Translational medicine and ALS clinical trials

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Washington University in St. Louis

Genetics and Genomics of Disease
October 6, 2014

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Disclosures:

Isis Pharmaceuticals provided the antisense oligos used in these studies and provides research support for my lab

Regulus Therapeutic has provided the antisense oligos used in the miRNA studies

Biogen Idec. provides research support for clinical studies.

Washington University, Isis Pharmaceuticals, Regulus Therapeutics have filed patents regarding the use of antisense oligonucleotides in neurodegenerative disease

Research Focus

Goal: Understand the pathophysiology of and develop novel therapeutic strategies for neurological diseases.

- SOD1 Familial ALS
  - To be described
- miRNAs –
  - Understanding miRNA changes in disease
  - Developing novel tools to understand cell type specific miRNAs
- C9ORF72 –
  - Using neurons directly converted from fibroblasts to understand disease
  - Understanding clinical phenotype and biomarkers
- Tau –
  - Understanding role of tau isoforms
  - Understanding how decreasing tau affects seizures (hyperexcitability)
  - Developing antisense oligo methods of reducing total tau mRNA or changing tau splicing patterns

Targets

- Huntingtin – Huntington's Disease
- Tau – Alzheimers Disease, FTD, PSP, CBD
- Prion protein – prion disease (Creutzfeld-Jacob)
- SMN – spinal muscular atrophy
- Dystrophin – muscular dystrophy (DMD)
- TDP-43 - FTD, ALS
- C9ORF72 – FTD, ALS
- Myostatin – muscle diseases
- TREM2 – AD, Parkinsons, FTD, ALS
- Many other pathways

Targeted Therapeutic Approaches

- Define a clear target
- Consider rationale for the therapeutic
  - Link to human disease?
  - Likely safe?
- Develop a method to engage that target
- Develop a method to measure the target in living humans
- Applies more broadly?
- Understand patient population
- Focused clinical trial

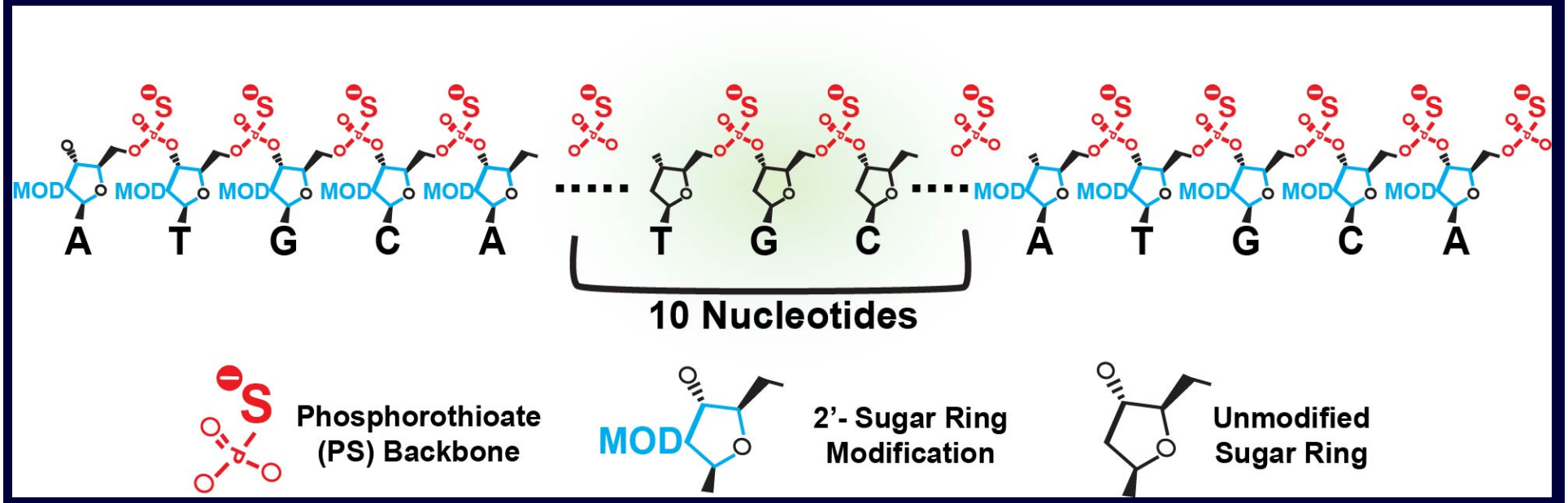
Methods to Increase/Replace Proteins

- Small molecules
- Viral delivery
- Change splicing (Small molecules/Antisense oligonucleotides

Methods to Clear/Improve Toxic Proteins

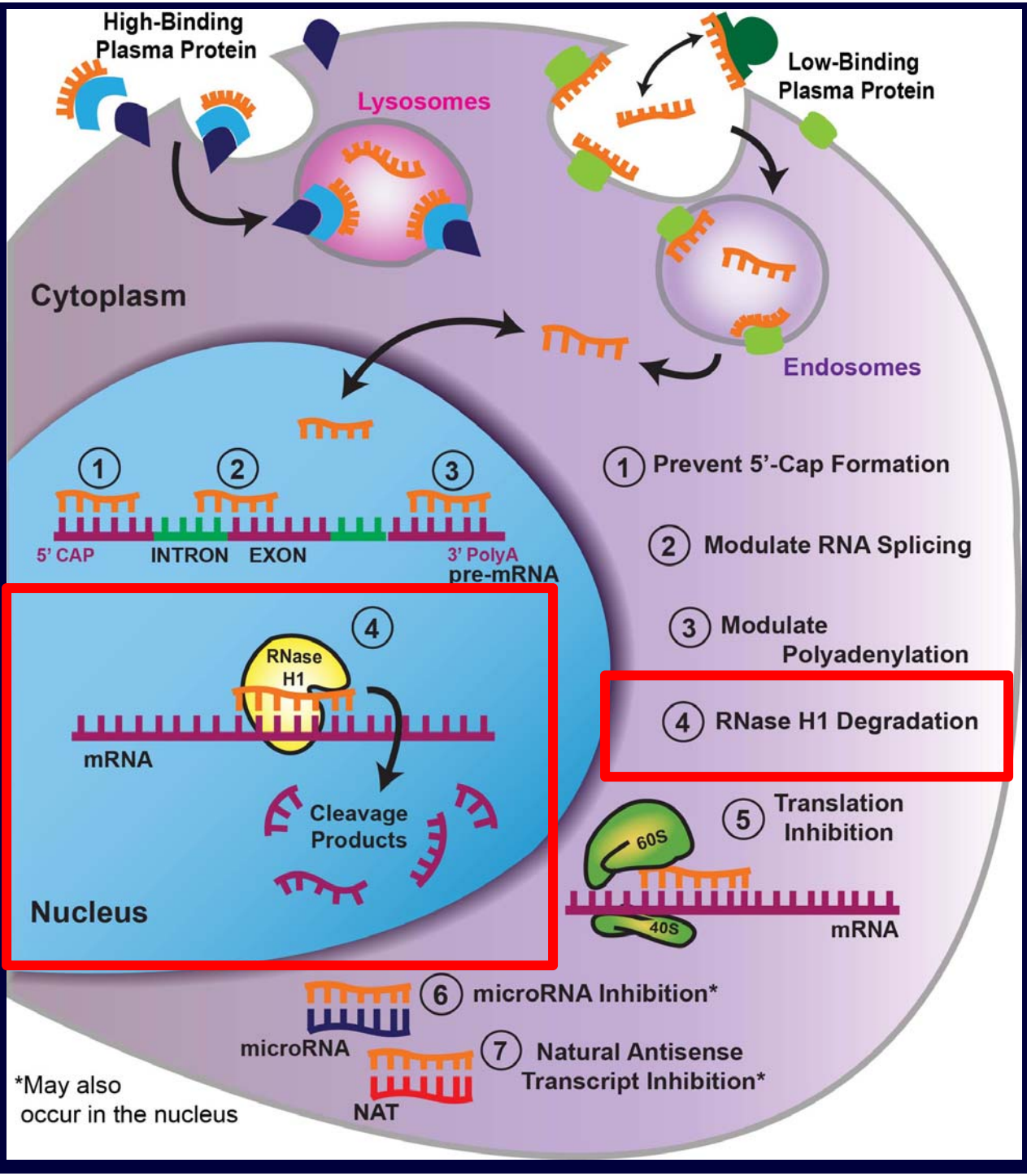
- Small molecules
- Use the immune system (vaccination or passive immunization)
- RNA interference
- Antisense oligonucleotides

Antisense Oligonucleotides

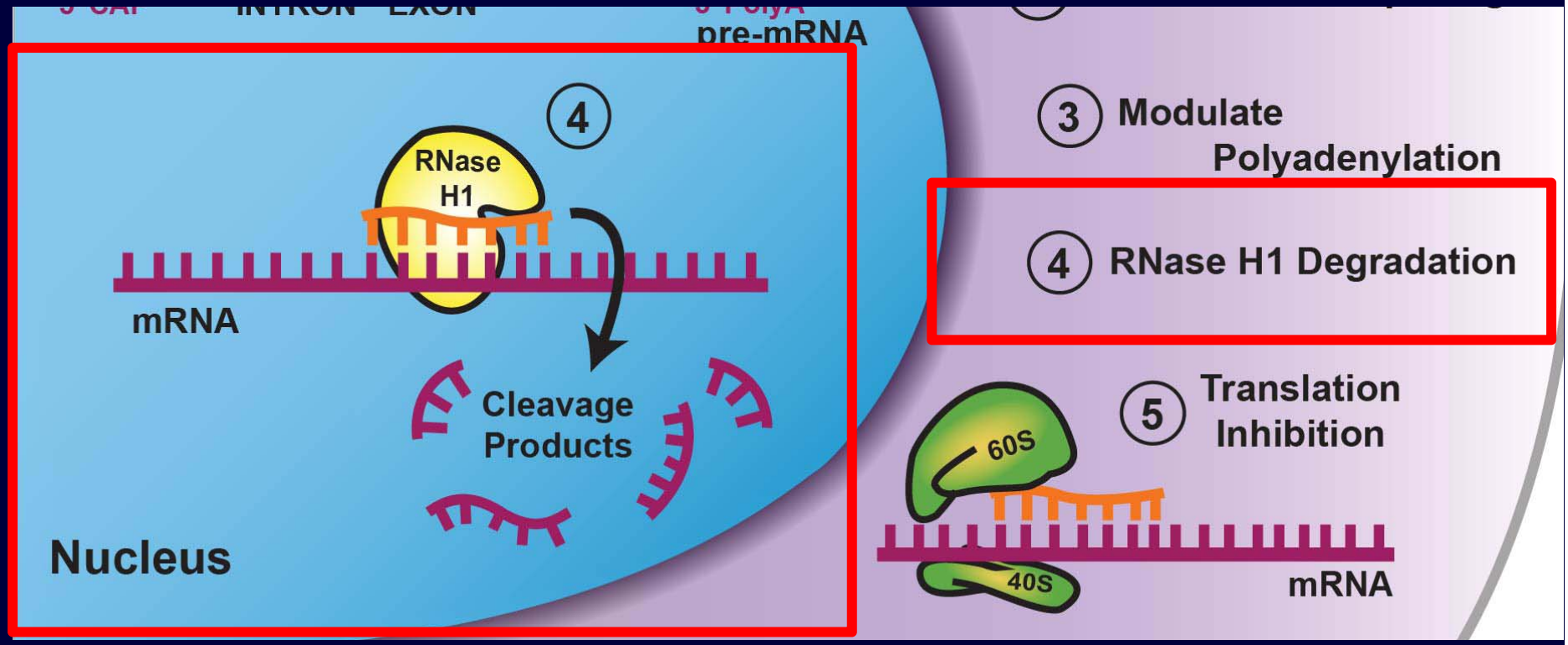


Current chemistries

- 10 fold increase in potency
- 10 fold increase in duration of action
- Marked decrease in toxicities
- Increase in therapeutic index
- Clinical experience 1000+ patients outside of CNS



DeVos and Miller, 2013



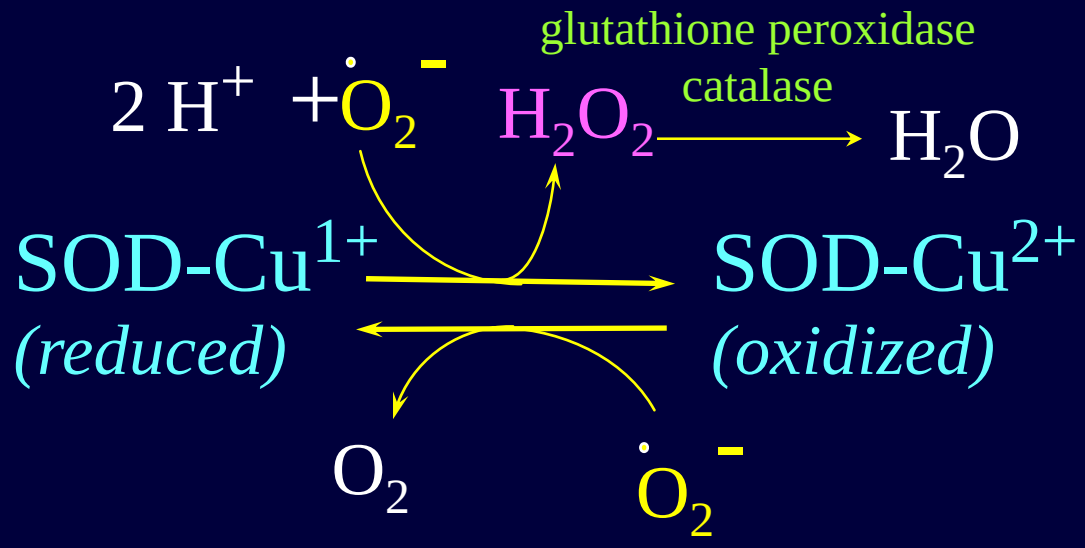
DeVos and
Miller, 2013

Amyotrophic Lateral Sclerosis

- Progressive degenerative disease
  - resulting in stiffness, weakness, and death in 2-5 years from respiratory failure
- No adequate current therapies
- Loss of neurons in the brain and spinal cord in the motor pathways
- 10% ALS familial / 90% Sporadic
- 15-20% of familial ALS caused by superoxide dismutase 1 (SOD1) mutations

Lou Gehrig

Properties of SOD1



- Soluble homodimers (153aa)
- Very stably folded protein
- Binds one Cu and one Zn; active site is Cu
- Abundant (~1% of brain protein)
- Ubiquitous, Cytosolic

Rationale for Decreasing SOD1 as a Therapy for SOD1-Mediated ALS

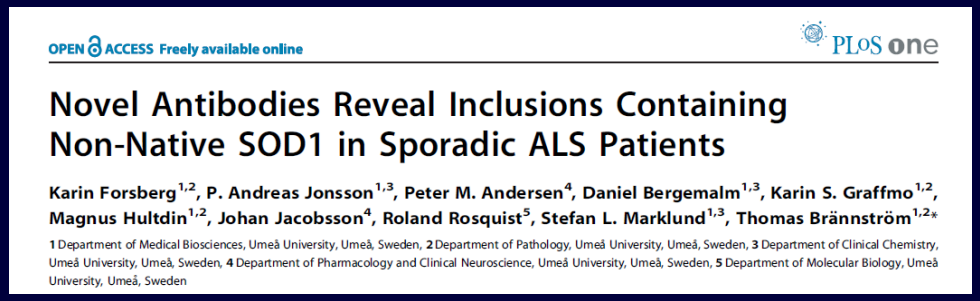
- Mutant Superoxide Dismutase 1 (SOD1) causes disease by acquisition of a toxic property that is independent of dismutase activity
- Decreasing SOD1 likely to ameliorate disease
- Likely safe to decrease SOD1

SOD1 in Sporadic ALS

nature neuroscience

Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS

Daryl A Bosco[1,8], Gerardo Morfini[2,7,8], N Murat Karabacak[3], Yuyu Song[2,7], Francois Gros-Louis[4], Piera Pasinelli[5], Holly Goolsby[6], Benjamin A Fontaine[1], Nathan Lemay[1], Diane McKenna-Yasek[1], Matthew P Frosch[6], Jeffrey N Agar[3], Jean-Pierre Julien[4], Scott T Brady[2,7] & Robert H Brown Jr[1]



OPEN ACCESS Freely available online

PLoS one

Novel Antibodies Reveal Inclusions Containing Non-Native SOD1 in Sporadic ALS Patients

Karin Forsberg[1,2], P. Andreas Jonsson[1,3], Peter M. Andersen[4], Daniel Bergemalm[1,3], Karin S. Graffmo[1,2], Magnus Hultdin[1,2], Johan Jacobsson[4], Roland Rosquist[5], Stefan L. Marklund[1,3], Thomas Brännström[1,2]*

1 Department of Medical Biosciences, Umeå University, Umeå, Sweden, 2 Department of Pathology, Umeå University, Umeå, Sweden, 3 Department of Clinical Chemistry, Umeå University, Umeå, Sweden, 4 Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden, 5 Department of Molecular Biology, Umeå University, Umeå, Sweden

LETTERS

nature biotechnology

Astrocytes from familial and sporadic ALS patients are toxic to motor neurons

Amanda M Haidet-Phillips[1,2,7], Mark E Hester[1,7], Carlos J Miranda[1,7], Kathrin Meyer[1], Lyndsey Braun[1], Ashley Frakes[1,2], SungWon Song[1,3], Shibi Likhite[1,3], Matthew J Murtha[1,3], Kevin D Foust[1], Meghan Rao[1], Amy Eagle[1], Anja Kammesheidt[4], Ashley Christensen[4], Jerry R Mendell[1,2], Arthur H M Burghes[5] & Brian K Kaspar[1–3,6]

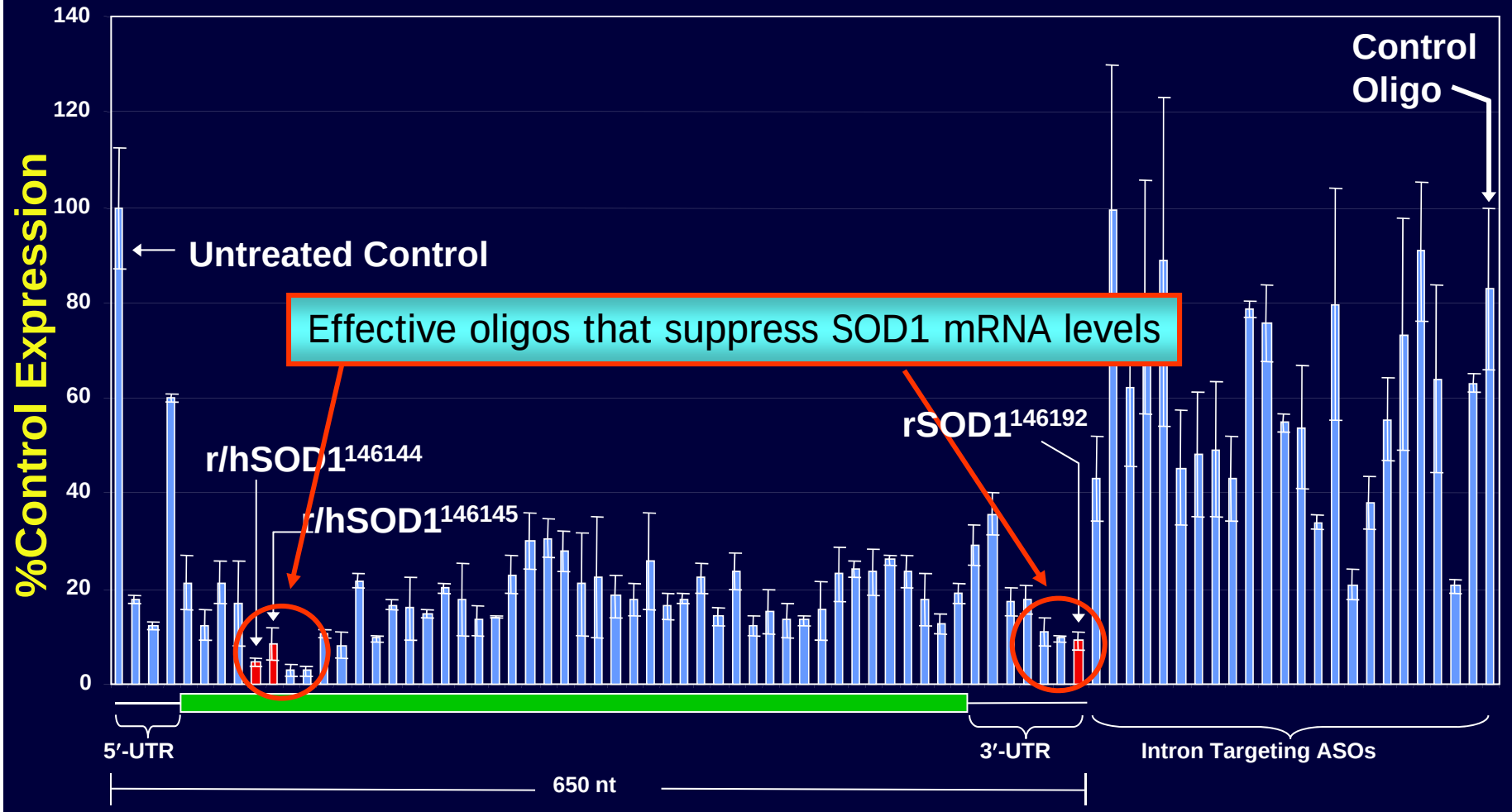
Oxidized/misfolded superoxide dismutase-1: the cause of all amyotrophic lateral sclerosis?

Kabashi E, Valdmanis PN, Dion P, Rouleau GA.

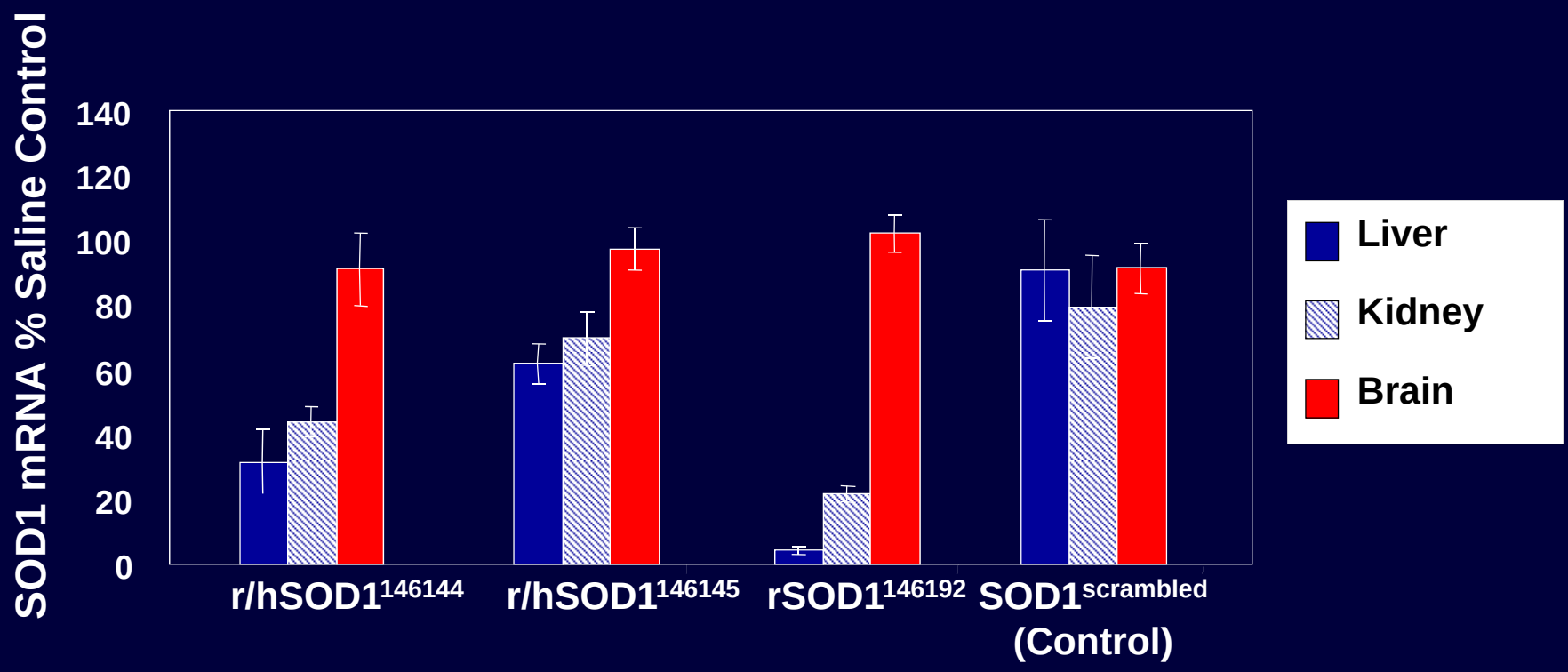
Gene Targeted Therapy for ALS

- Preclinical SOD1 Antisense oligo data
  - decrease SOD1 in vivo
  - distribute widely
  - neuroprotective
- Phase I Clinical Trial
- Other SOD1 studies to enable Phase II

Inhibition of SOD1 mRNA after antisense oligo treatment in vitro

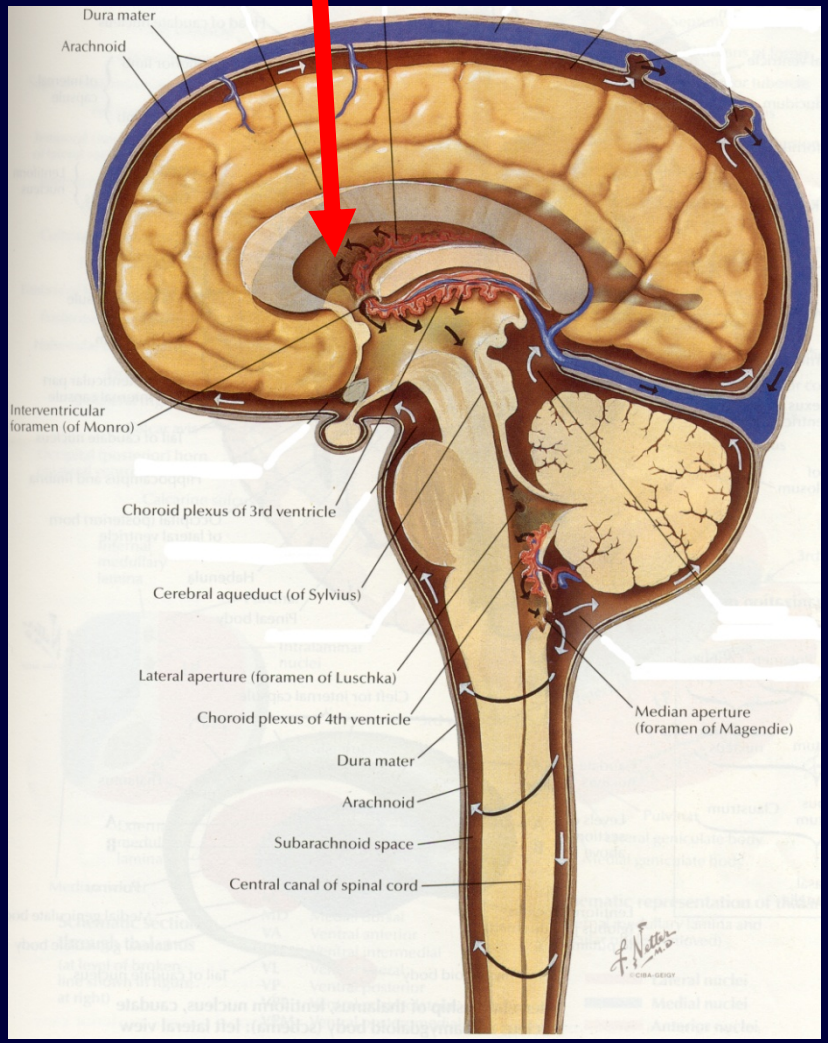


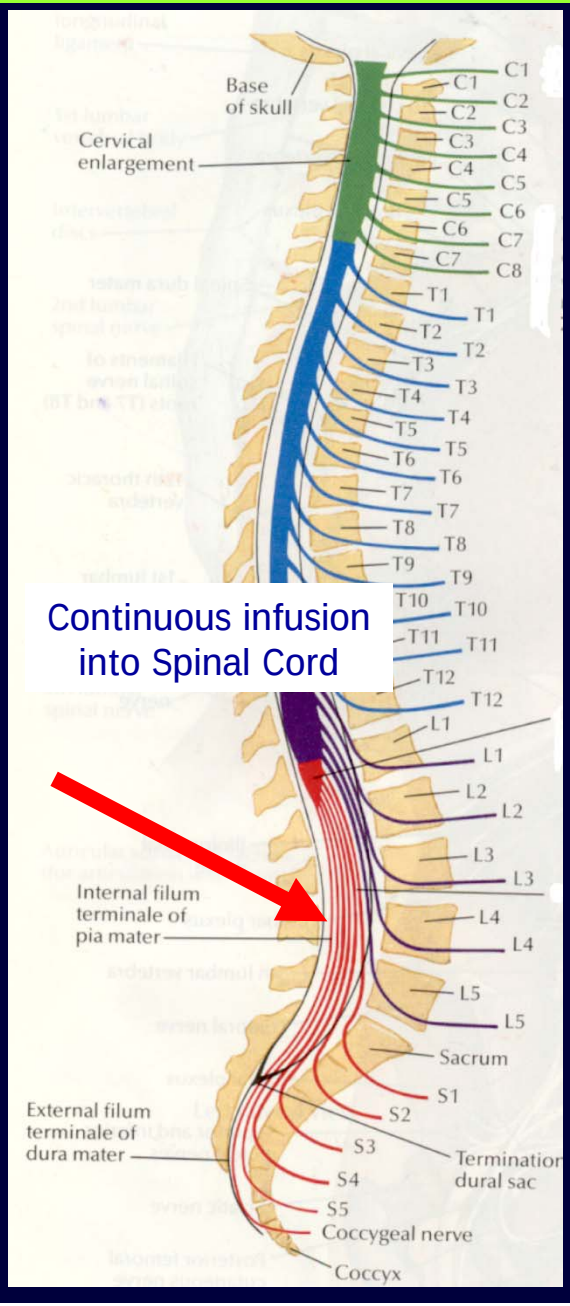
Intraperitoneal Administration of Antisense Oligo



Delivery of Oligos into CNS

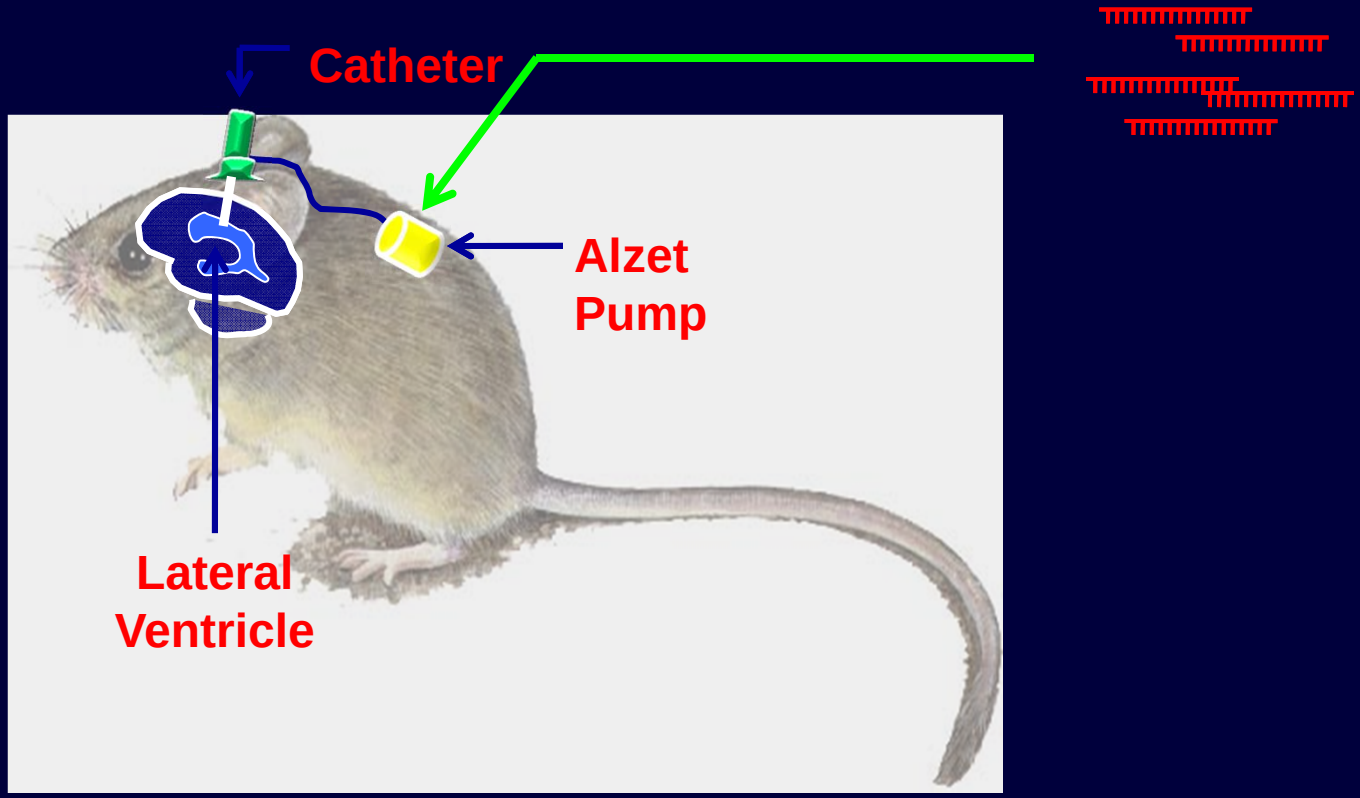
Continuous infusion into right lateral ventricle





Delivery of Oligos to Rats/Mice

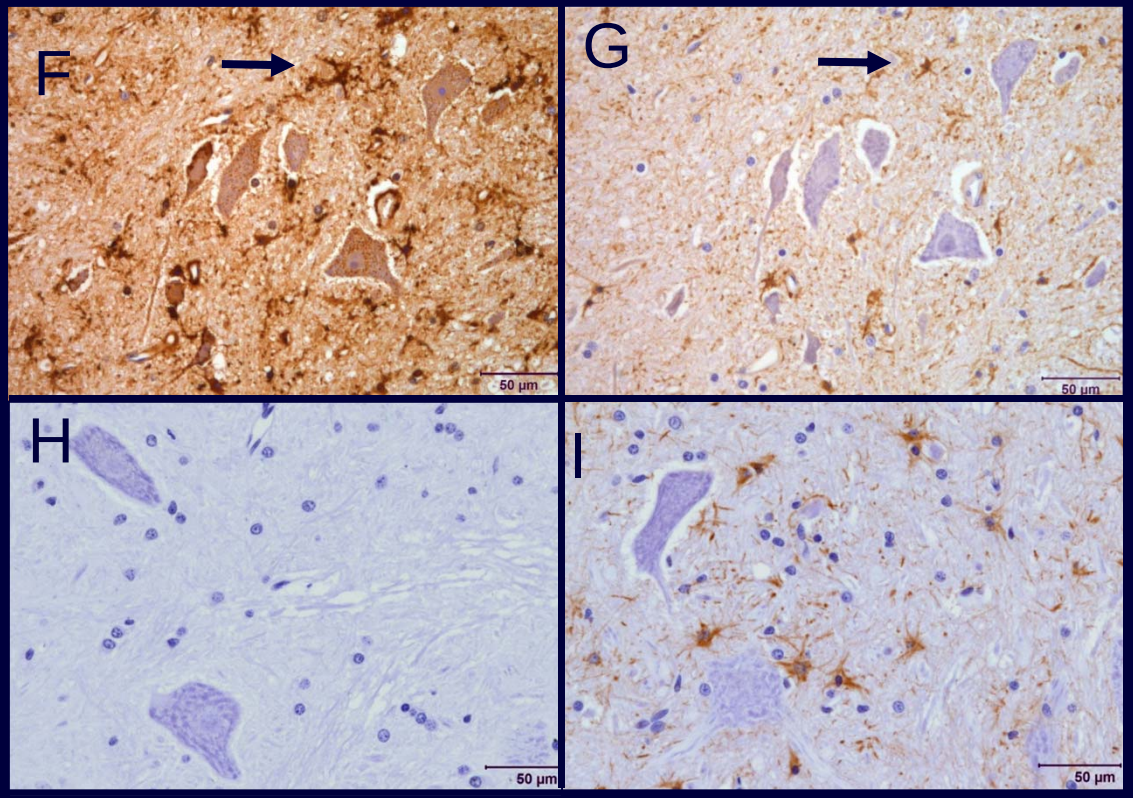
Anti-sense Oligonucleotides (ASOs)



Delivery by intraventricular administration to Rhesus monkey spinal cord

Anti Oligo

Anti-GFAP



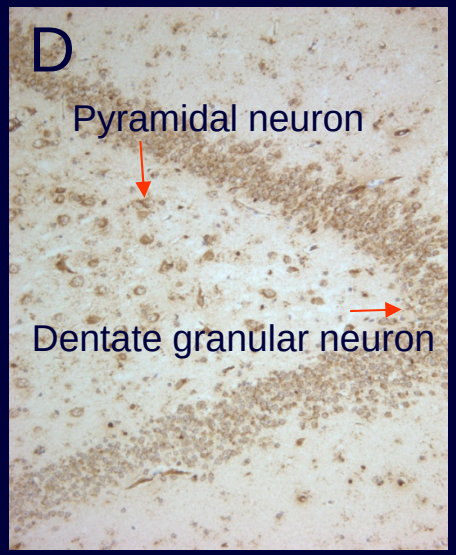
Oligo Treated

Saline Treated

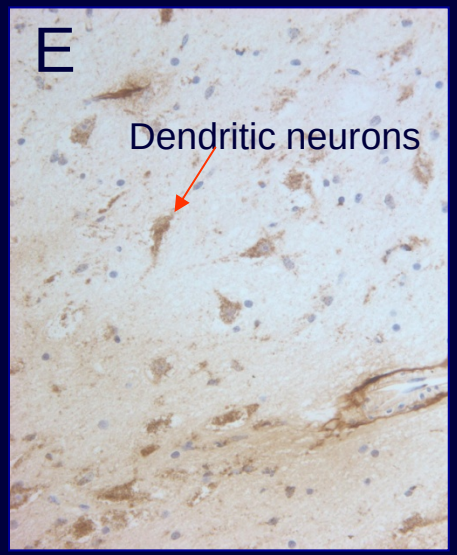
Lumbar Ventral Horn

Intraventricular infusion delivers oligos widely

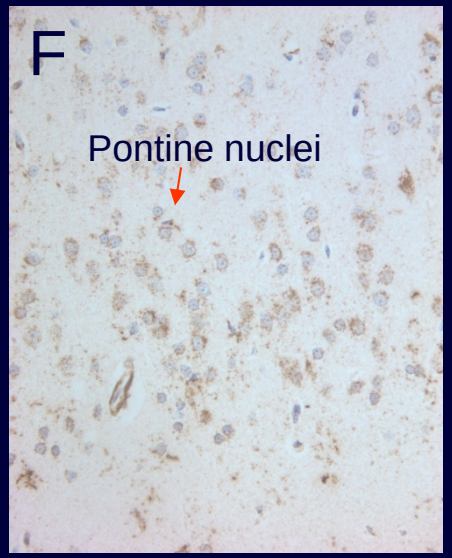
Hippocampus



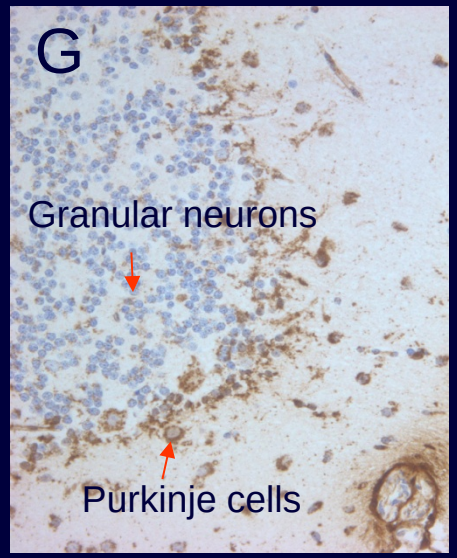
Substantia nigra



Brainstem (Pons)



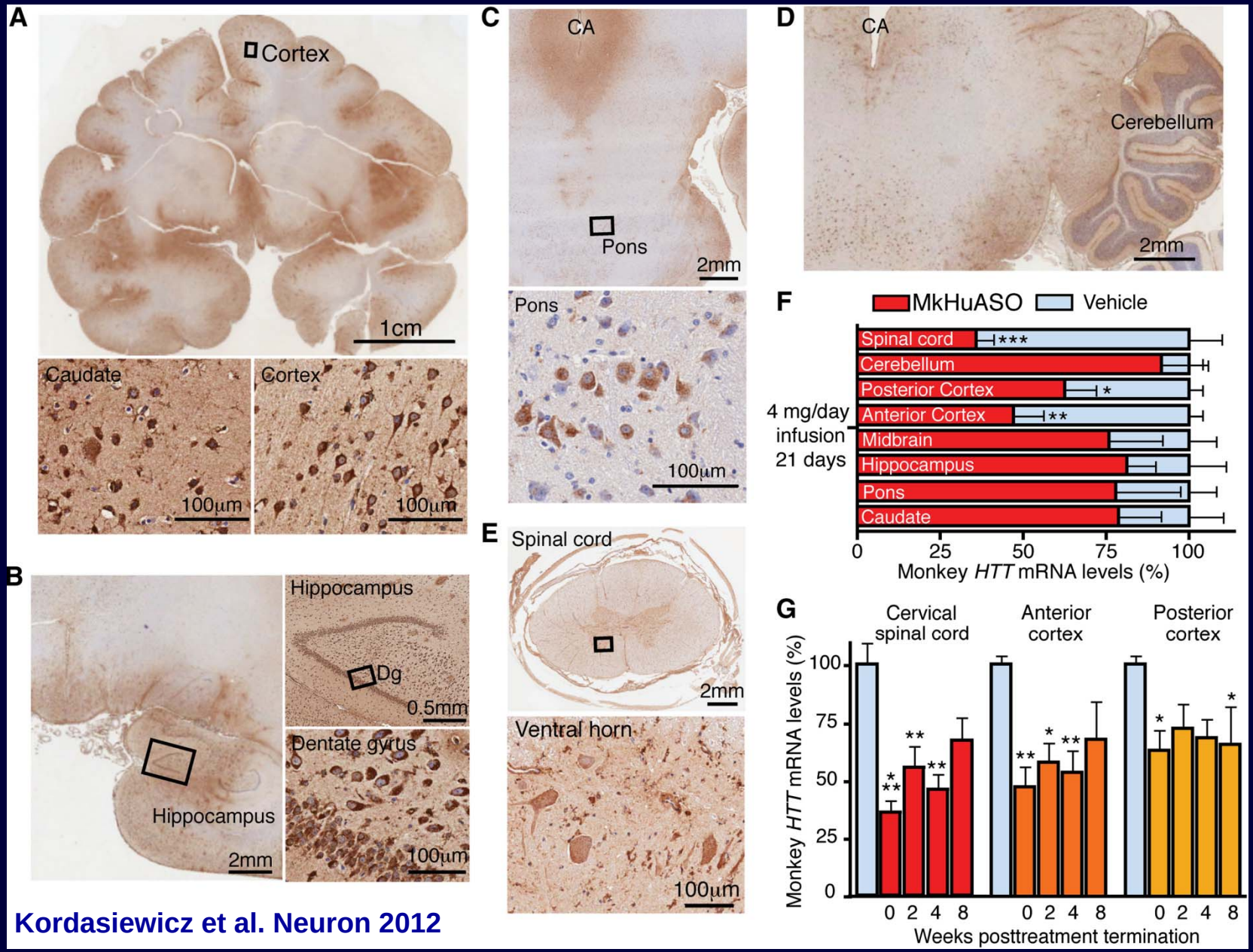
Cerebellum



Rhesus monkey brain

Anti oligo antibody: monoclonal antibody that specifically recognizes modified oligos
100 micrograms infused per day intraventricularly for 14 days

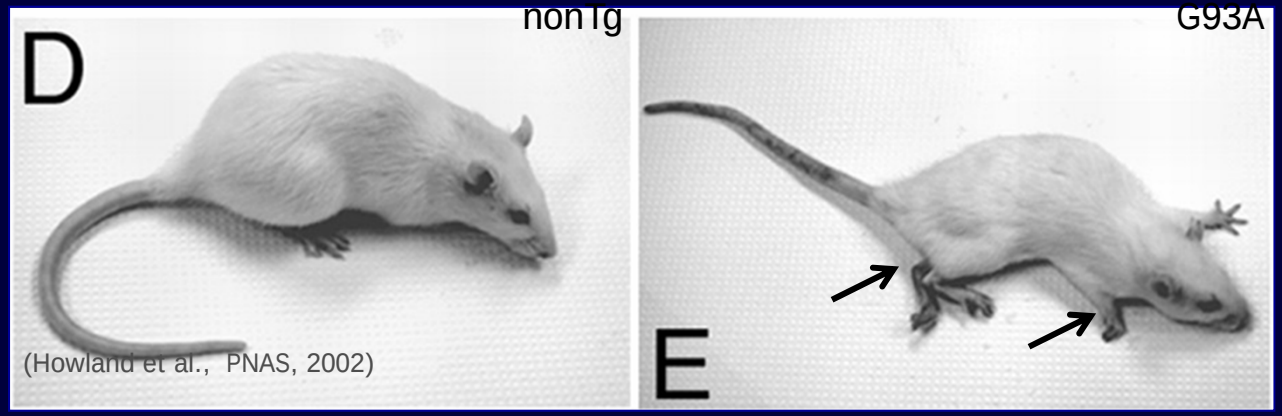
CSF infusion delivers SOD1 Antisense oligos widely



Kordasiewicz et al. Neuron 2012

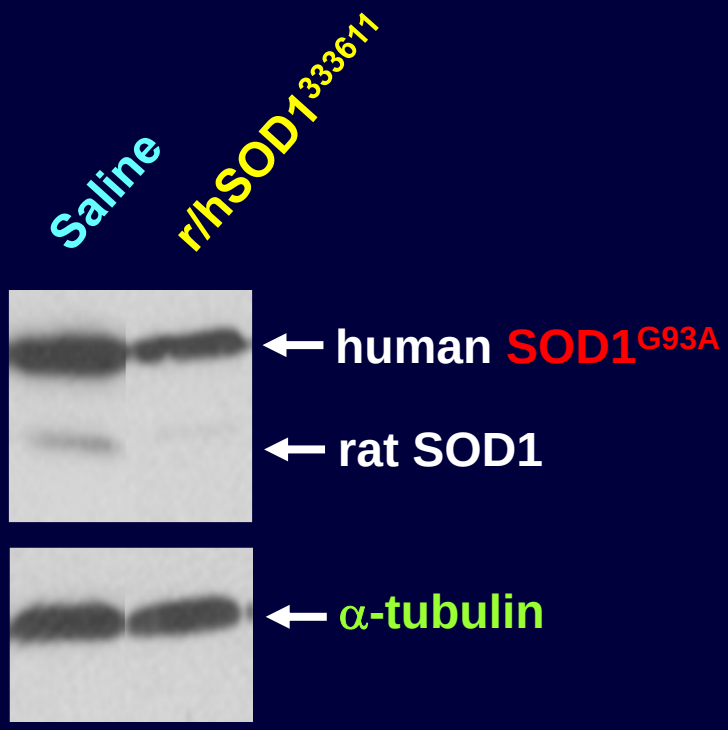
Mutant SOD1 Causes ALS-like phenotype in Rodents

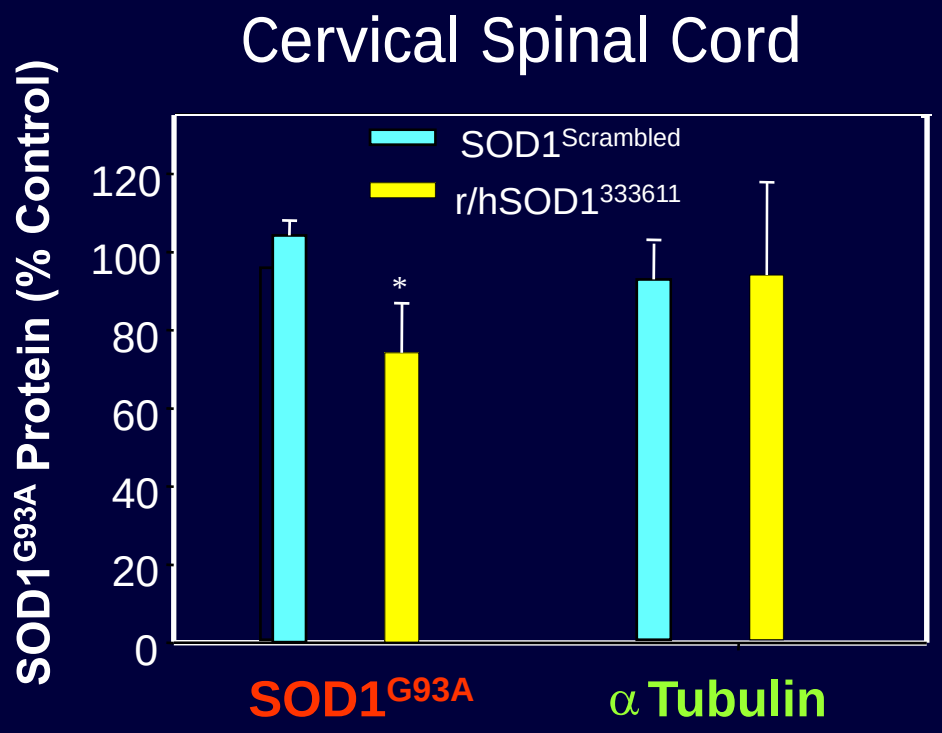
- Mice, rats develop weakness and atrophy
- $SOD1^{G93A}$ Rat



Richard Smith, Don Cleveland

Antisense SOD1 oligos decrease SOD1 protein in SOD1^{G93A} rat

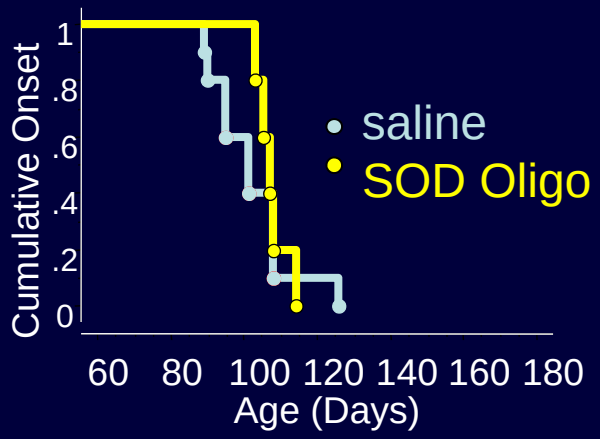




N=6, +/- SD

Treatment with SOD1 Oligo Extends Survival in SOD1^{G93A} Rat

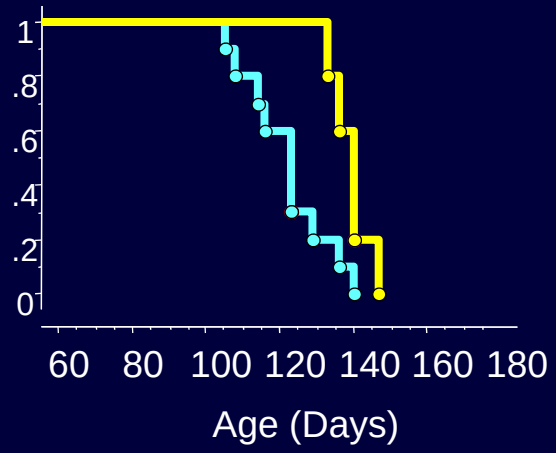
Onset



Saline: 102+/11

SOD1 Oligo: 107+/-4

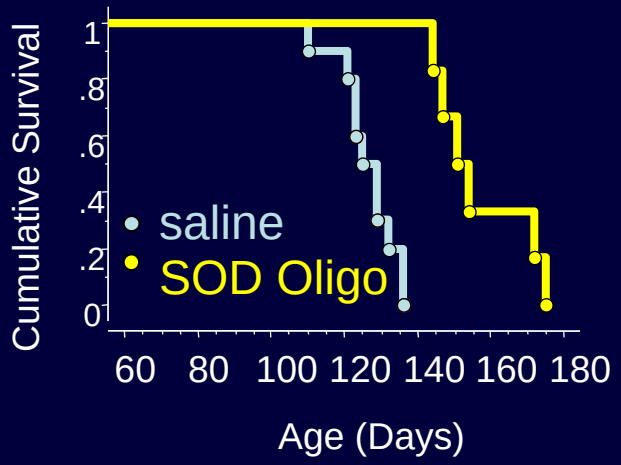
Early Disease



122+/-11

139+/-5

Survival



126+/-8

156+/-12

Doubling of survival *after* onset

N=12

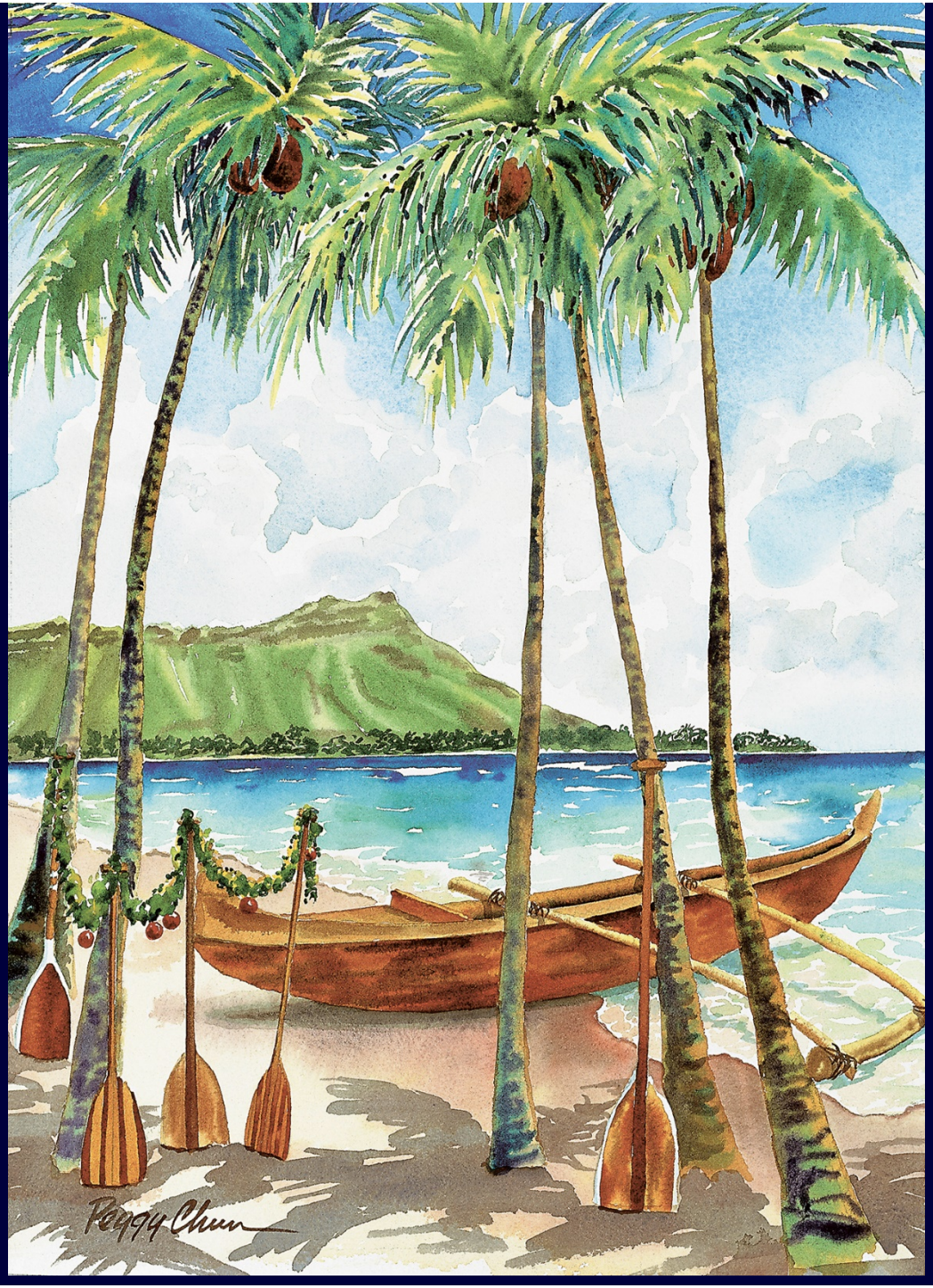
An antisense oligonucleotide against *SOD1* delivered intrathecally for patients with *SOD1* familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study

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

www.thelancet.com/neurology **Published online March 29, 2013**

Antisense Oligonucleotide in CNS in Humans

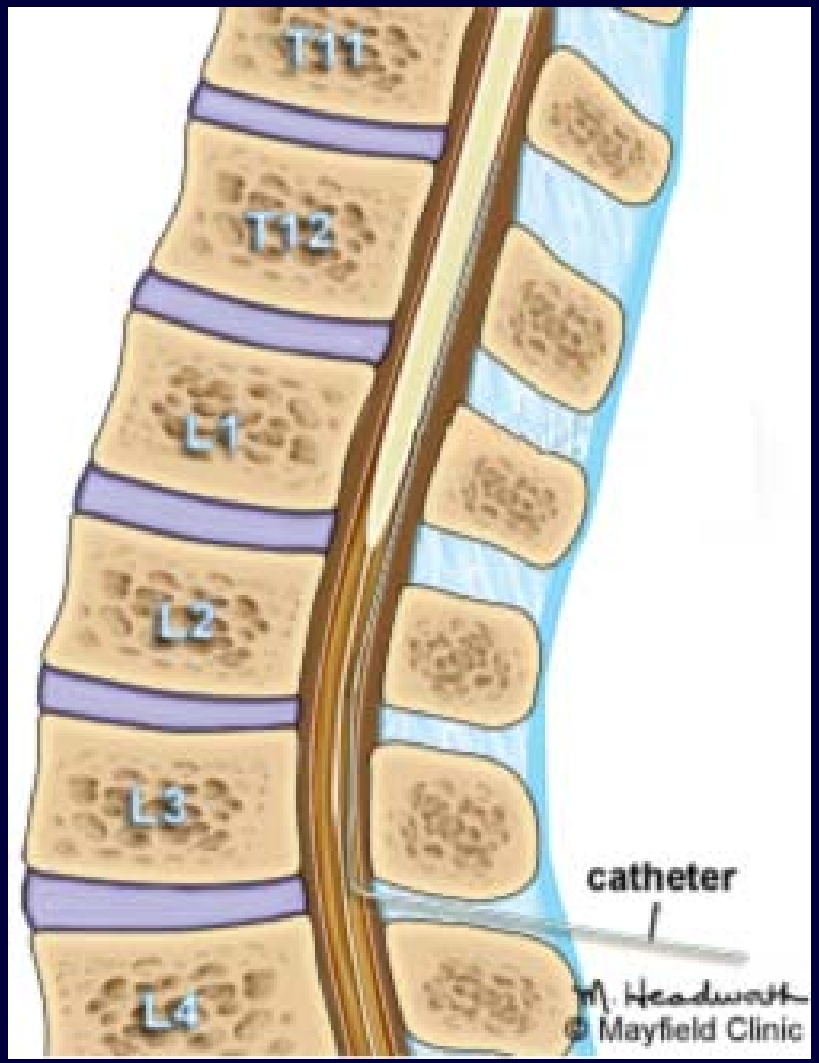
- 32 subjects, 21 individuals


Peggy Chun

Antisense Oligonucleotide in CNS in Humans

- 32 subjects, 21 individuals
- Received single, dose of Antisense oligonucleotide designed to lower SOD1 levels
- Intrathecal infusion for 12 hours
- Randomized, double-blind, placebo
- Doses (0.15 mg, 0.50 mg, 1.50 mg, 3.00 mg)

Intrathecal Infusion



Treatment-emergent Adverse Events

Adverse events listed are those that occurred with a frequency >5% (i.e. occurring in >1 ISIS-SOD1$_{Rx}$ patient) or were CTCAE grade 3 or greater in severity

Adverse Event Term	**ISIS-SOD1$_{Rx}$** % (# events)	**Placebo** % (# events)
Post-LP Syndrome	33% (8)	38% (5)
Back Pain	17% (4)	50% (4)
Nausea	13% (3)	0% (0)
Vomiting	8% (2)	0% (0)
Headache	8% (2)	13% (1)
Fall	8% (2)	0% (0)
Dizziness	8% (2)	0% (0)
Cerebral Infarct	0% (0)	13% (1)
Pneumonia	0% (0)	13% (1)
Cough	0% (0)	13% (1)

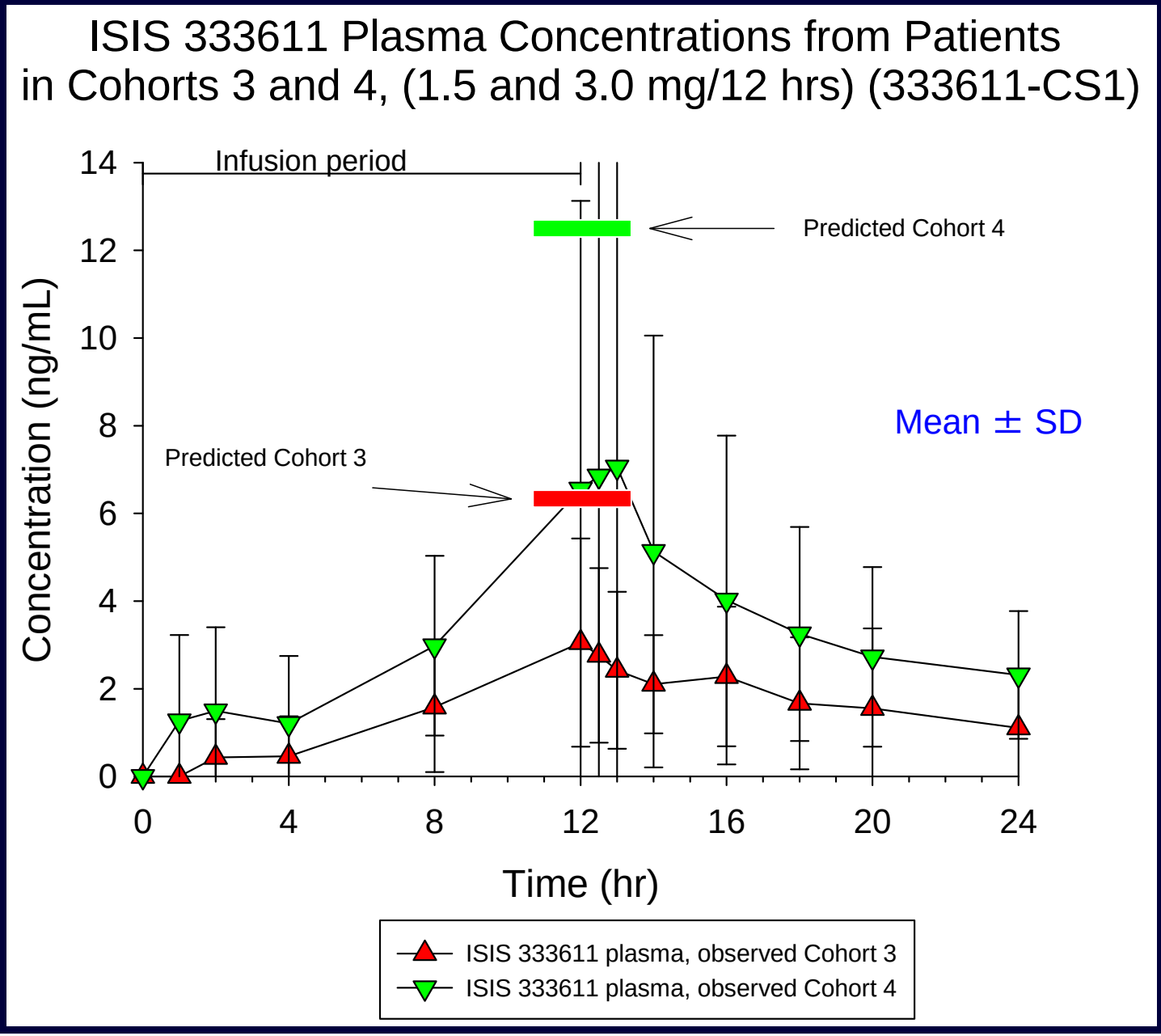
Post-LP syndrome, back pain, and nausea/vomiting incidences are not unexpected given the 17G Tuohy needle used for the infusion

ISIS-SOD1$_{Rx}$ Adverse Events are not Dose-Related

Adverse Event Term	**ISIS-SOD1$_{Rx}$** % (# events)	**Cohort Frequency** # events in Cohorts (1, 2, 3, 4)
Post-LP Syndrome	33% (8)	(4, 2, 1, 1)
Back Pain	17% (4)	(2, 1, 1, 0)
Nausea	13% (3)	(2, 0, 1, 0)
Vomiting	8% (2)	(2, 0, 0, 0)
Headache	8% (2)	(0, 2, 0, 0)
Fall	8% (2)	(1, 1, 0, 0)
Dizziness	8% (2)	(1, 0, 0, 1)

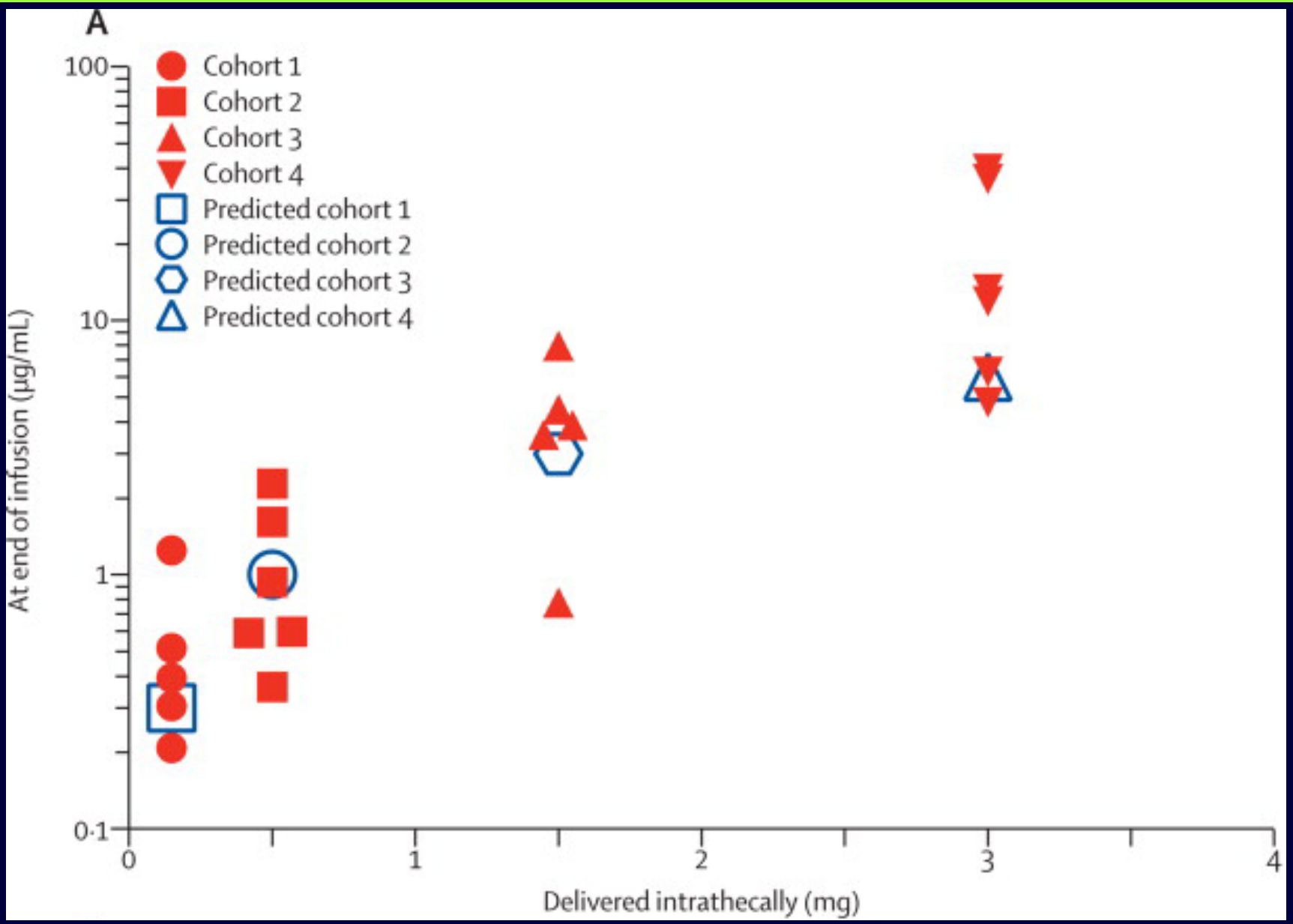
Pharmacokinetics

Plasma Concentrations Peak at End of 12-hr Infusion



Cohort 1,2 were <LLOD

Pharmacokinetics - CSF



Conclusions

- SOD1 ASO was very well tolerated at doses up to 3 mg;
  - No safety or tolerability concerns related to ASO were identified
- Dose dependent CSF and plasma concentrations were observed;
  - observed drug concentrations were reasonably consistent with expected values (generally within 2-fold)
- Results from this study suggest that antisense oligonucleotide delivery to the CNS may be a viable therapeutic strategy for neurological disorders

Antisense Oligos: C9ORF72

Targeting RNA Foci in iPSC-Derived Motor Neurons from ALS Patients with a *C9ORF72* Repeat Expansion

Dhruv Sareen,[1,2] Jacqueline G. O'Rourke,[1] Pratap Meera,[3] A. K. M. G. Muhammad,[1] Sharday Grant,[1] Megan Simpkinson,[1] Shaughn Bell,[1] Sharon Carmona,[1] Loren Ornelas,[1] Anais Sahabian,[1] Tania Gendron,[4] Leonard Petrucelli,[4] Michael Baughn,[5] John Ravits,[5] Matthew B. Harms,[6] Frank Rigo,[7] C. Frank Bennett,[7] Thomas S. Otis,[3] Clive N. Svendsen,[1,2] Robert H. Baloh[1,8]*

Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for amyotrophic lateral sclerosis and frontotemporal dementia

Clotilde Lagier-Tourenne[1,2,*], Michael Baughn[1,*], Frank Rigo[5], Shuying Sun[2,3], Patrick Liu[3], Hai-Ri Li[3], Jie Jiang[2,3], Andy Watt[5], Seung Chun[5], Melanie Katz[5], Jinsong Qiu[3], Ying Sun[1,3], Shuo-Chien Ling[1,3], Qiang Zhu[2,3], Magdalini Polymenidou[2,3,8], Kevin Drenner[1,2], Jonathan W. Artates[2,3], Melissa M. McAlonis[2,3], Sebastian Markmiller[3], Kasey R. Hutt[3], Donald P. Pizzo[4], Janet Cady[7], Matthew B. Harms[7], Robert H. Baloh[6], Scott R. VandenBerg[4], Gene W. Yeo[3], Xiang-Dong Fu[3], C. Frank Bennett[5], Don W. Cleveland[1,2,3,+], and John Ravits[1,+]

RNA Toxicity from the ALS/FTD *C9ORF72* Expansion Is Mitigated by Antisense Intervention

Christopher J. Donnelly,[1,5] Ping-Wu Zhang,[1,5] Jacqueline T. Pham,[3] Aaron R. Heusler,[4] Nipun A. Mistry,[1,5] Svetlana Vidensky,[1,5] Elizabeth L. Daley,[1,5] Erin M. Poth,[2] Benjamin Hoover,[1,5] Daniel M. Fines,[1,5] Nicholas Maragakis,[1] Pentti J. Tienari,[6] Leonard Petrucelli,[7] Bryan J. Traynor,[1,8] Jiou Wang,[2,4] Frank Rigo,[9] C. Frank Bennett,[9] Seth Blackshaw,[2] Rita Sattler,[1,5,10,*] and Jeffrey D. Rothstein[1,2,3,5,10,*]

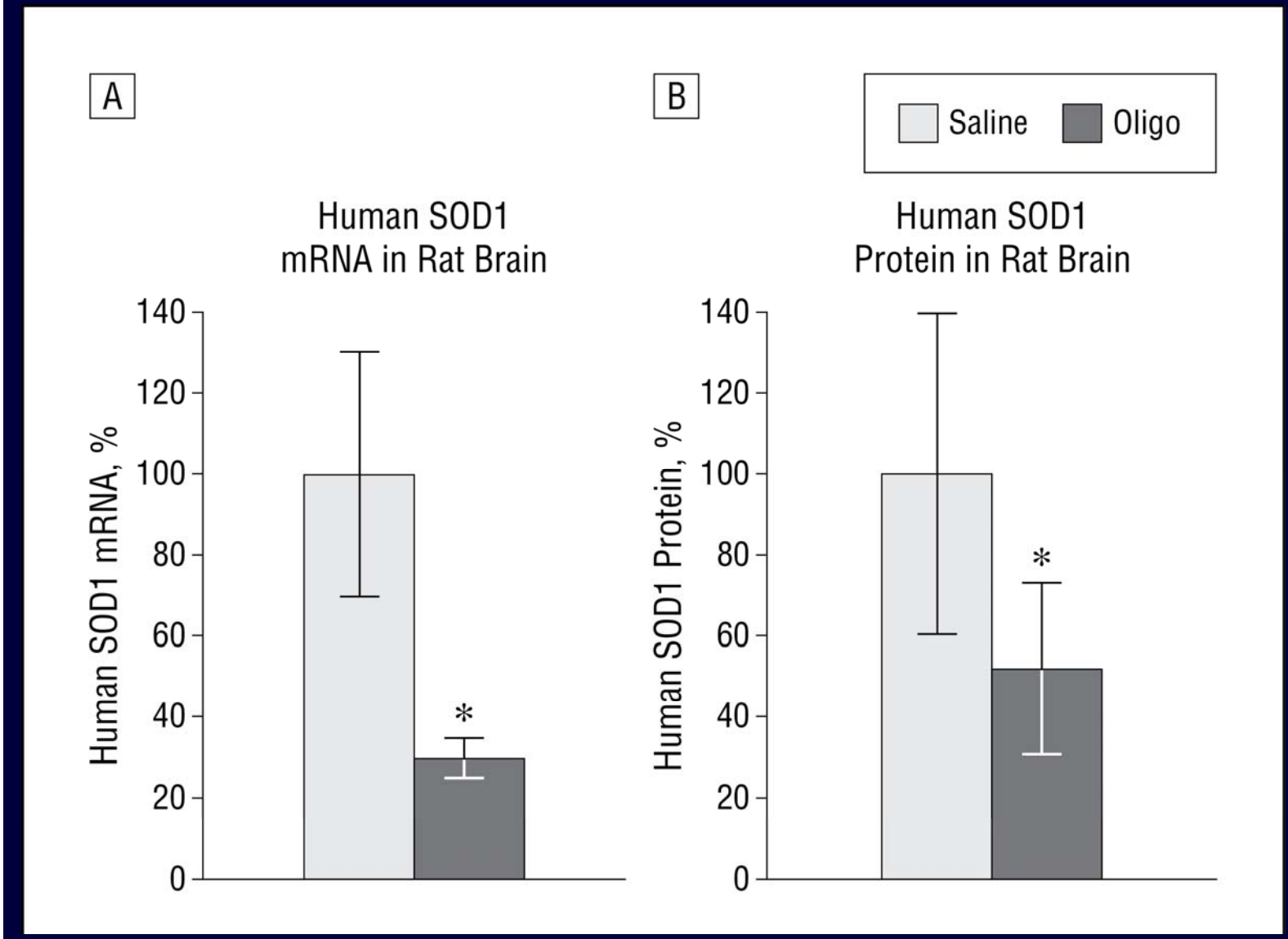
Planning for SOD1 Phase II

- Natural history of SOD1
- SOD1 as a pharmacodynamics marker?

SOD1 as a Biomarker in CSF

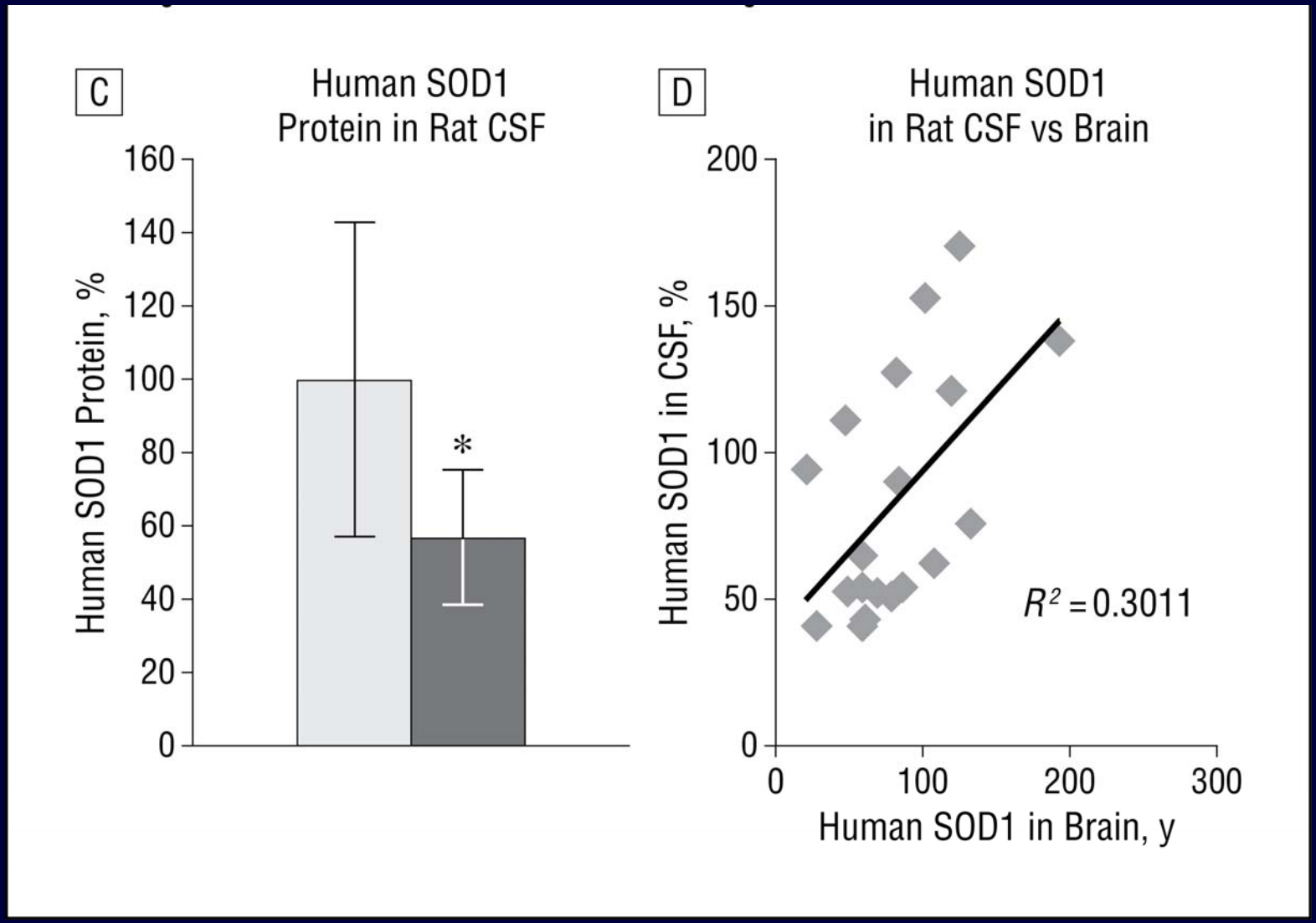
- Does SOD1 in CSF reflect brain SOD1?
- Is SOD1 stable over time?

Antisense Oligo Decreases SOD1 in CSF



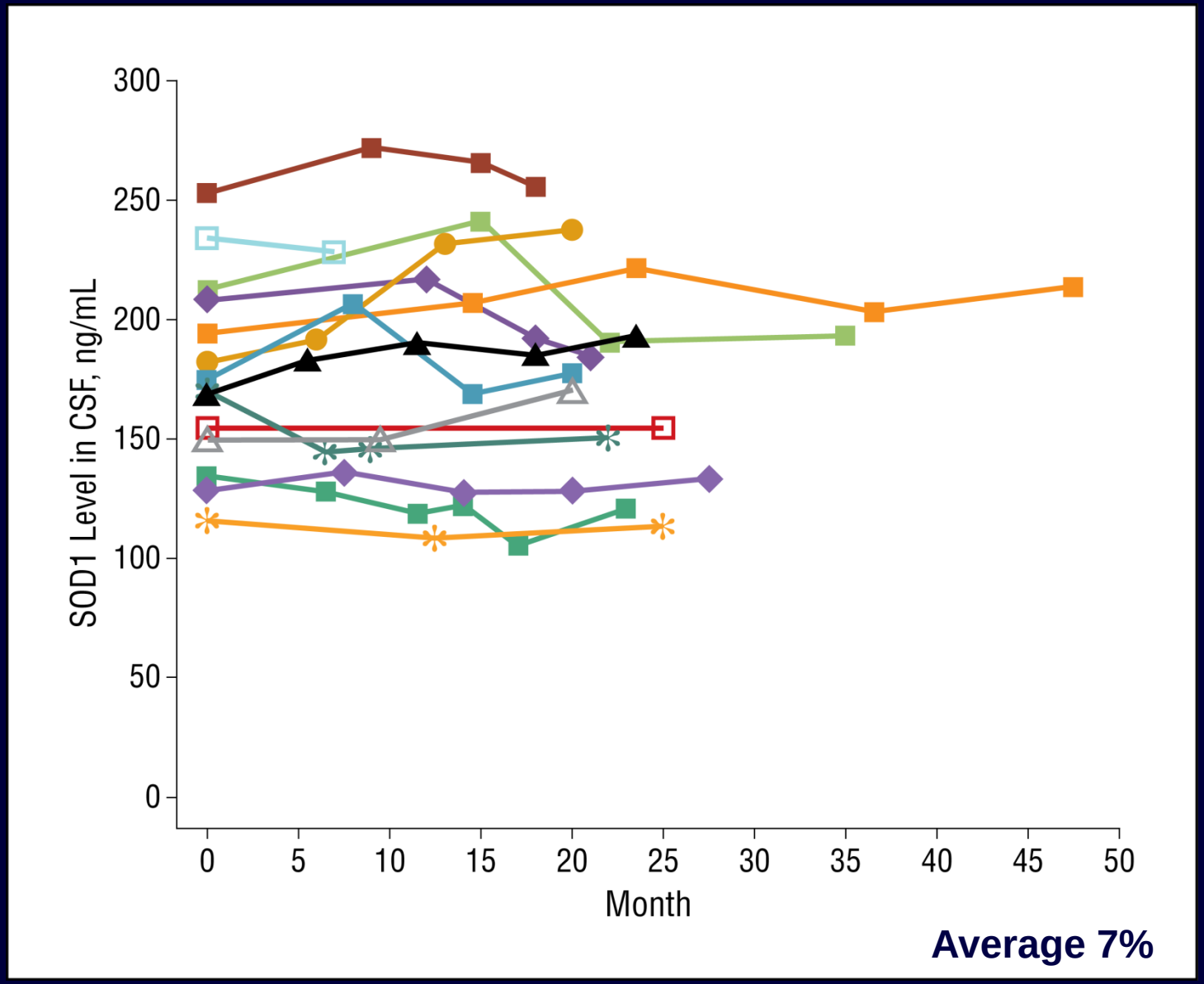
Winer et al. , JAMA Neurology 2013

Antisense Oligo Decreases SOD1 in CSF



Winer et al. , JAMA Neurology 2013

SOD1 in CSF Varies Little Over Time



Bob Bowser
David Lacomis

CSF SOD1 as a Pharmacodynamic Marker

- SOD1 Knockdown in brain leads to knockdown in CSF
- SOD1 CSF varies little with repeat measurements

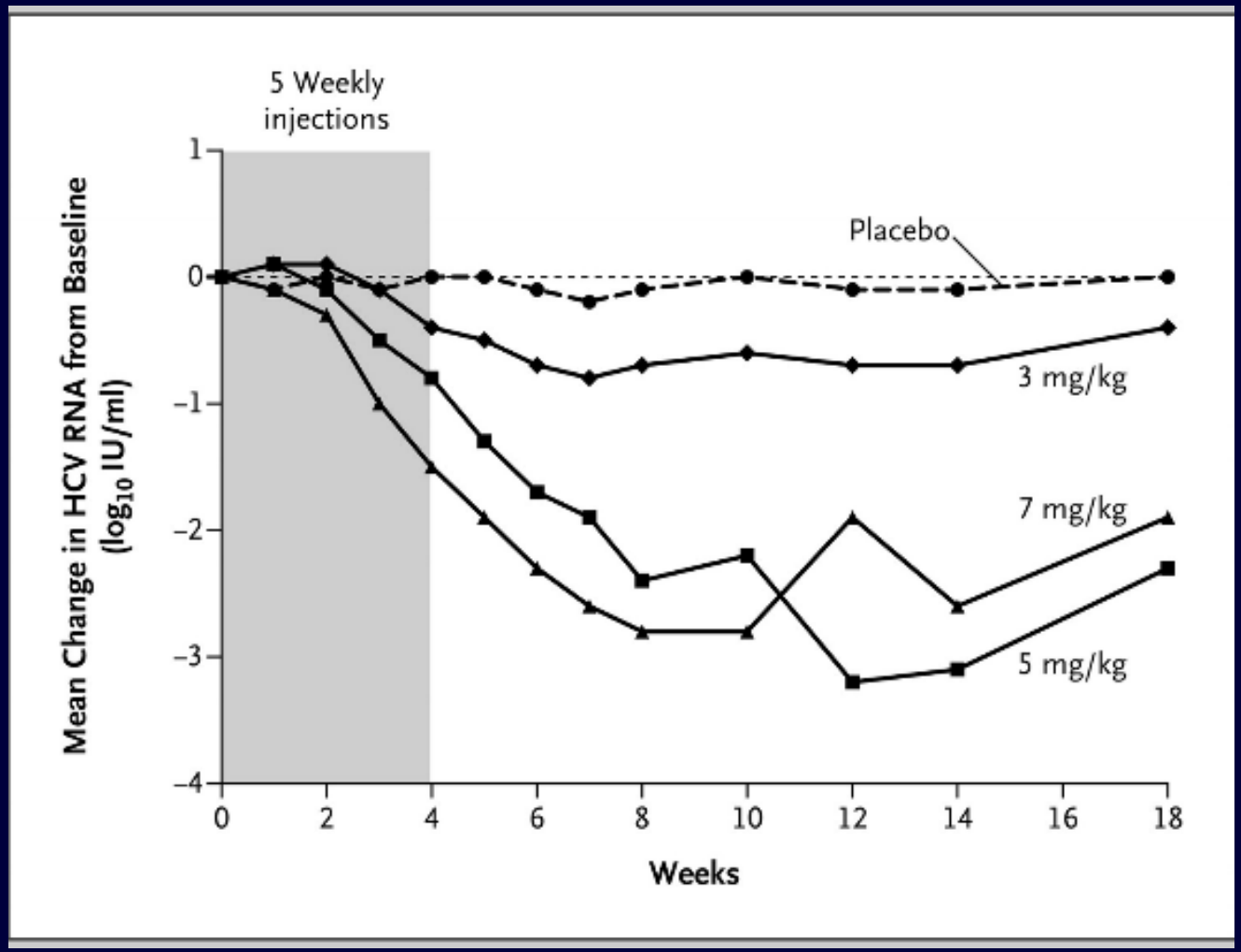
SOD1 half life?

microRNAs

- Discovered in 1993
  - 2nd discovered in 2000
- Translational repressors; 18-22nt long
- Partial complementarity
  - Seed region
  - Typically 200-300 mRNAs

miRNA Antisense Oligonucleotide Safety:

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

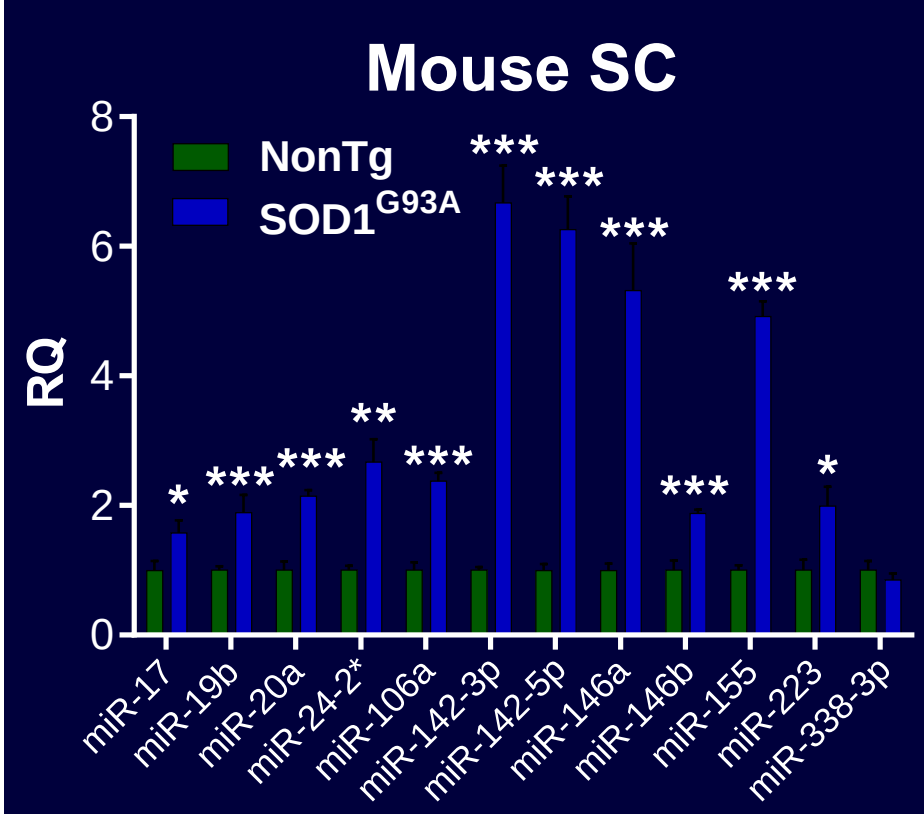


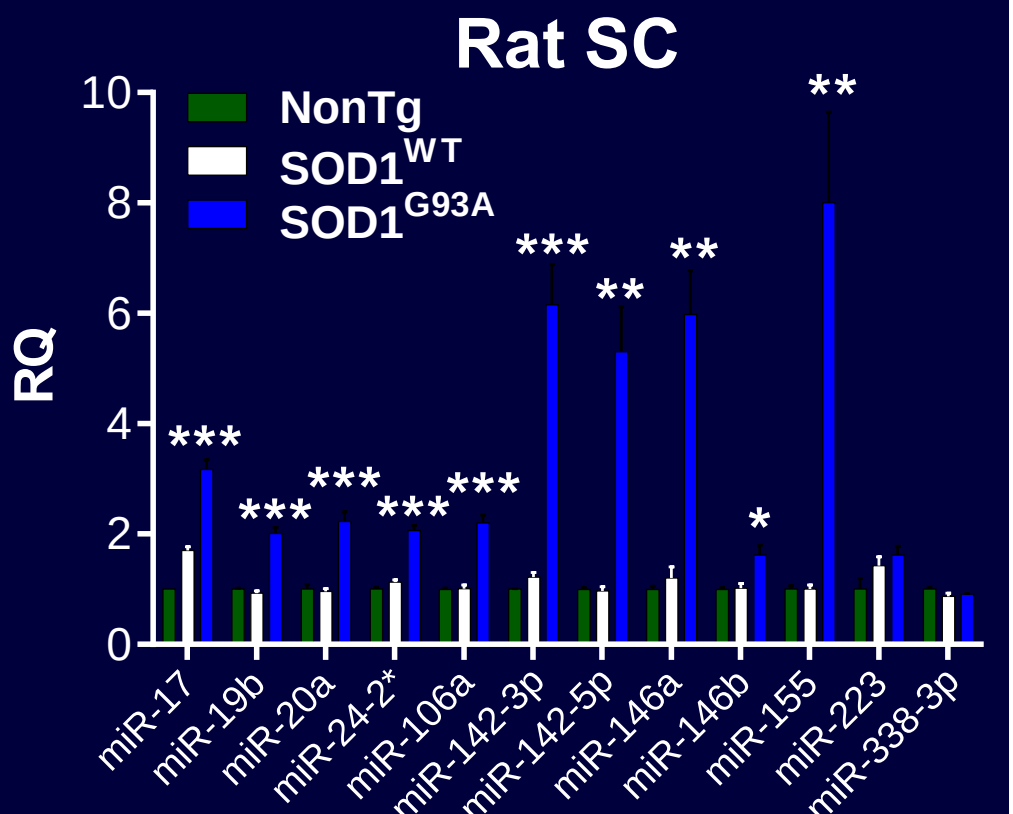
Janssen et al, NEJM 2013.

miRNAs as Targets for ALS Therapeutics

- Identify dysregulated microRNAs in ALS
  - In rodent model and in patients
- Develop method for inhibiting these miRNAs throughout CNS
- Determine if these miRNAs negatively or positively influence disease progression

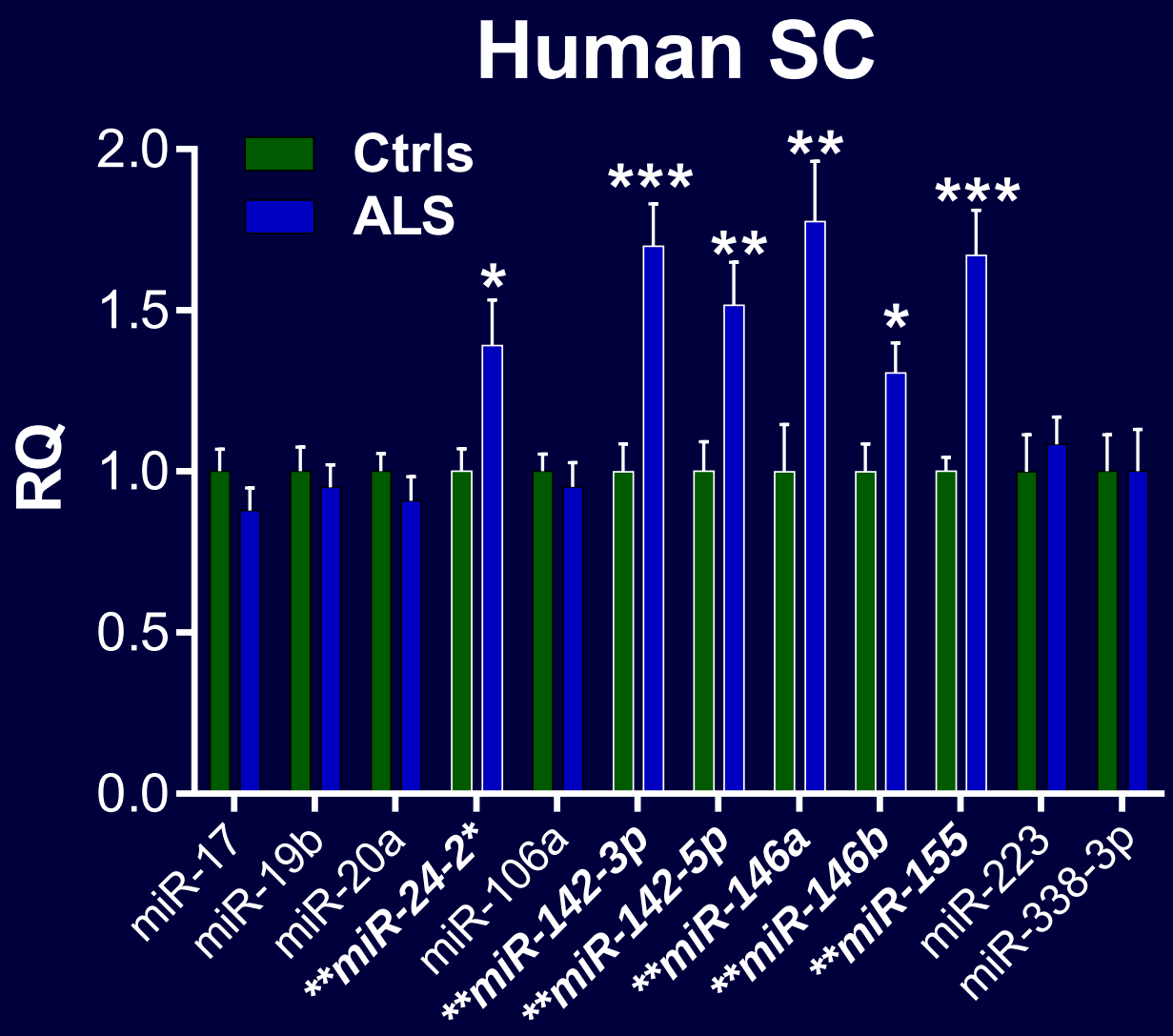
10 array hits confirmed





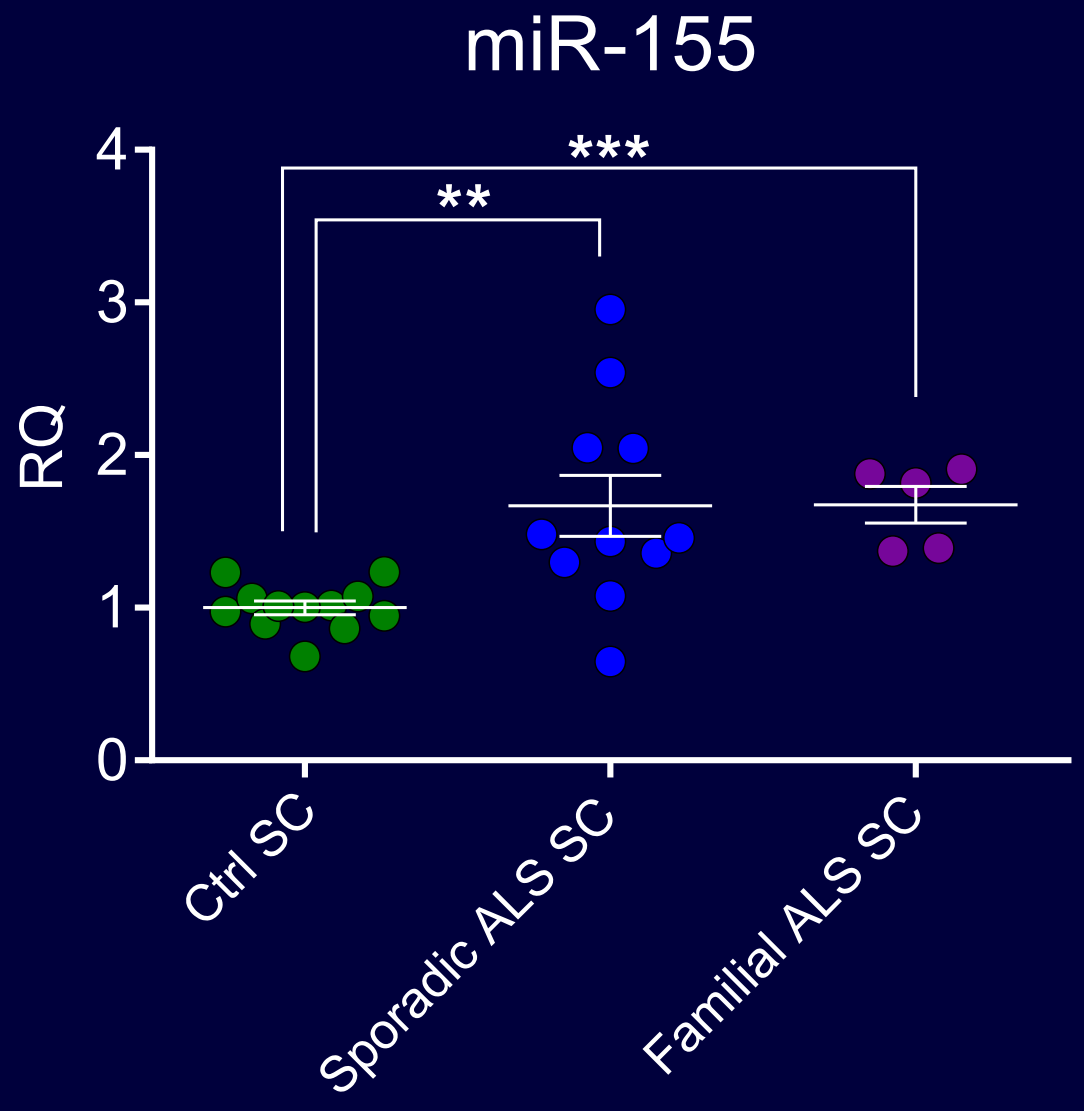
Symptomatic SOD1^{G93A} mice and rats

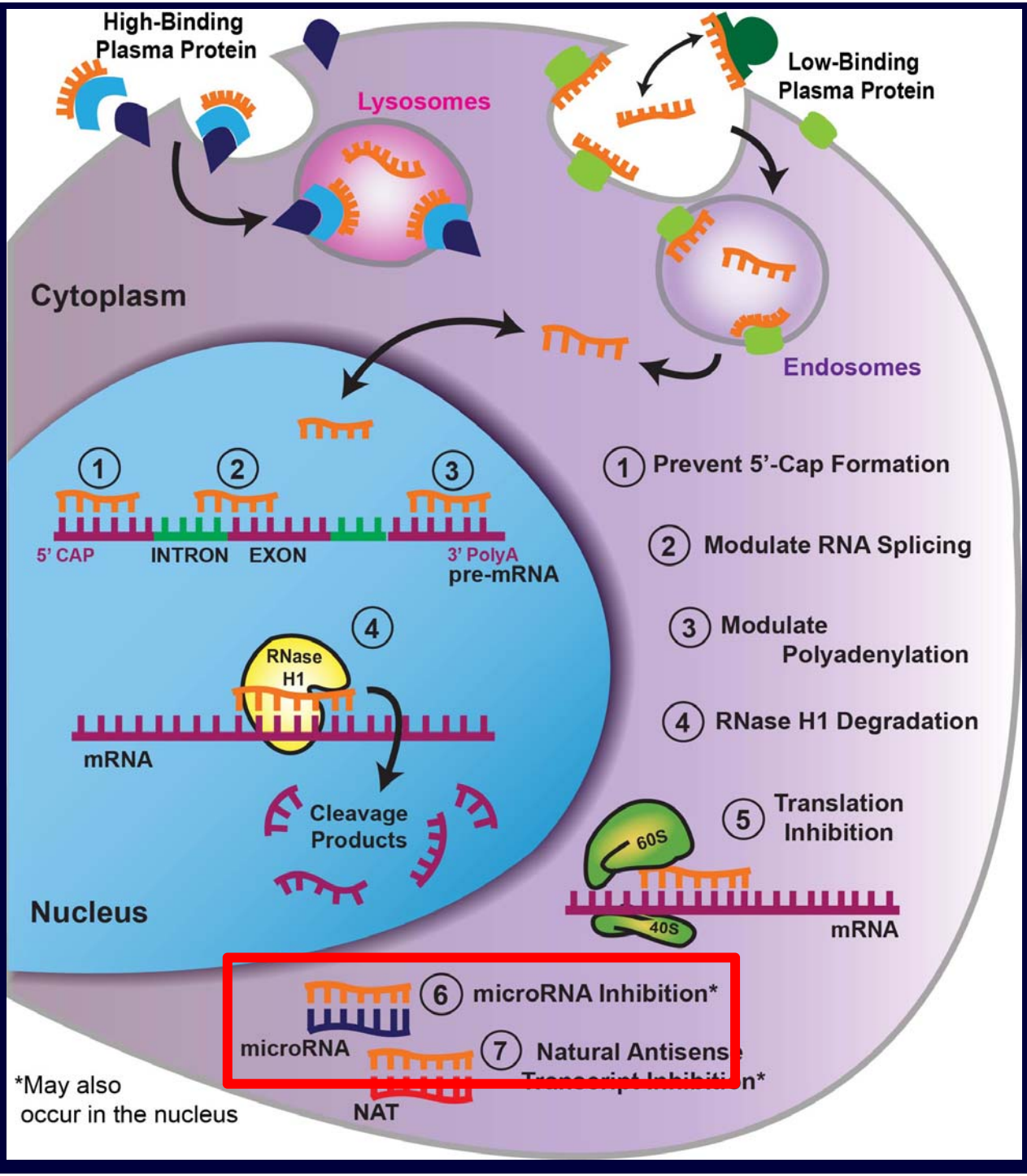
Human Tissues Identifies 6 Best Targets



Koval et al. Hum Mol Genet 2013

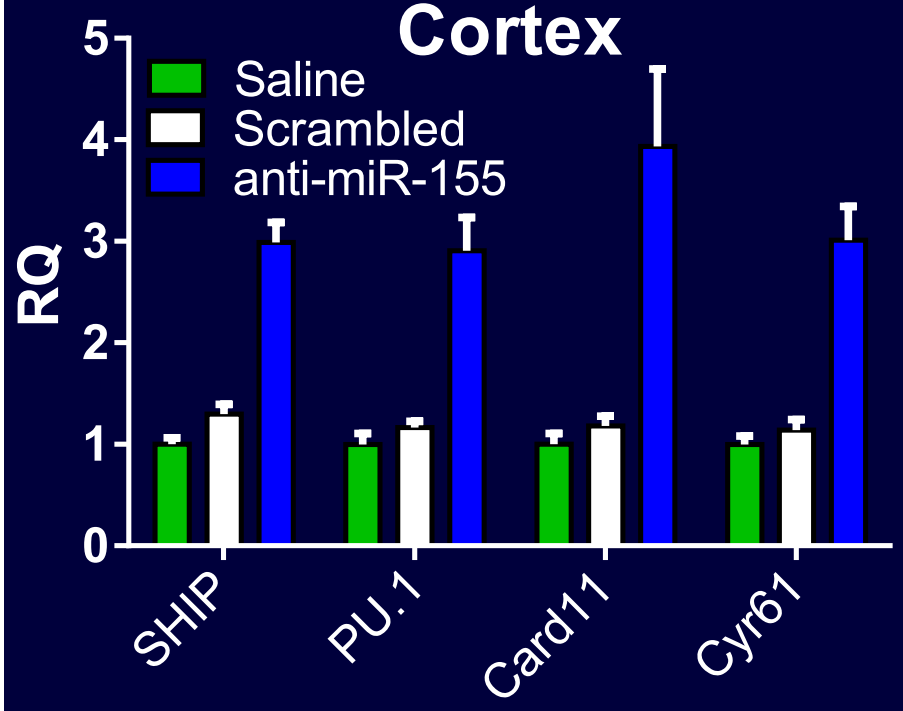
MiR-155 is increased in human ALS

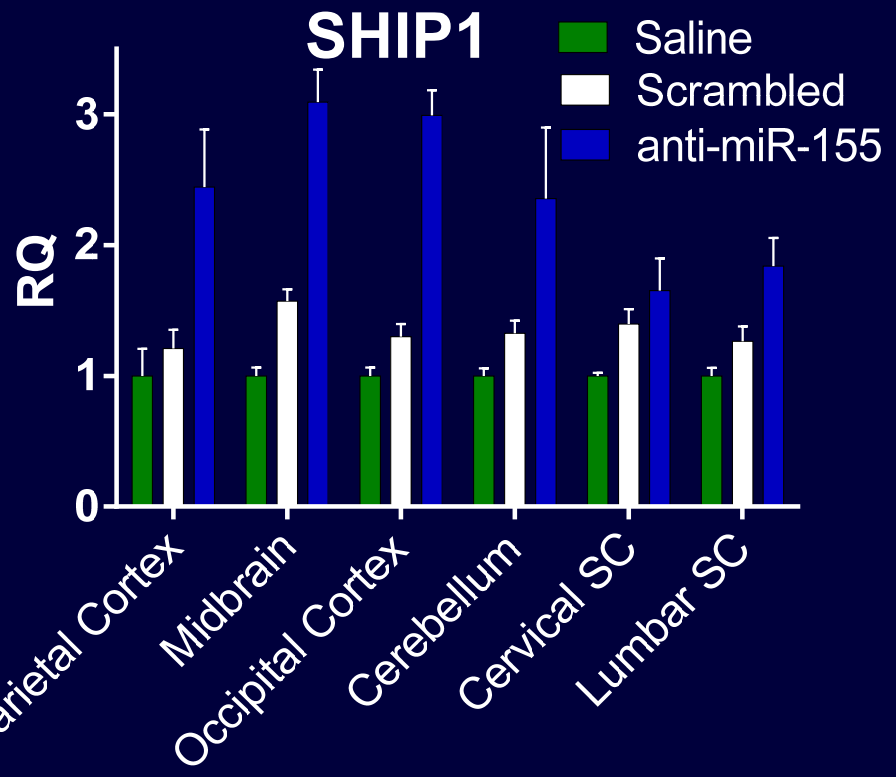




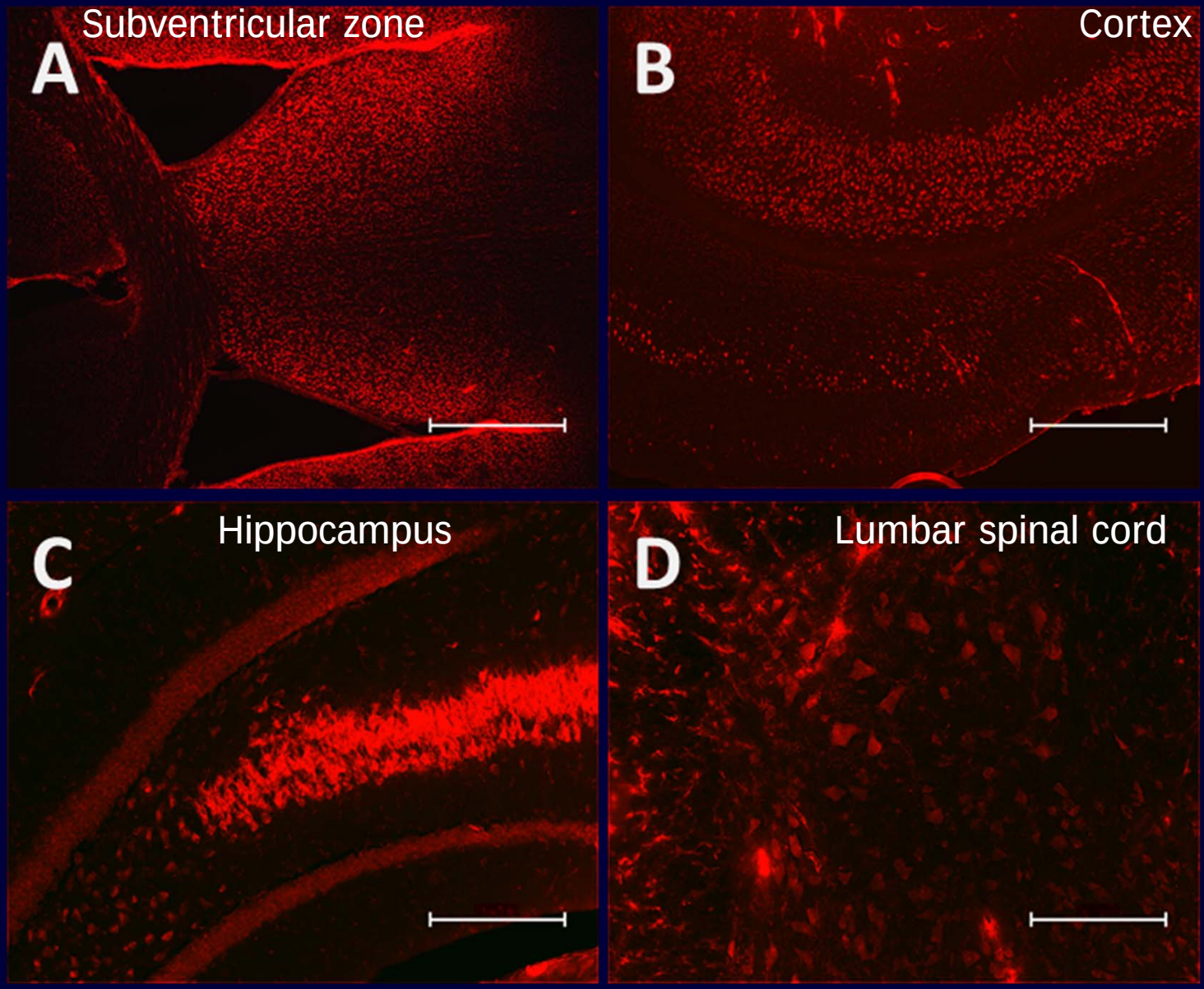
DeVos and Miller, 2013

anti-miR-155 is functional throughout CNS

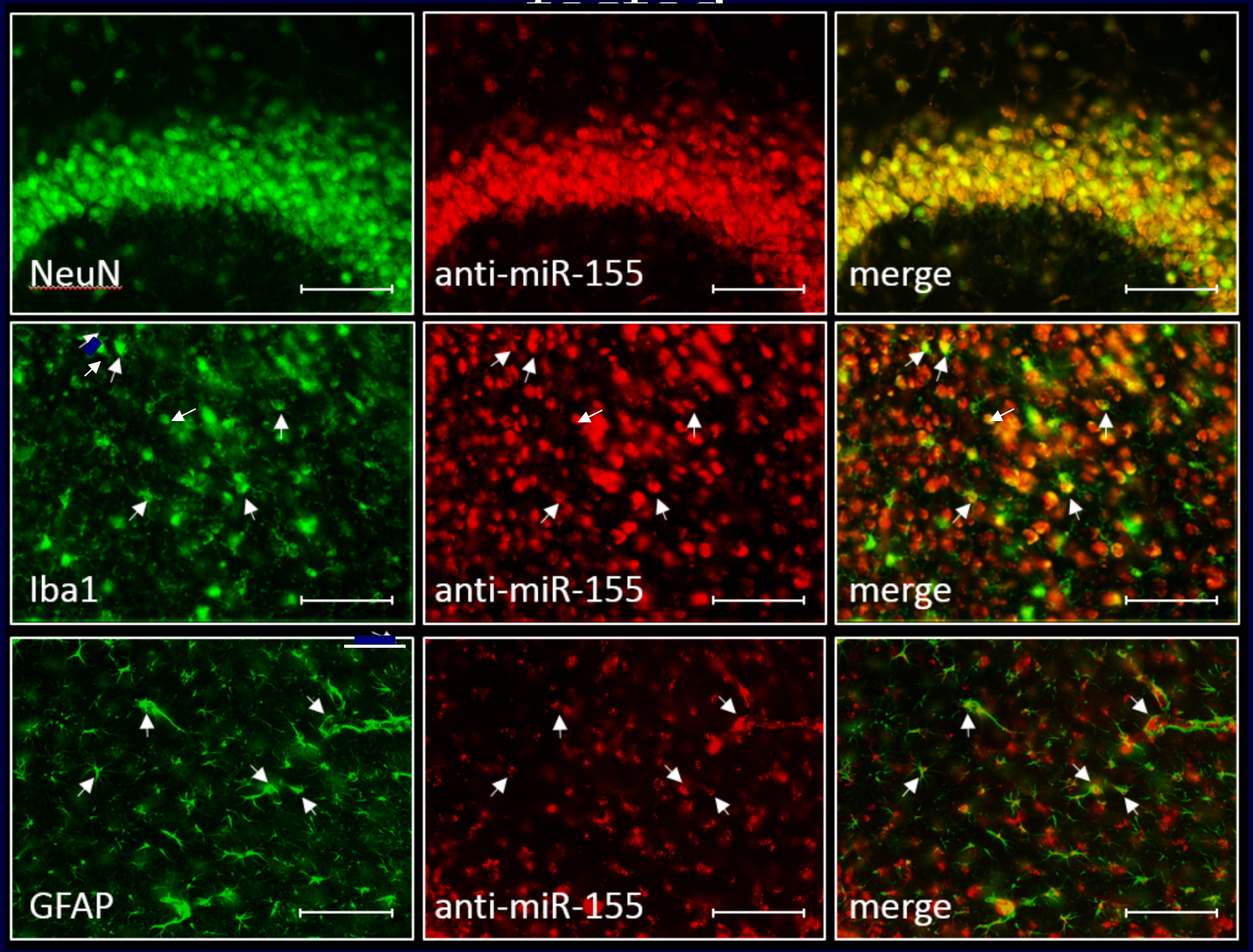




Cy3-anti-miR-155 distributes throughout CNS

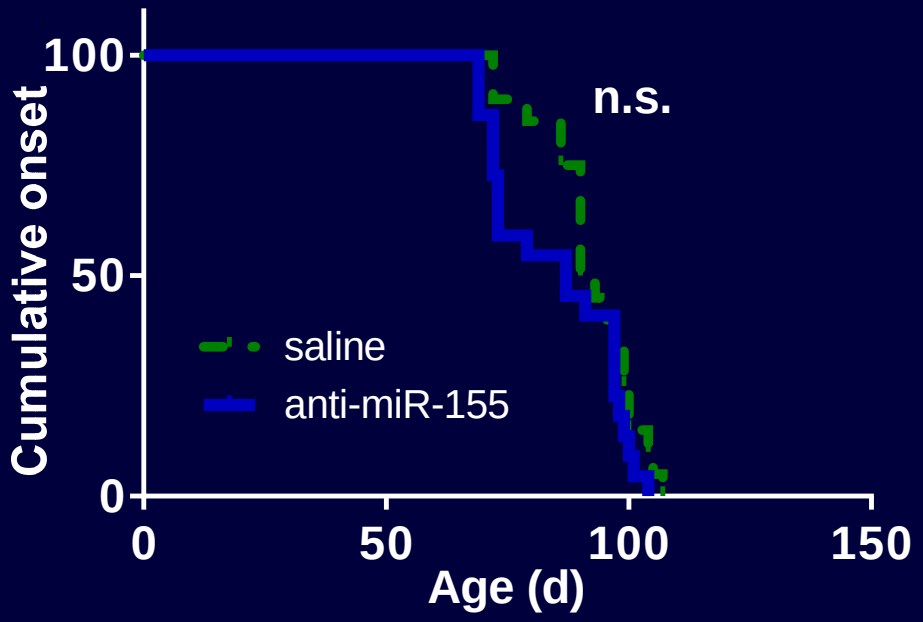


Anti-miR-155 is present in all cell types tested

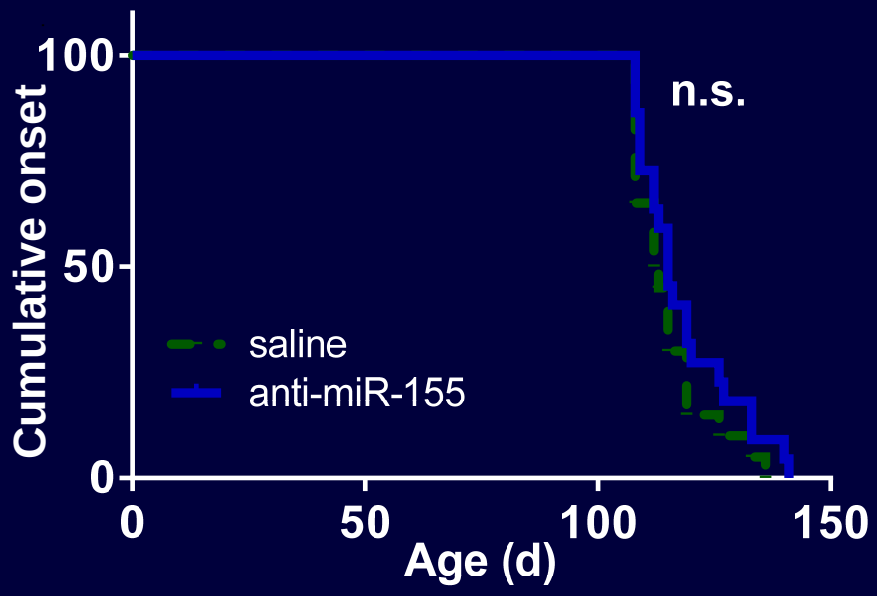


Anti-Mir-155 Does not Change Onset

Weight Peak

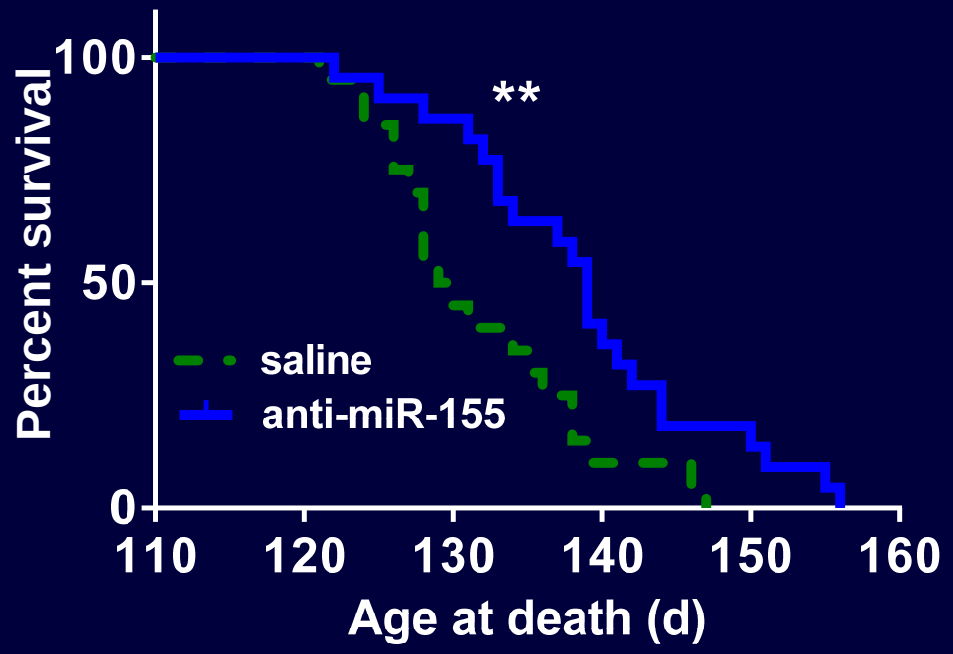


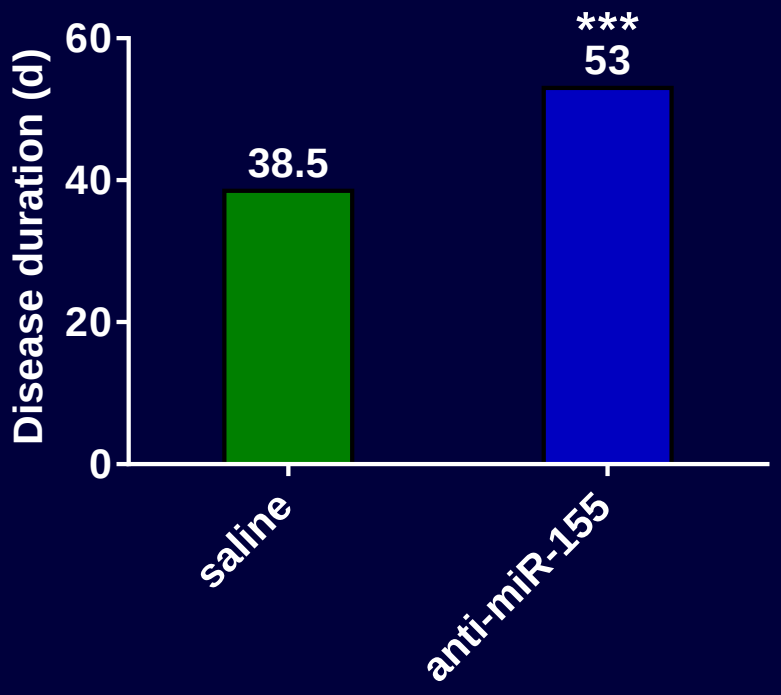
Neuroscore 1



SOD1^{G93A} mice, treated at 60 days of age both intraventricularly and intraperitoneally

Anti-miR-155 Extends Disease Duration





10 day extension p=0.007

38% increase p<0.001

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 exciting therapeutic target
  - miR-155 negatively contributes to disease
  - Implications for both sALS and fALS
  - Significant increase in survival
  - Can read miR-155 in peripheral blood cells

Remaining questions

- Mechanism of how miR-155 influences disease
- Which CNS cells express miR-155?
- Other miRNAs?
- miR-155 clinical trial?

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