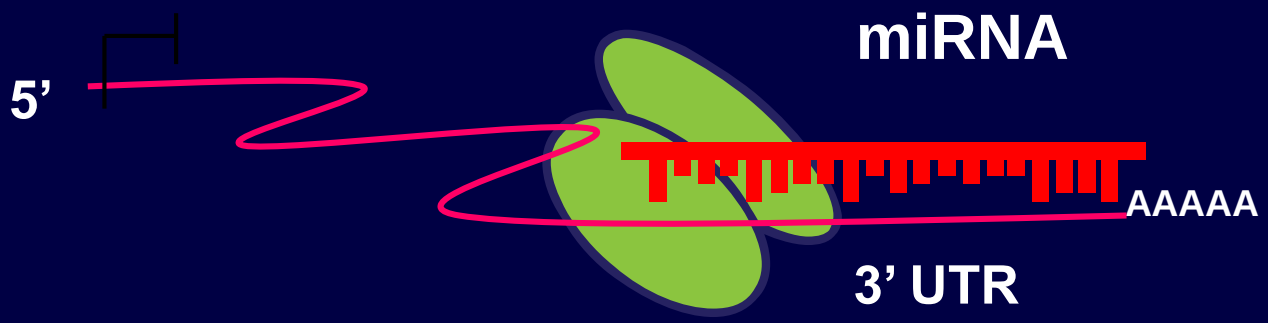
Reversal in Animal Models

- CMAP in SOD1 animal models
  - McCampbell et al. JCI 2018
- Behavioral function in Huntington's models
  - Kordasiewicz et al. Neuron 2012
- Pathology in Tau models
  - Devos et al. Science Translational Medicine, 2017
- Physiology in Ataxin 2 models
  - Scoles et al. Nature 2017

Success in Targeted Clinical Trials

- SMN for Spinal Muscular Atrophy
  - Approved by FDA
- SMN viral therapy for Spinal Muscular Atrophy
  - Approved by FDA
- TTR for Amyloid Neuropathy
  - ASO and siRNA approved by FDA
- Huntingtin for Huntington's Disease
  - NEJM 2019
- SOD1 Antisense
  - Recent press release AAN 2019
- C9ORF72 Antisense

miRNAs



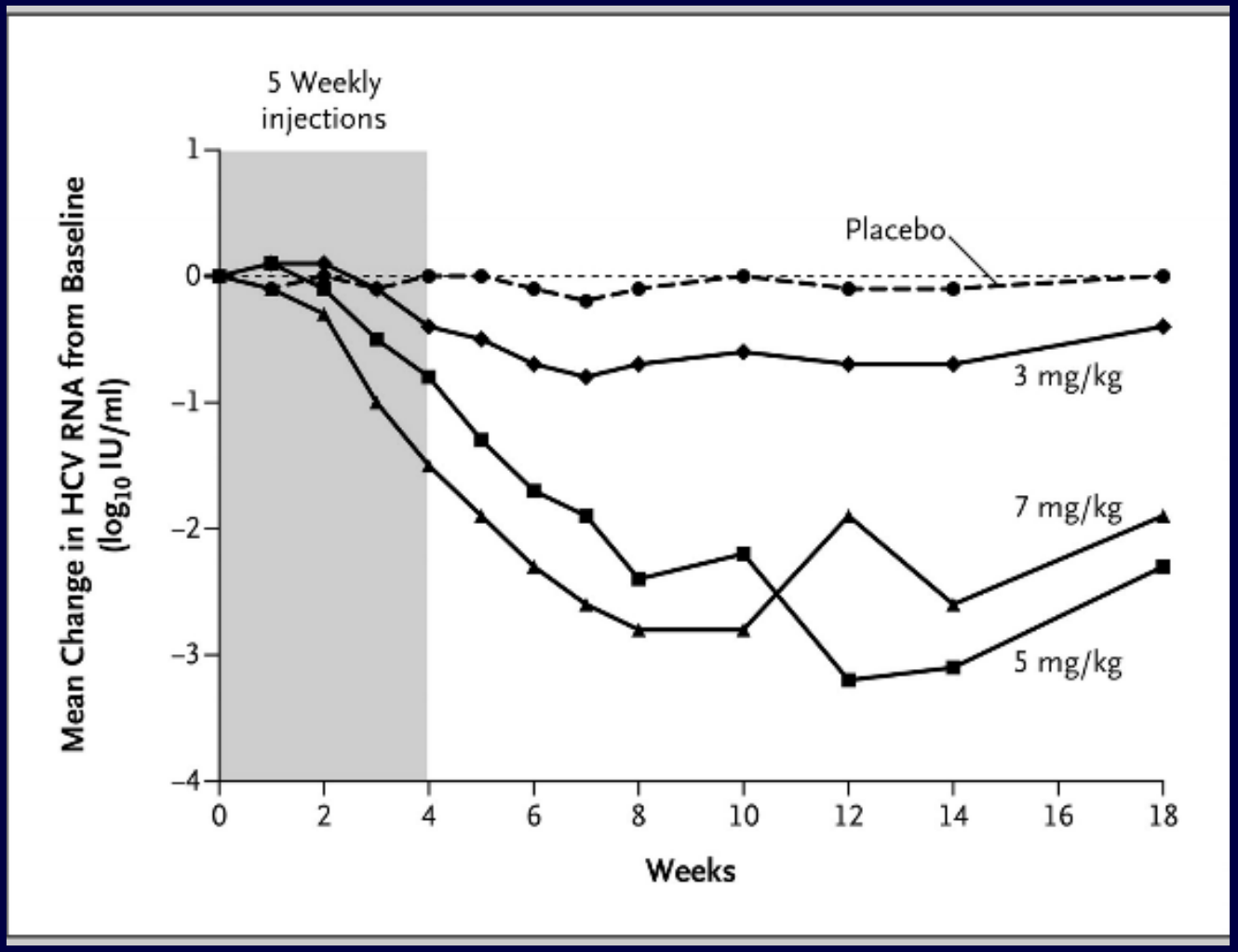
Short regulatory RNAs that translationally repress target mRNA

Each miRNA regulates about 100-200 mRNAs

Schoch and Miller Neuron 2017.

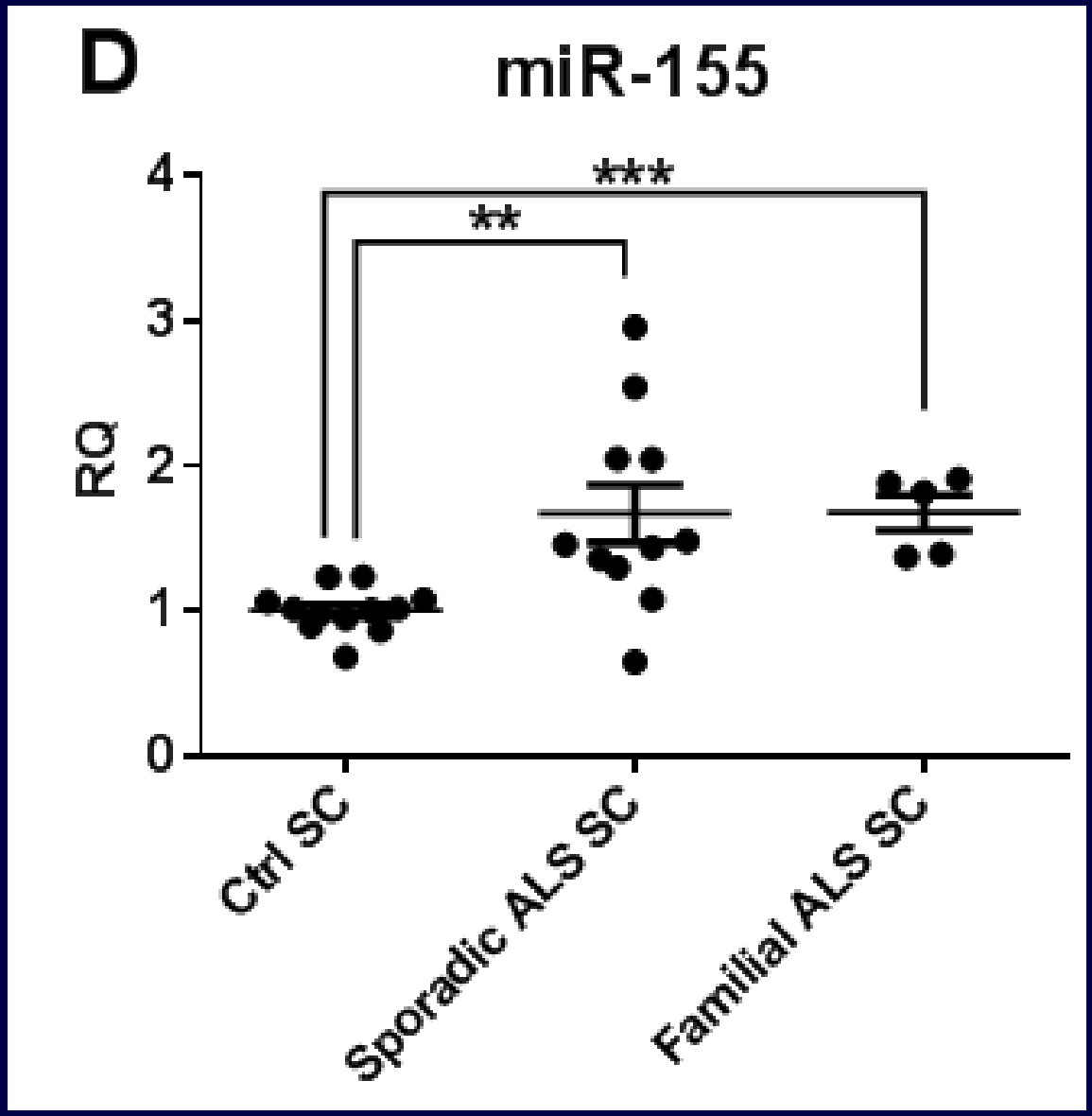
Inhibiting miRNA Strongly Influences Disease

- Phase 2a by Santaris Pharma, 36 patients with chronic HCV genotype 1 infection.



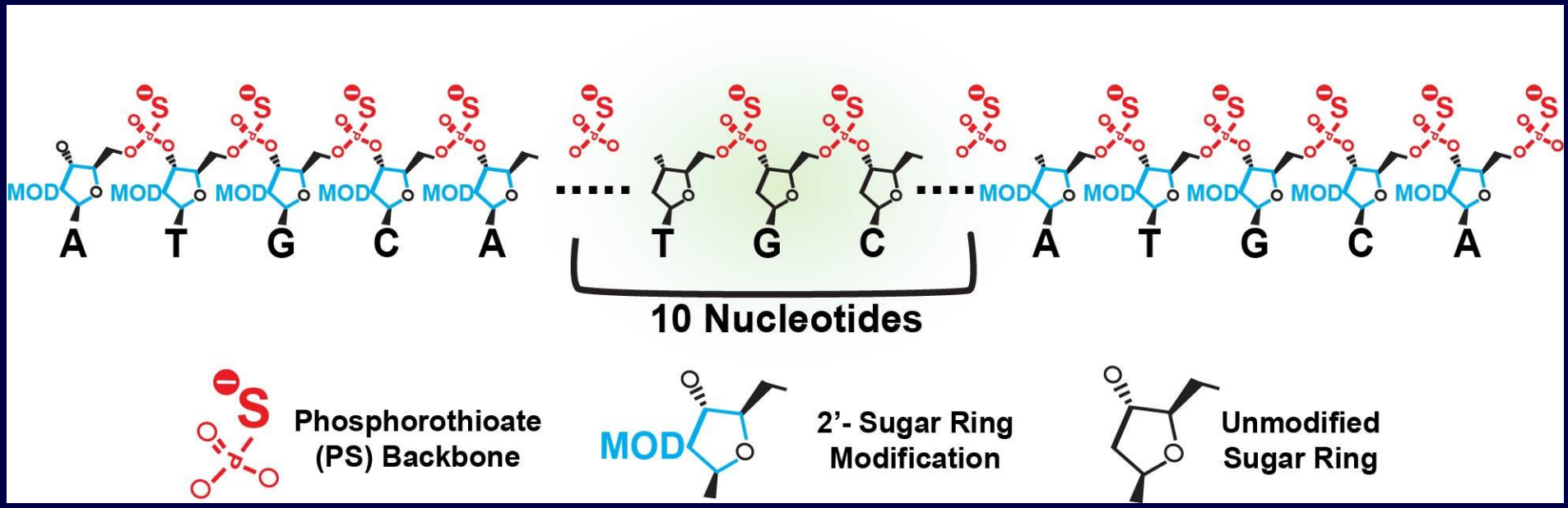
Janssen et al, NEJM 2013.

miR-155 Increased in ALS

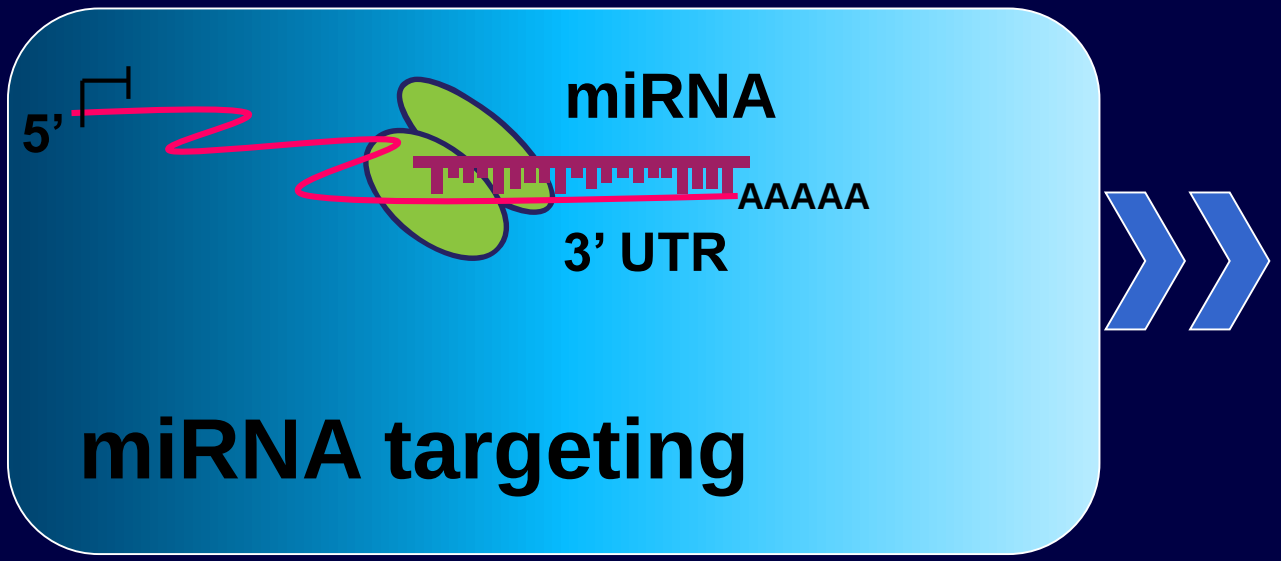


Koval et al. Human Molecular Genetics, 2013

Antisense Oligonucleotides

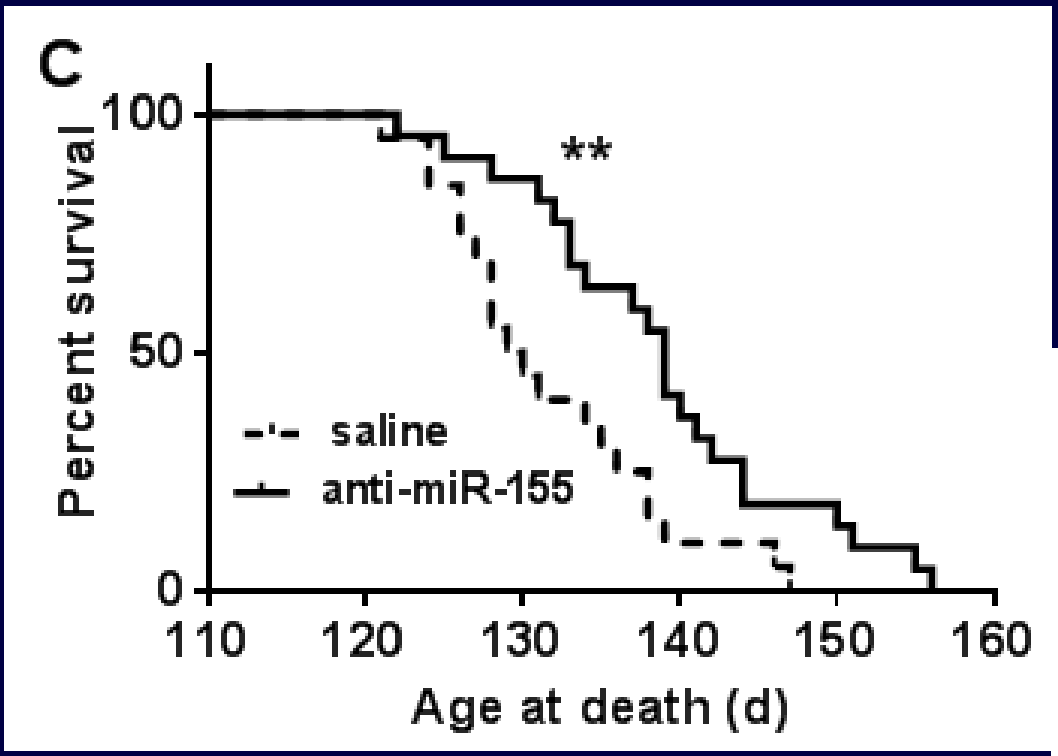


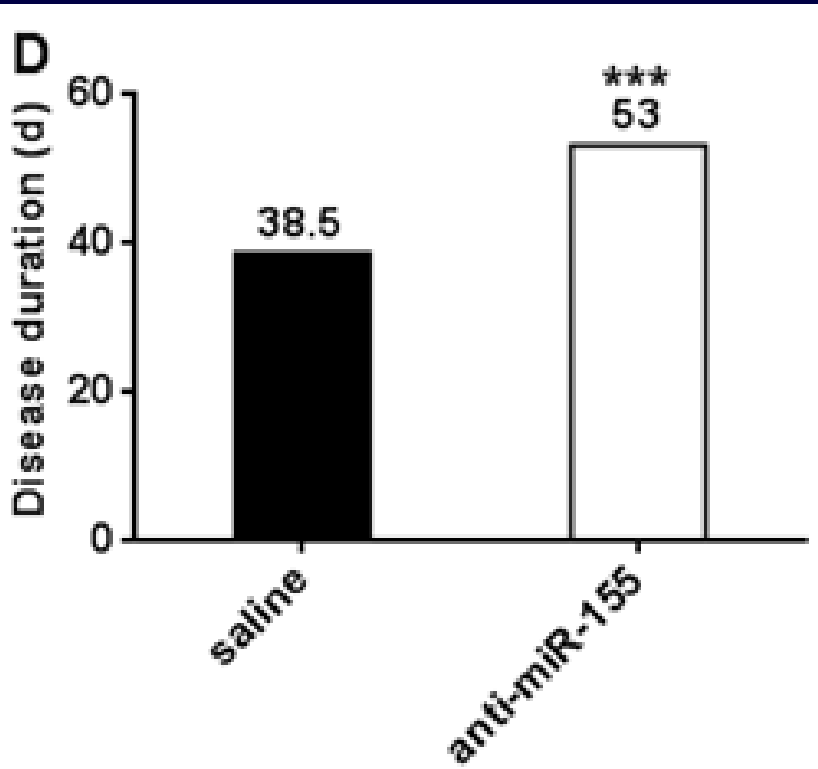
Devos and Miller, 2013



Schoch and Miller, Neuron 2017

Anti-miR-155 prolongs disease duration





Koval et al. Human Molecular Genetics, 2013

Conclusions

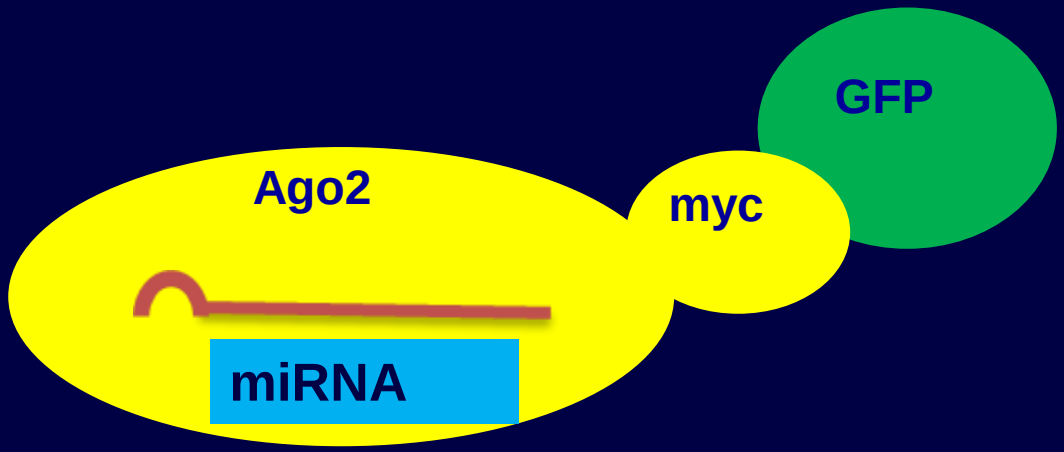
- miRNAs are dysregulated in ALS in both the rodent model and in patients
- miRNAs can be inhibited broadly in the CNS with antisense oligonucleotides
- miR-155 remains an interesting therapeutic target
  - miR-155 negatively contributes to disease
  - Implications for both sALS and fALS
  - Can read miR-155 in peripheral blood cells
  - Butovsky, Annals Neuro 2015, similar findings

Koval et al. Human Molecular Genetics, 2013

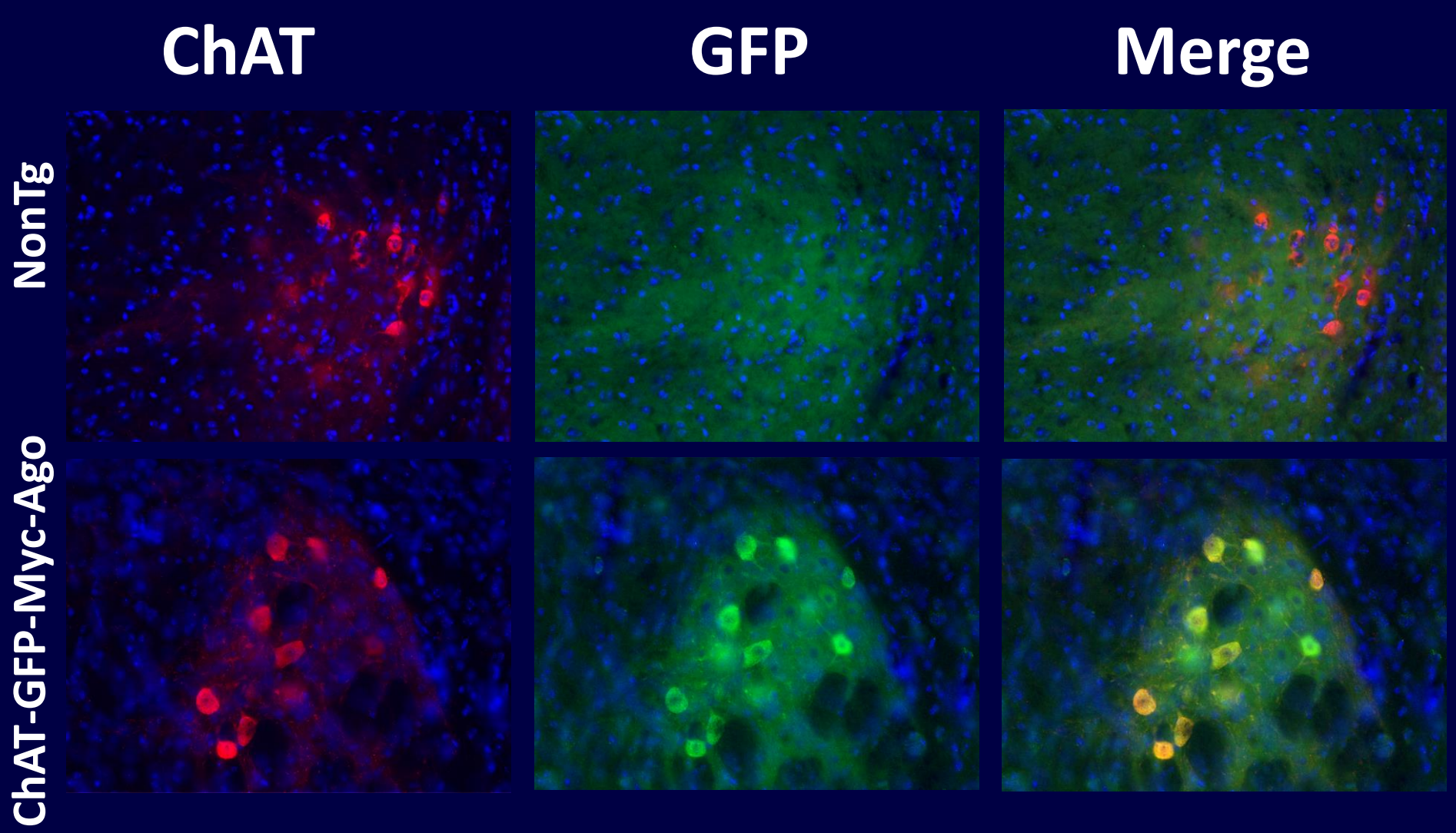
Motor Neuron-Enriched miRNAs

- Do MN-enriched miRNAs inform MN biology?
- Will dysregulation of MN-enriched miRNAs inform ALS disease mechanisms or markers?
- MNs only 4% of spinal cord volume

miRNA Affinity Purification

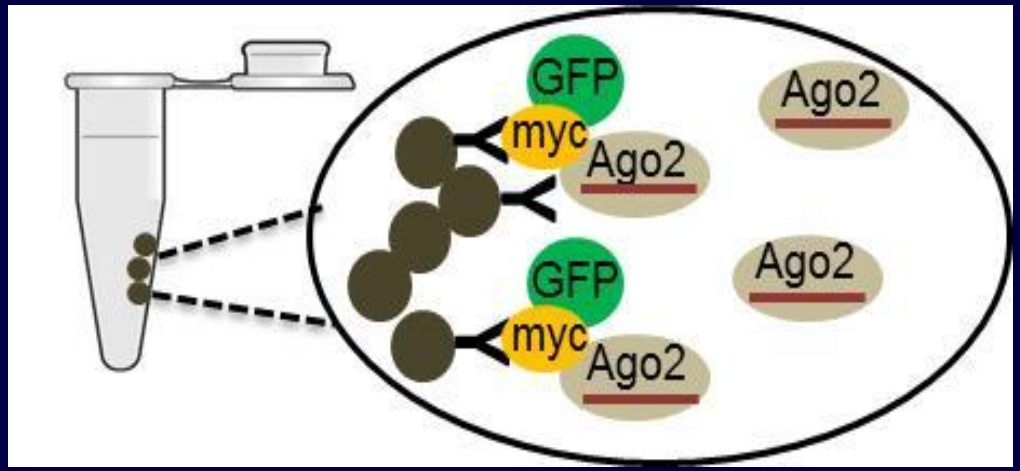


Collaboration with
Joe Dougherty
Washington University


ChAT
GFP
Merge
NonTg
ChAT-GFP-Myc-Ago

Isolation of Cell-Enriched miRNAs

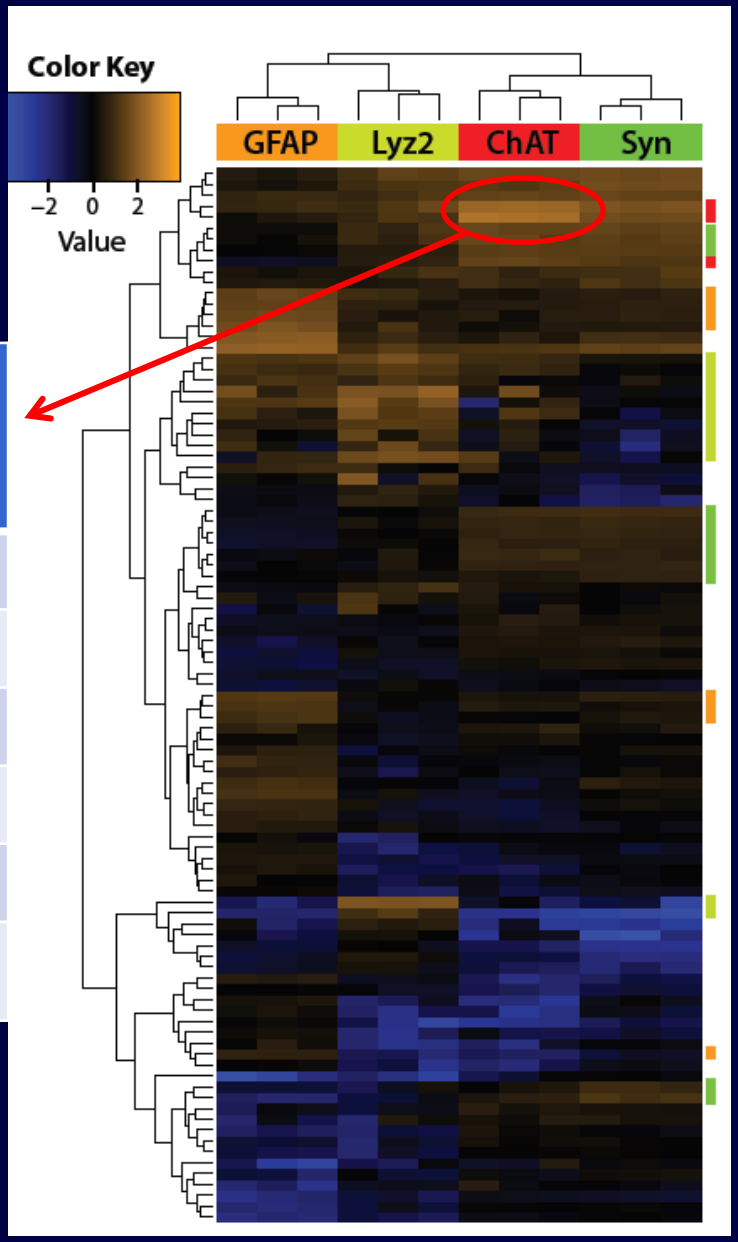
Myc IP



miRNAs from only motor neurons

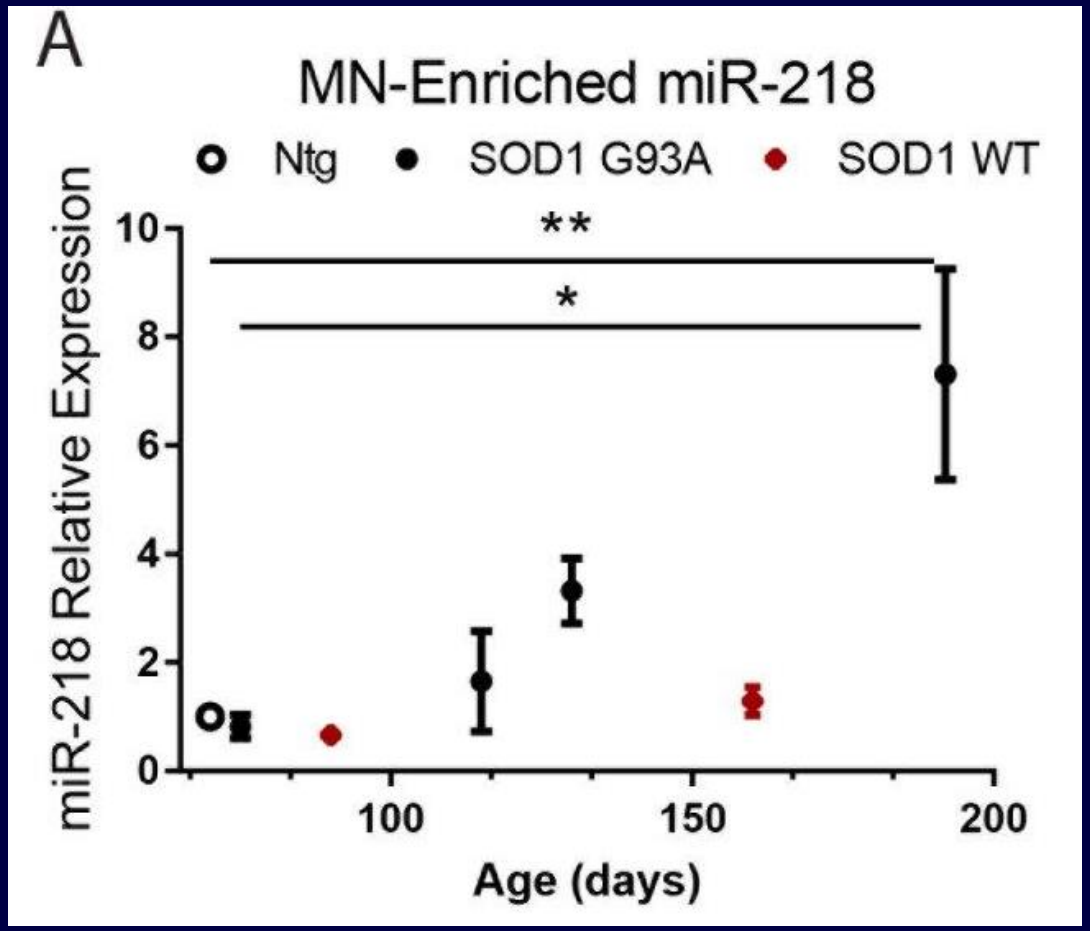
Identification of MN-enriched miRNAs

miRNA	Fold-Change (vs all other neurons)	P-value
miR-218	11.9	0.0002
miR-218-2	11.9	<0.0001
miR-138	3.2	<0.0001
miR-133a	2.8	<0.0001
miR-1193	3.7	0.0008
miR-34b-3p	3.1	0.045



Hoye et al. 2017. *J Neuroscience*

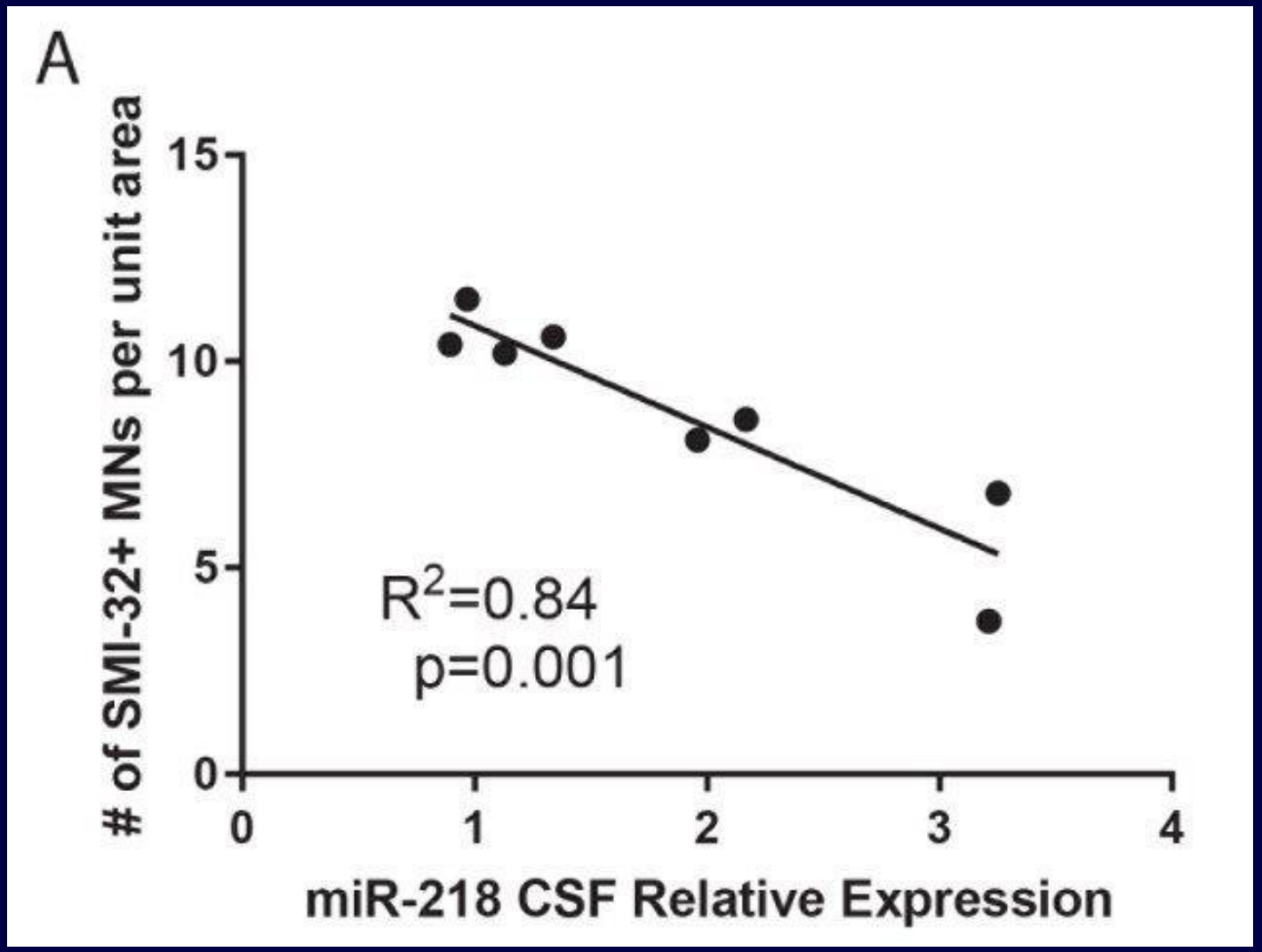
MN-enriched miRNAs are increased in ALS rat model CSF



Other neuronal miRNA, for example miR-124 and miR-132 not changed in CSF

Hoye et al. 2017. *J Neuroscience*

Marker of Motor Neuron Loss



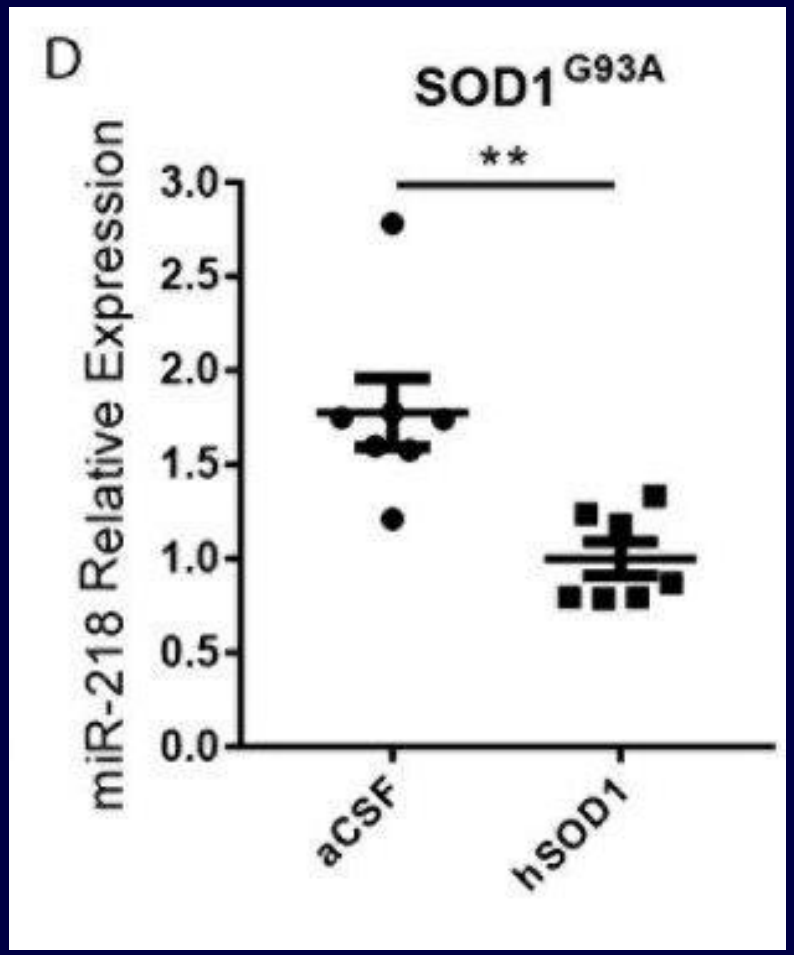
Hoye et al. 2017. *J Neuroscience*

Motor Neuron Biomarker

Distinguishes between motor neuron diseases and other diseases

Marker of health of motor neurons, defining response to therapy, and perhaps progression rates.

miR-218 is a Responsive Biomarker



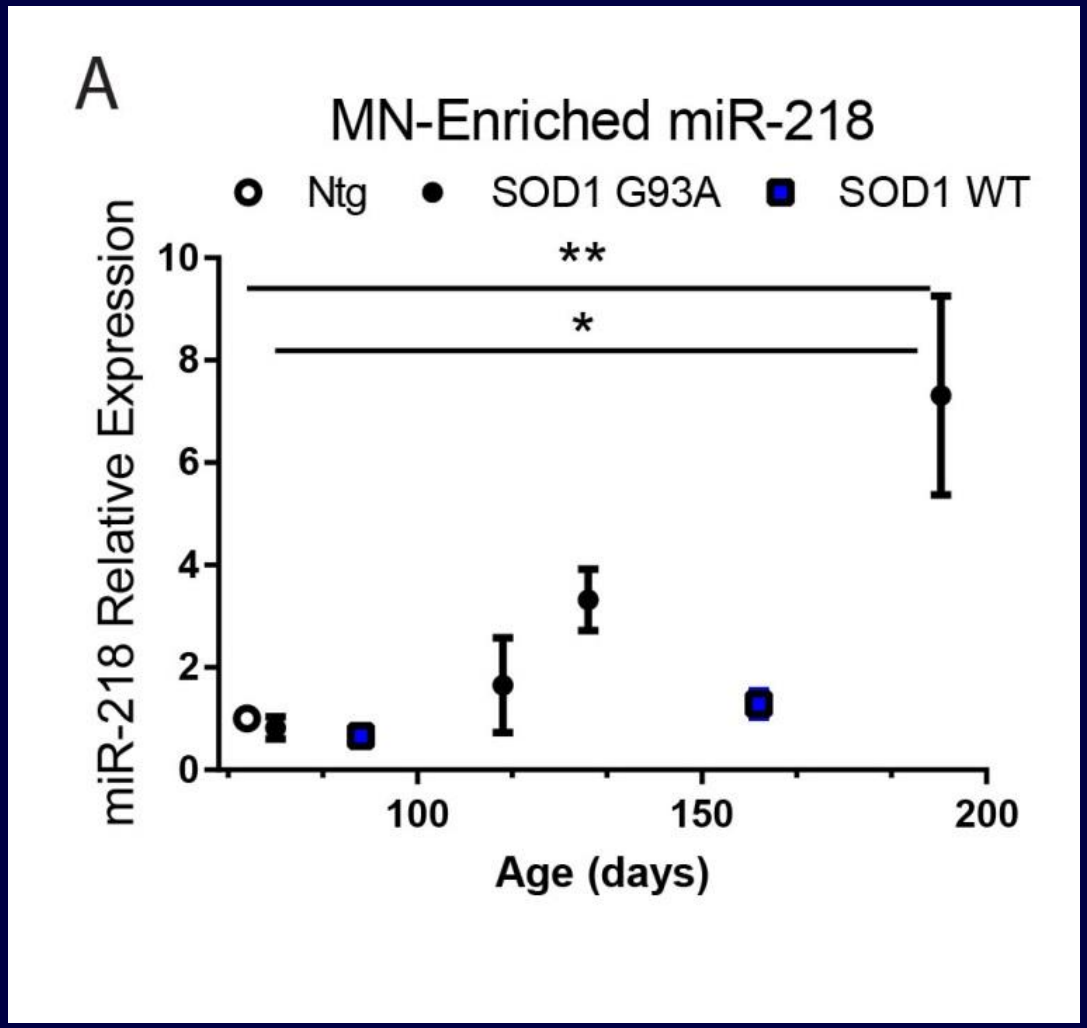
miR-124, miR-132 levels not changed
miR-218 not changed in non-transgenics treated with ASO or in scrambled treated SOD1G93A

Hoye et al. 2017. *J Neuroscience*

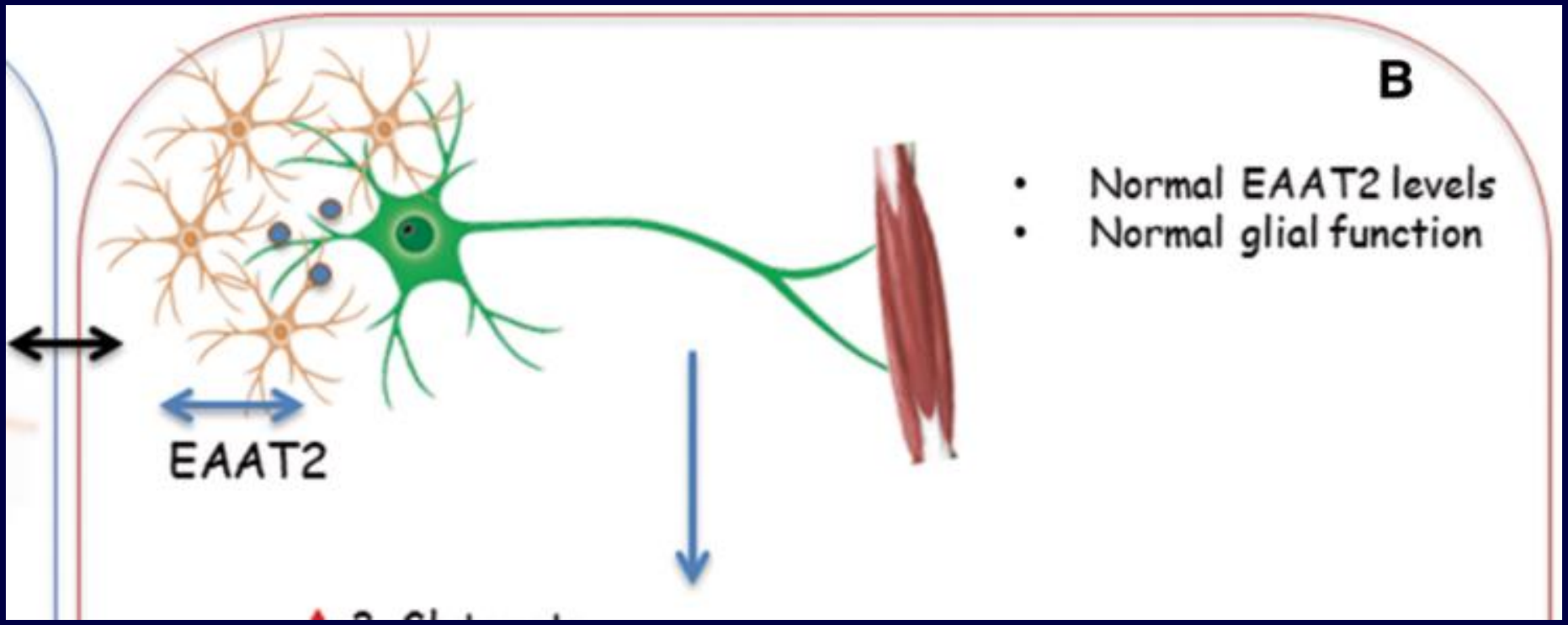
Conclusions

- Motor neuron enriched miRNAs are likely to increase understanding of motor neuron disease
- miR-218 is a motor neuron biomarker
- If true in humans, major advance for clinical trials in ALS

Is miR-218 more than a biomarker?

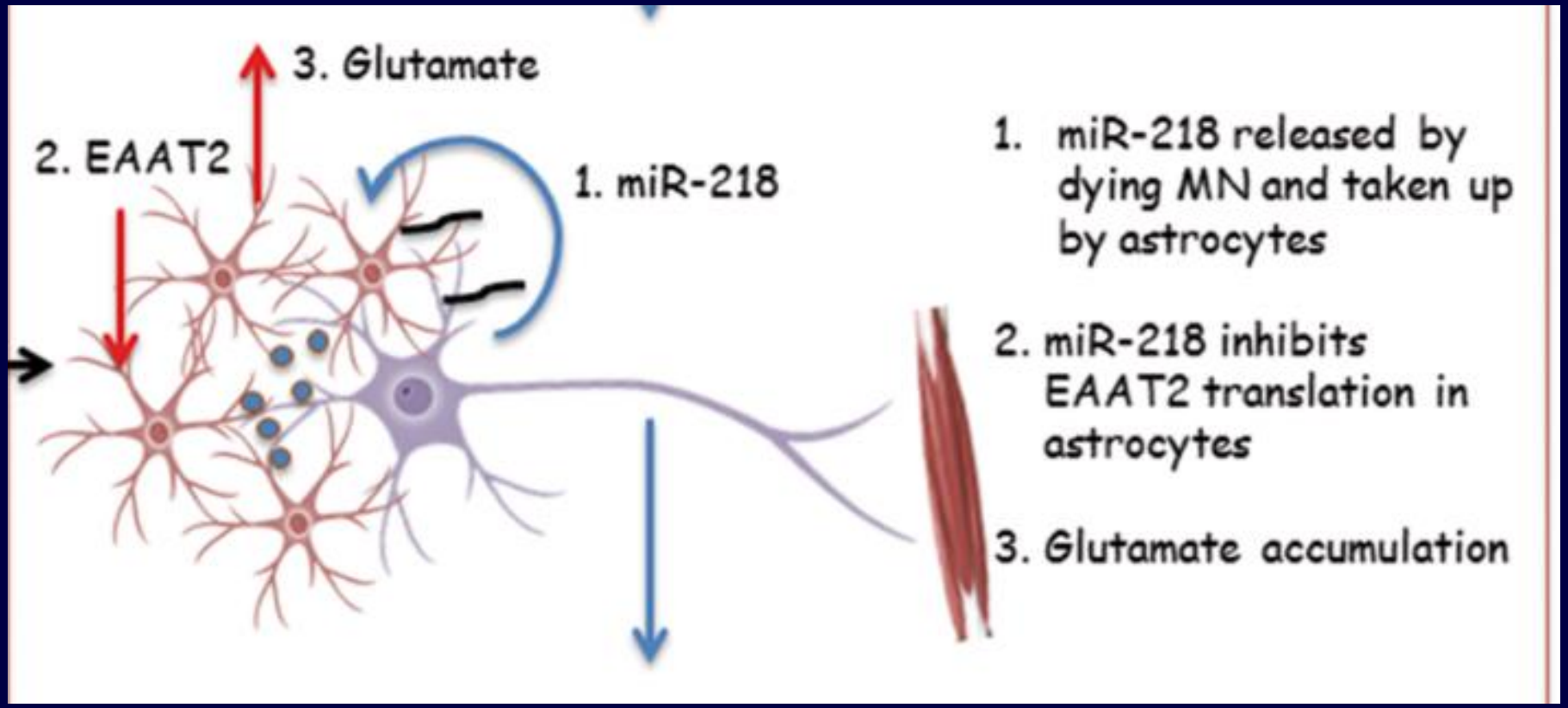


EAAT2 in Astrocytes Maintains Neuronal Health



Hoye et al. Brain 2018
Summary figure from commentary in Brain by Laura Ferraiuolo and Pamela J Shaw

miR-218 from Motor Neurons Taken Up by Astrocytes



Hoye et al. Brain 2018
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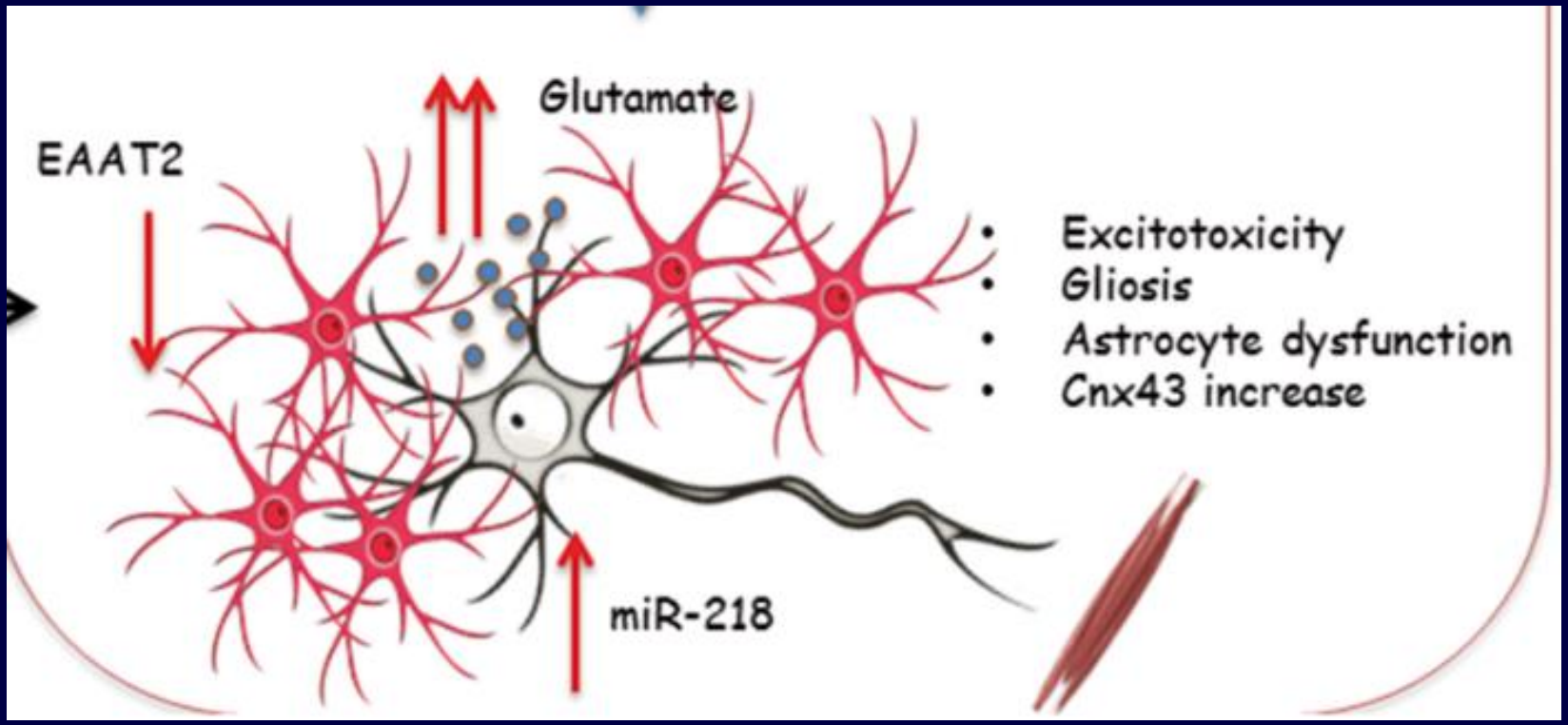
miR-218 Causes Increased Astrocyte Reactivity



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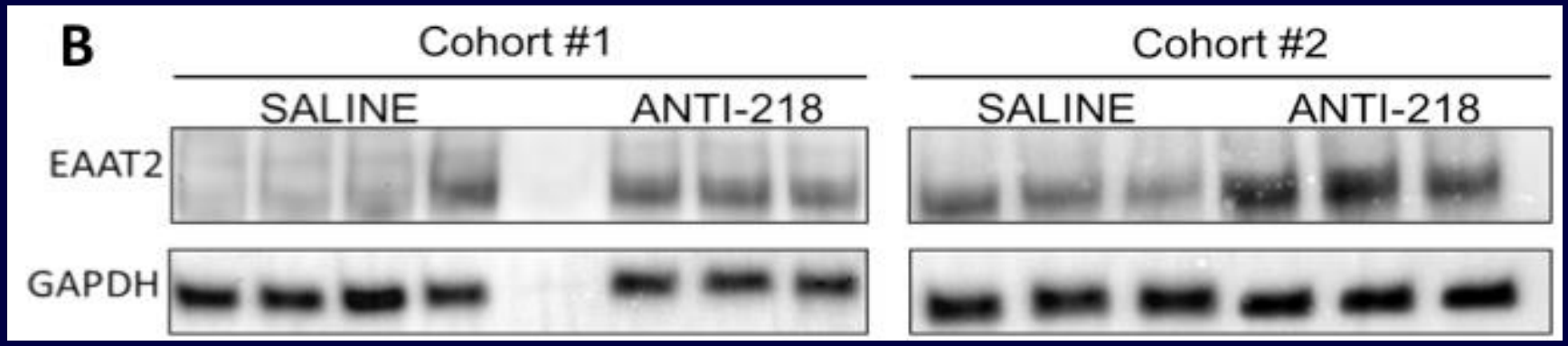
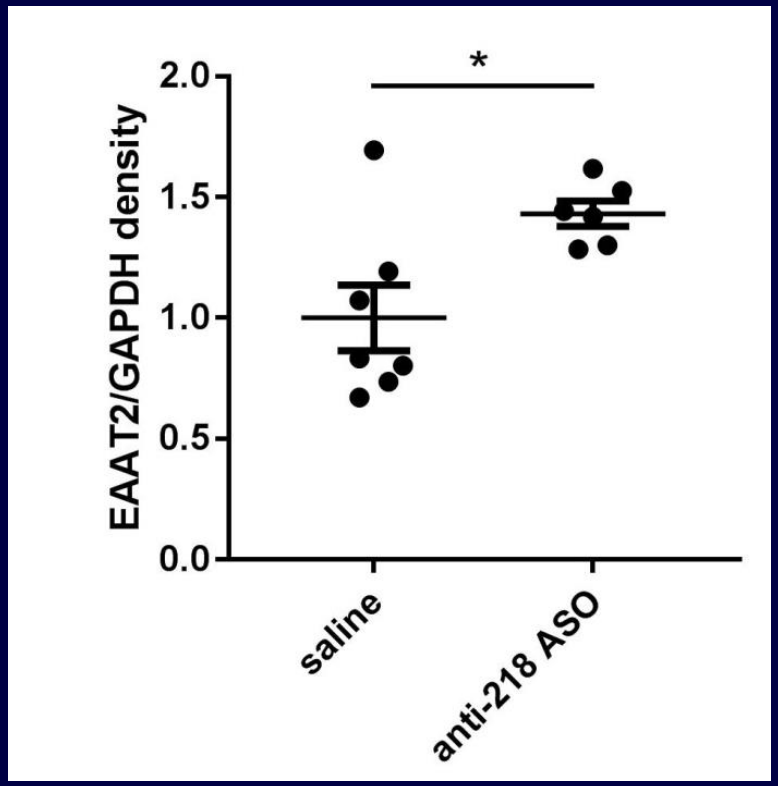
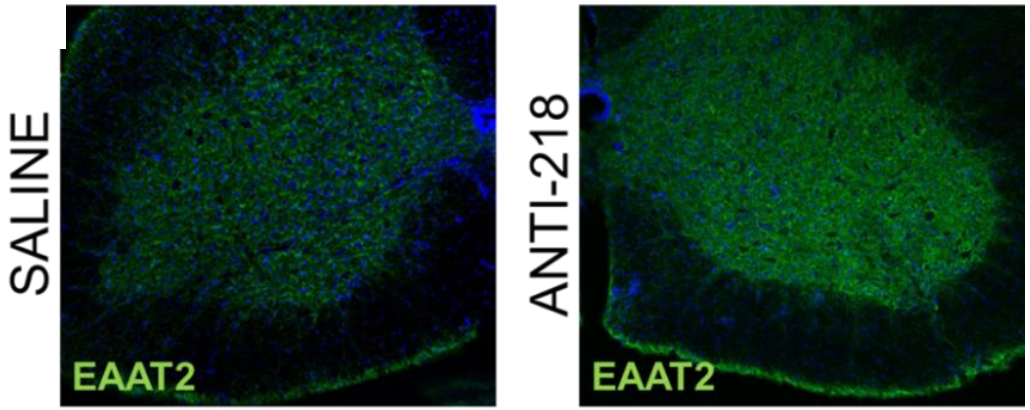
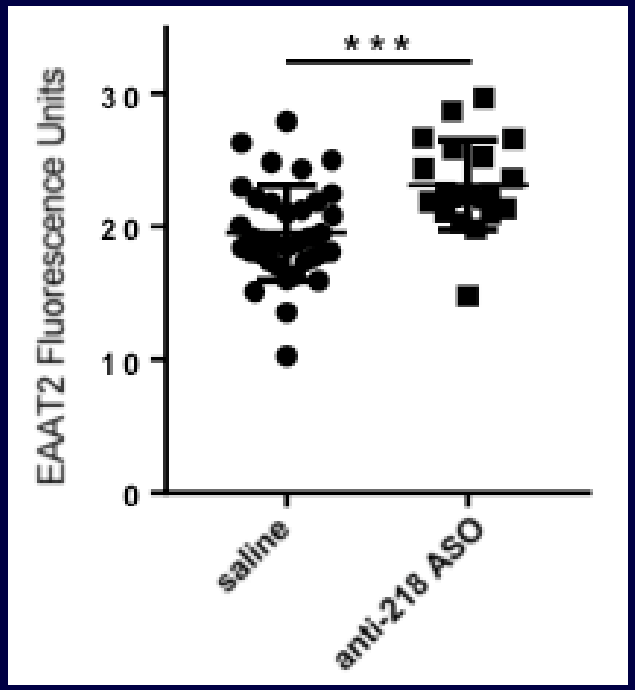
miR-218 inhibition prevents EAAT2 loss





miR-218 inhibition prevents EAAT2 loss

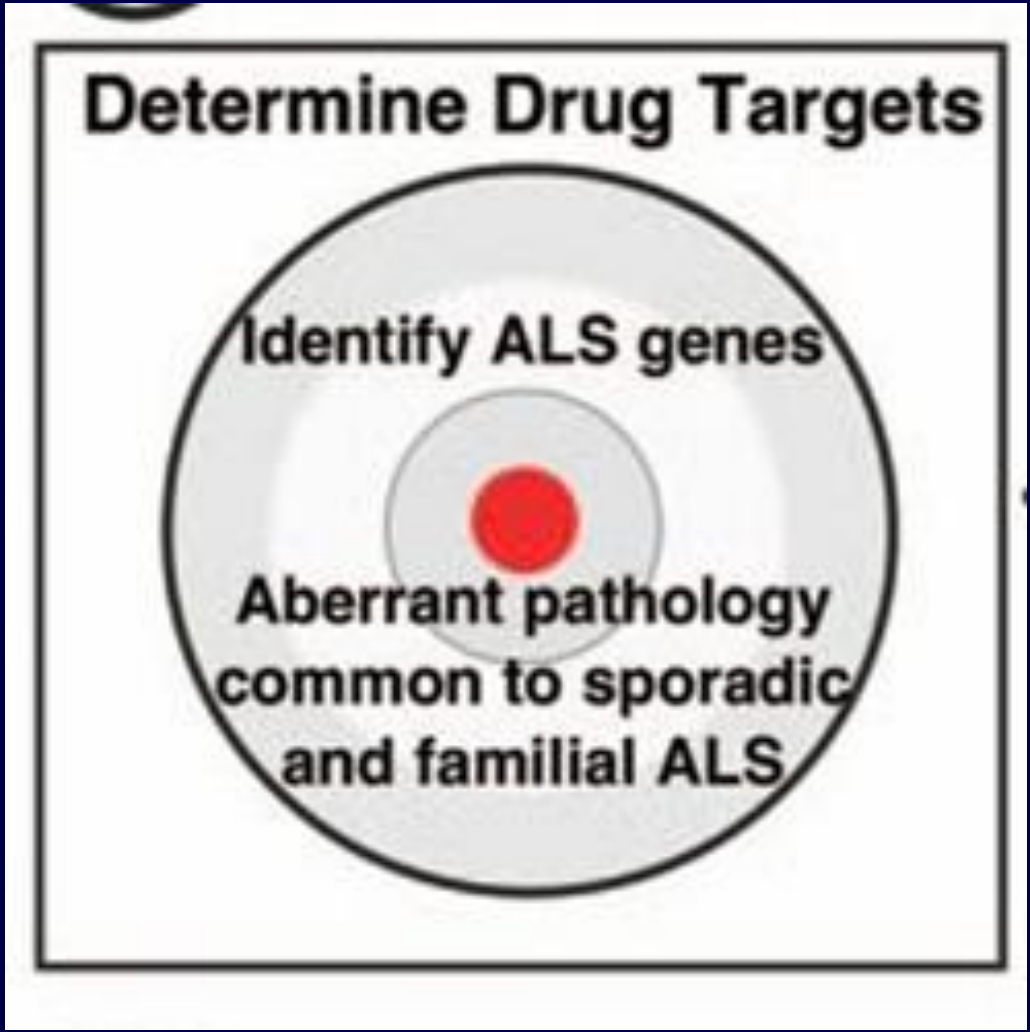




Motor Neuron-Enriched miRNAs:

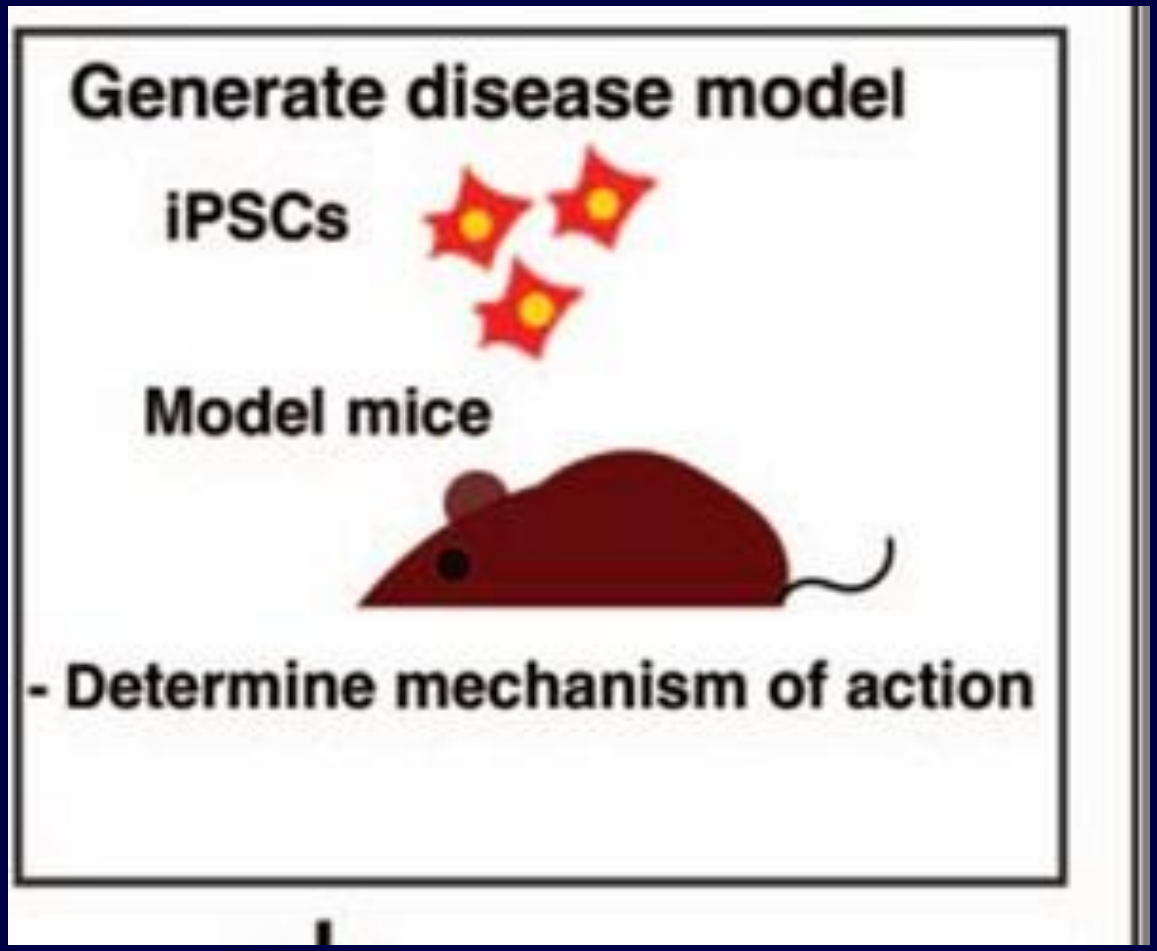
- Are functionally important for defining postnatal MN identity
- Are released from dying MNs in ALS and can be taken up by neighboring astrocytes to exacerbate feed-forward neurodegeneration

Cycle of Genetic Therapy Development



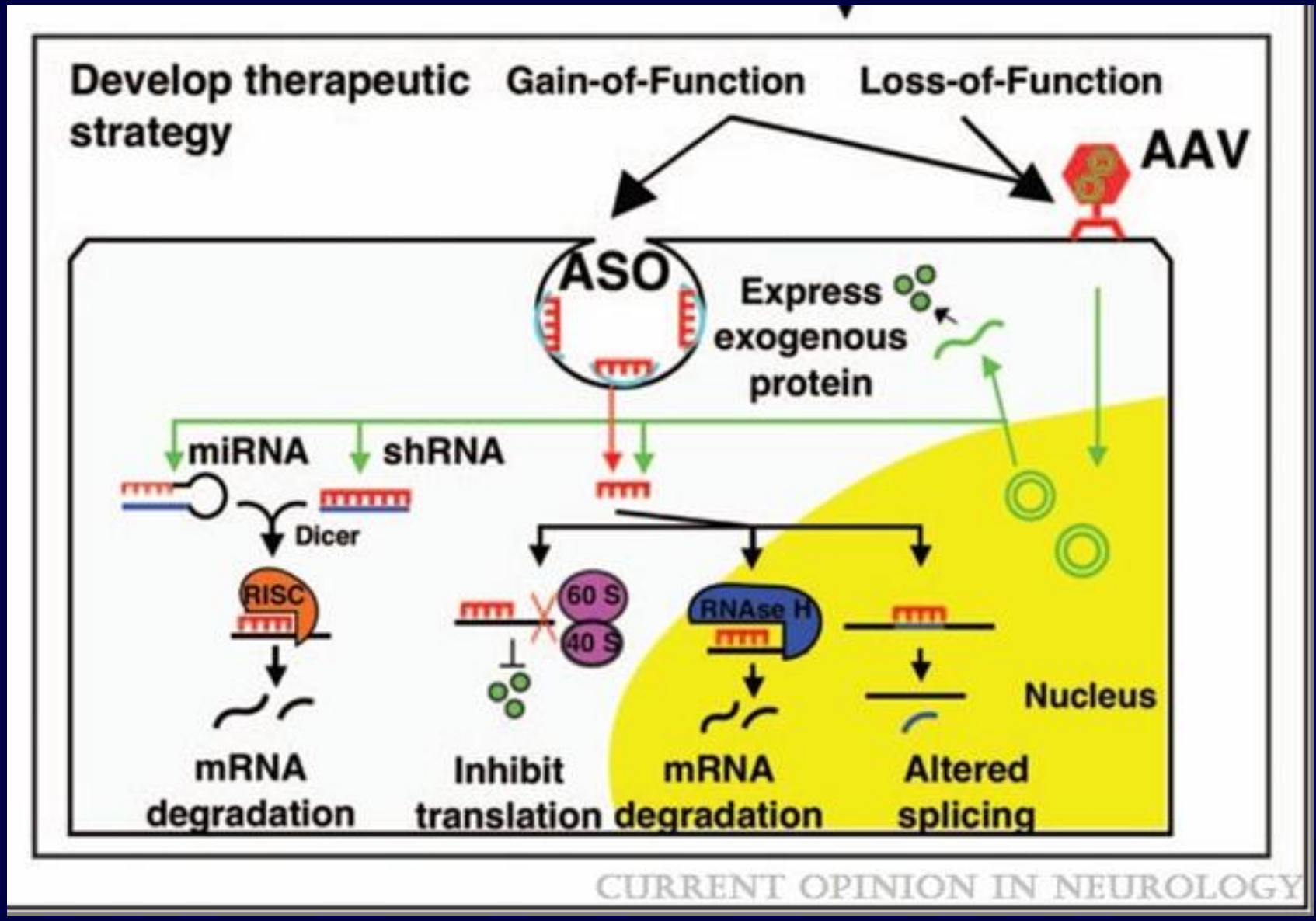
Ly and Miller, Current Opinion in Neurology, 2018

Cycle of Genetic Therapy Development



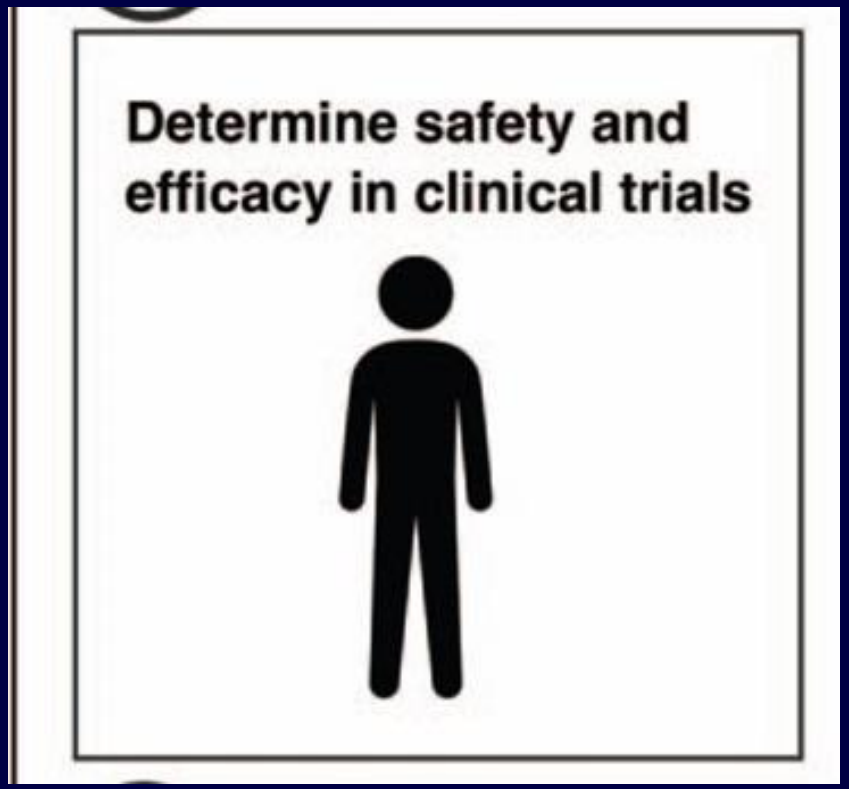
Ly and Miller, Current Opinion in Neurology, 2018

Cycle of Genetic Therapy Development



Ly and Miller, Current Opinion in Neurology, 2018

Cycle of Genetic Therapy Development



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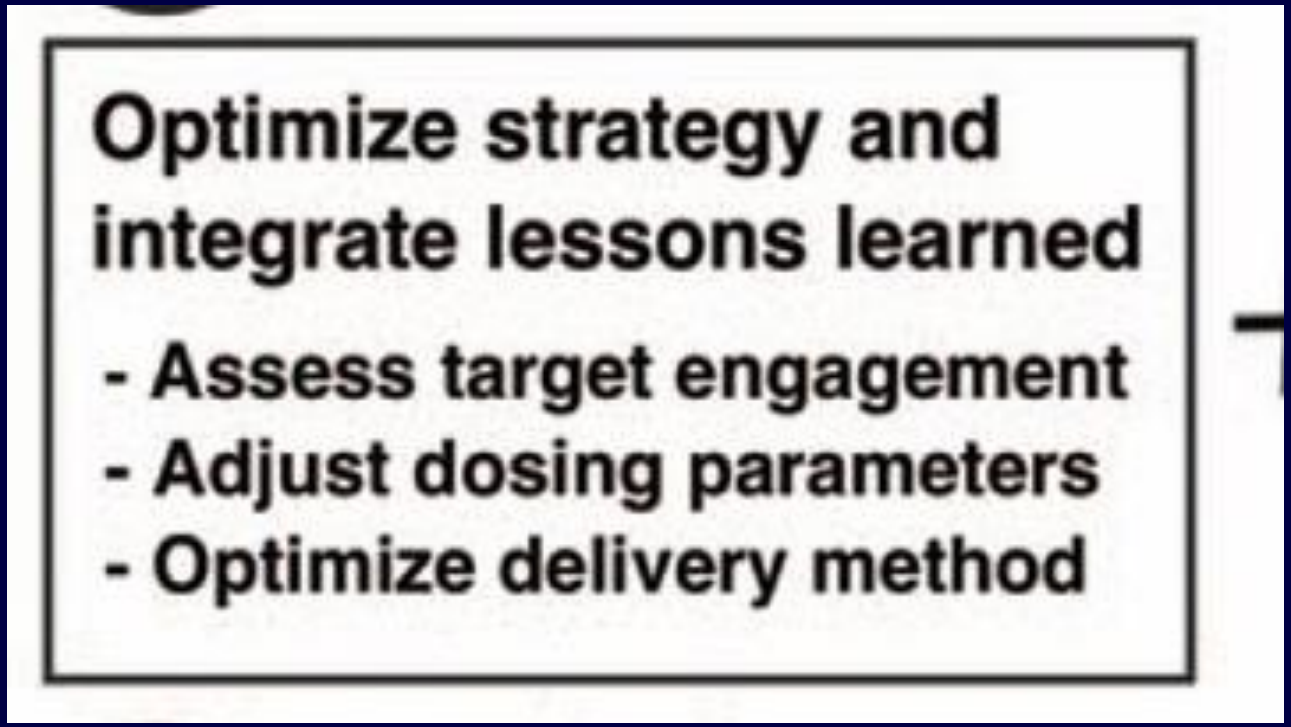
Cycle of Genetic Therapy Development



Ly and Miller, Current Opinion in Neurology, 2018

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Matt Crisp
Erica Koval
Mark Shabsovich

MGH Merit Cudkowiciz

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Melissa Regan
Jeff Rothstein

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UCSD Don Cleveland, Richard Smith

Biogen

Ionis Pharmaceuticals

Regulus Therapeutics

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Packard Center
ALS Association
MDA
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Project5 for ALS
Univ. MO SCIRP

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