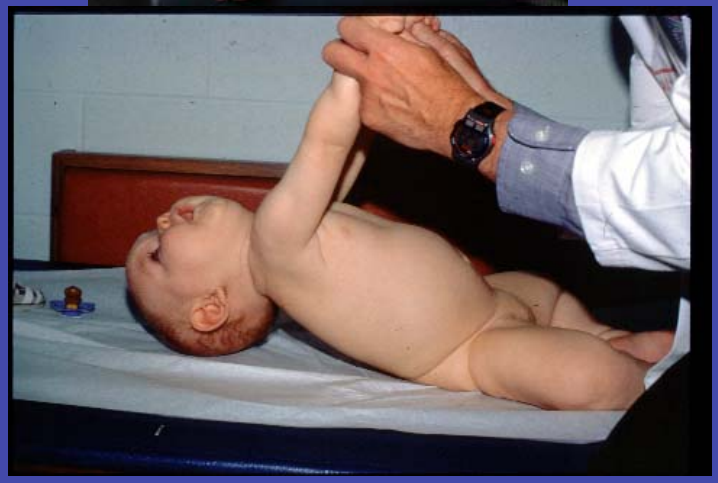
Proximal Spinal Muscular Atrophy

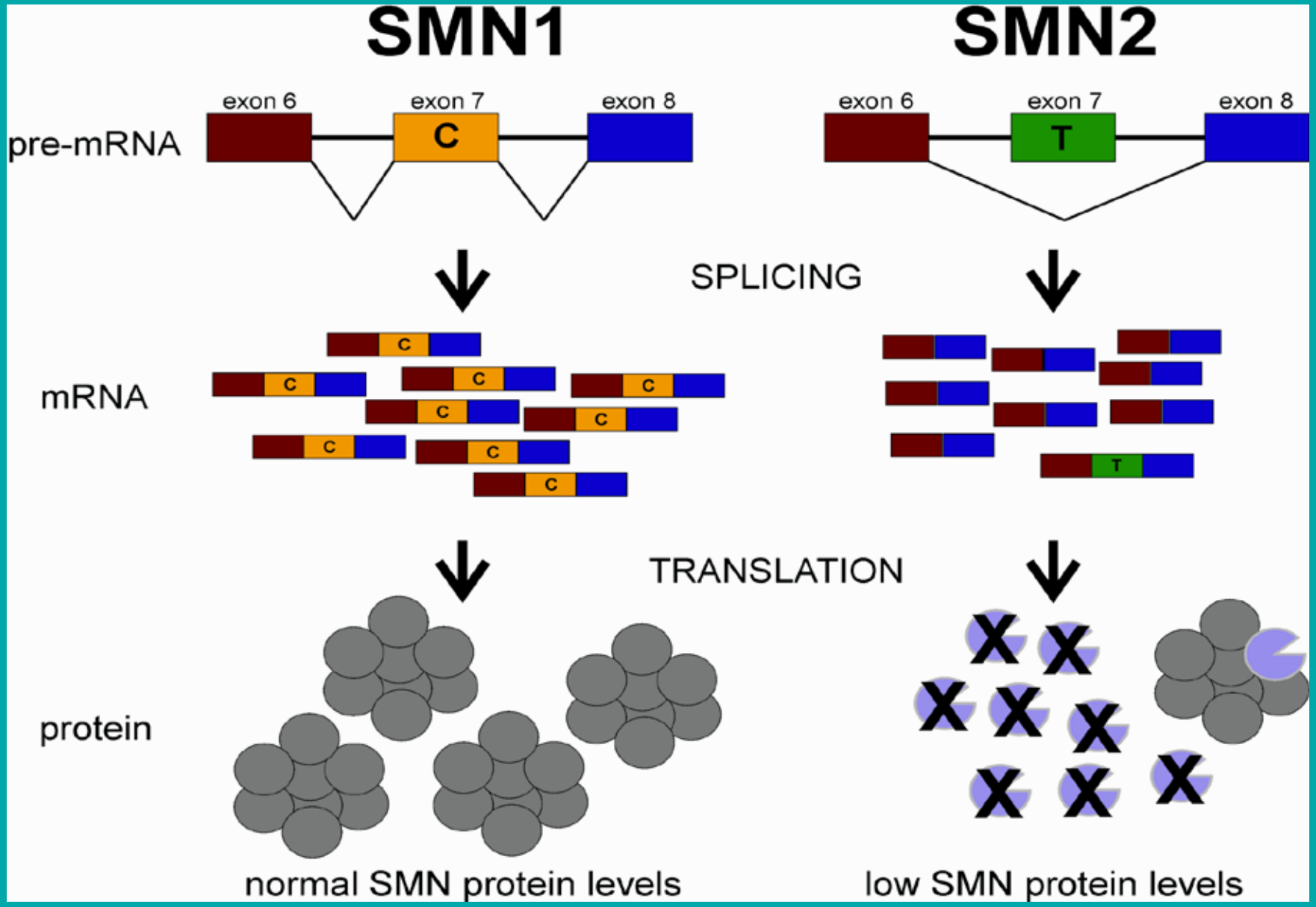
- autosomal recessive motor neuron disease
- incidence: 1:10,000 live births
- carrier frequency: 1:35
- loss of spinal α motor neurons in anterior horn of spinal cord
- atrophy of limb and trunk muscles
- clinical grades
  - Type I (Werdnig-Hoffmann): cannot sit or walk; death>2 yr
  - Type II: able to sit but cannot walk unaided; death<4 yr
  - Type III (Kugelberg-Welander): can walk unaided initially; progressive proximal muscle weakness; normal lifespan
  - Type IV (adult onset): very mild phenotype; normal lifespan

SMA Type 1
Acute Werdnig-Hoffman

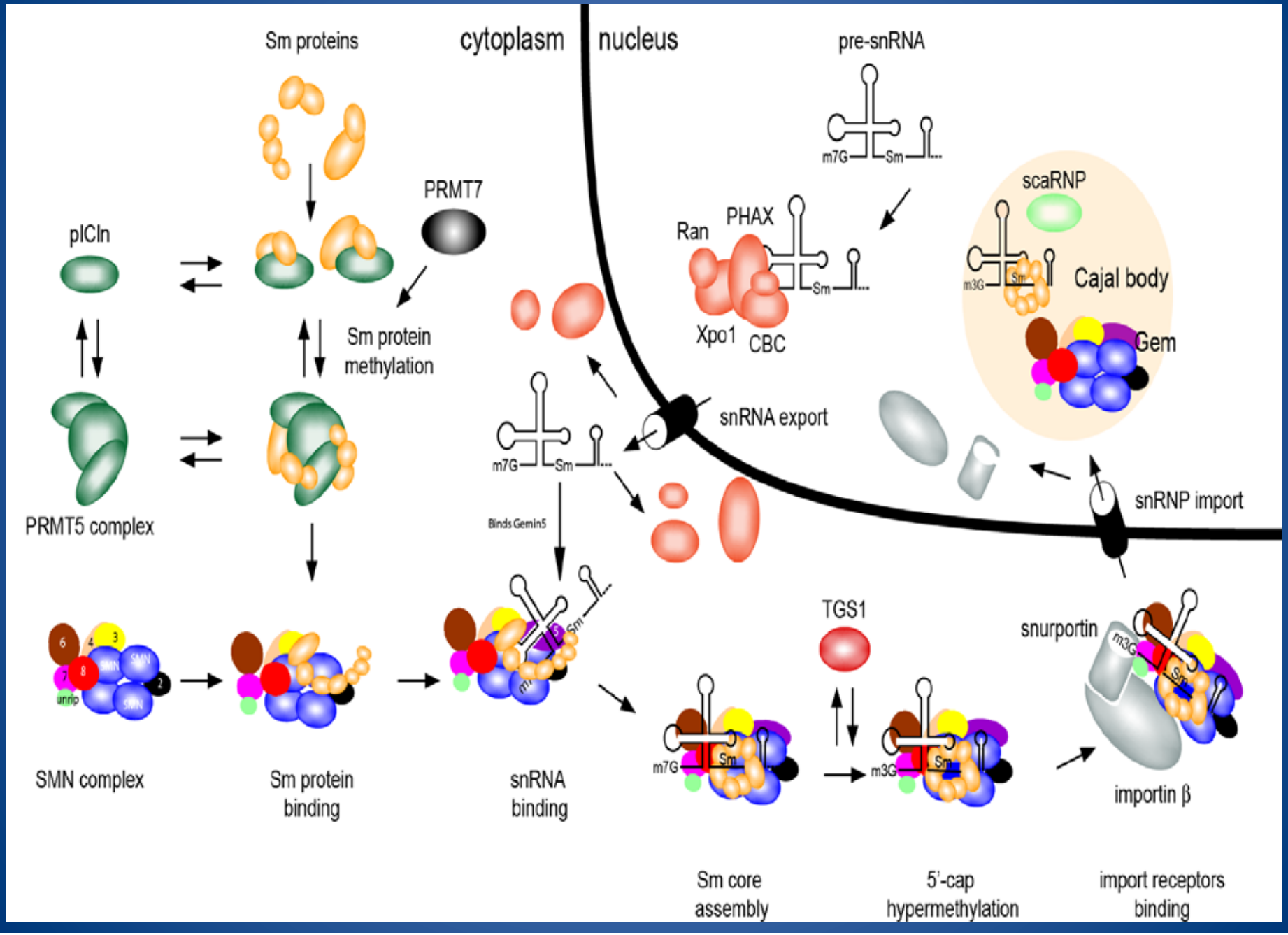
- Onset < 6 mos.
- Severe hypotonia, weakness; legs > arms
  - Poor head control
- Bulbar muscle weakness
  - Weak cry, suck, swallow
  - Tongue fascics in 50%
- Reflexes usually absent
- Bell-shaped chest
  Respiratory distress
  - **Death < 2 years**



SMA is caused by loss of SMN1 but retention of SMN2



SMN is in a complex that assembles Sm protein onto snRNA



SMA is caused by loss of SMN1 and retention of SMN2, thus not enough SMN. SMA mice lack mouse Smn and have 2 Copies of SMN2.

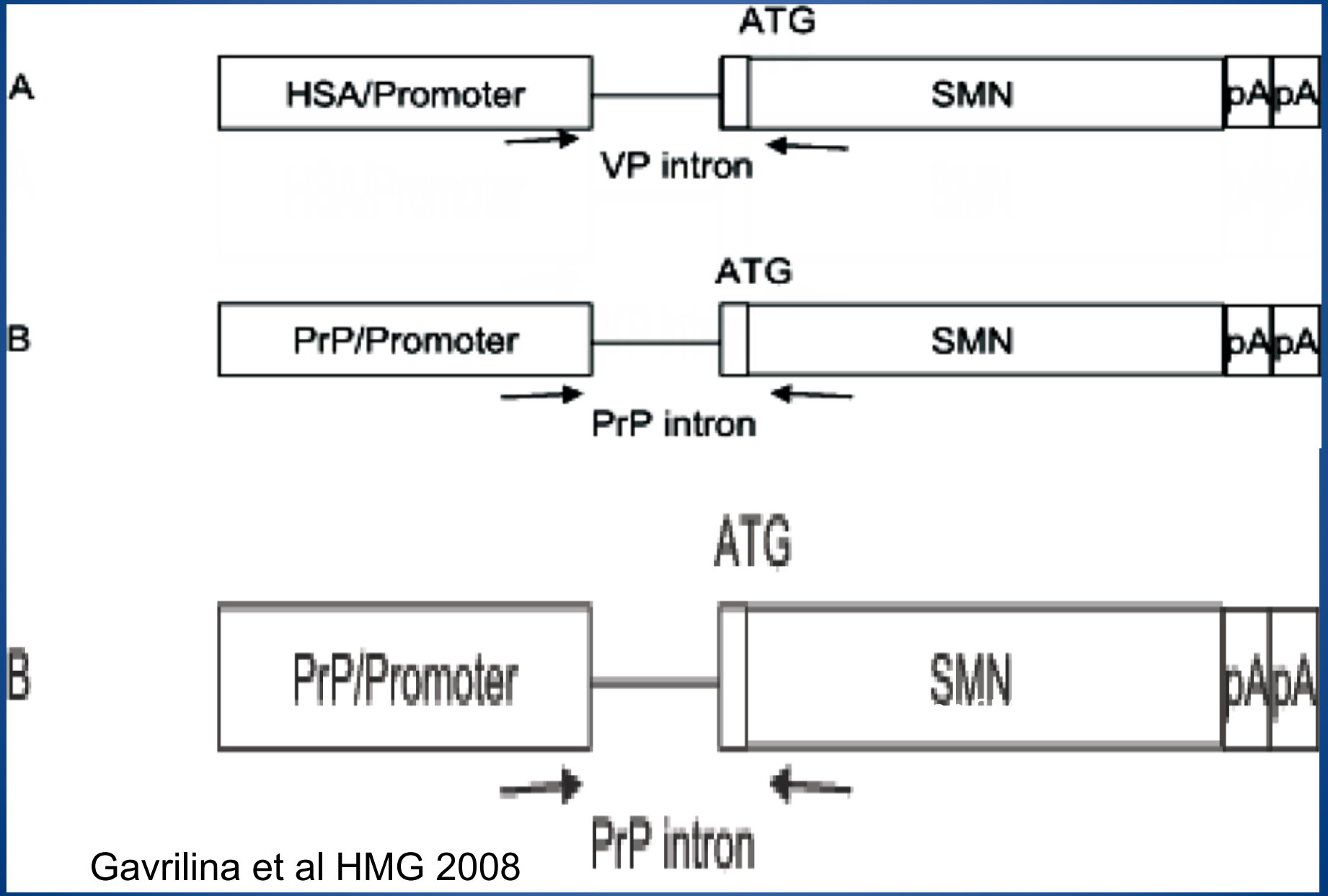
SMN questions for therapy

?

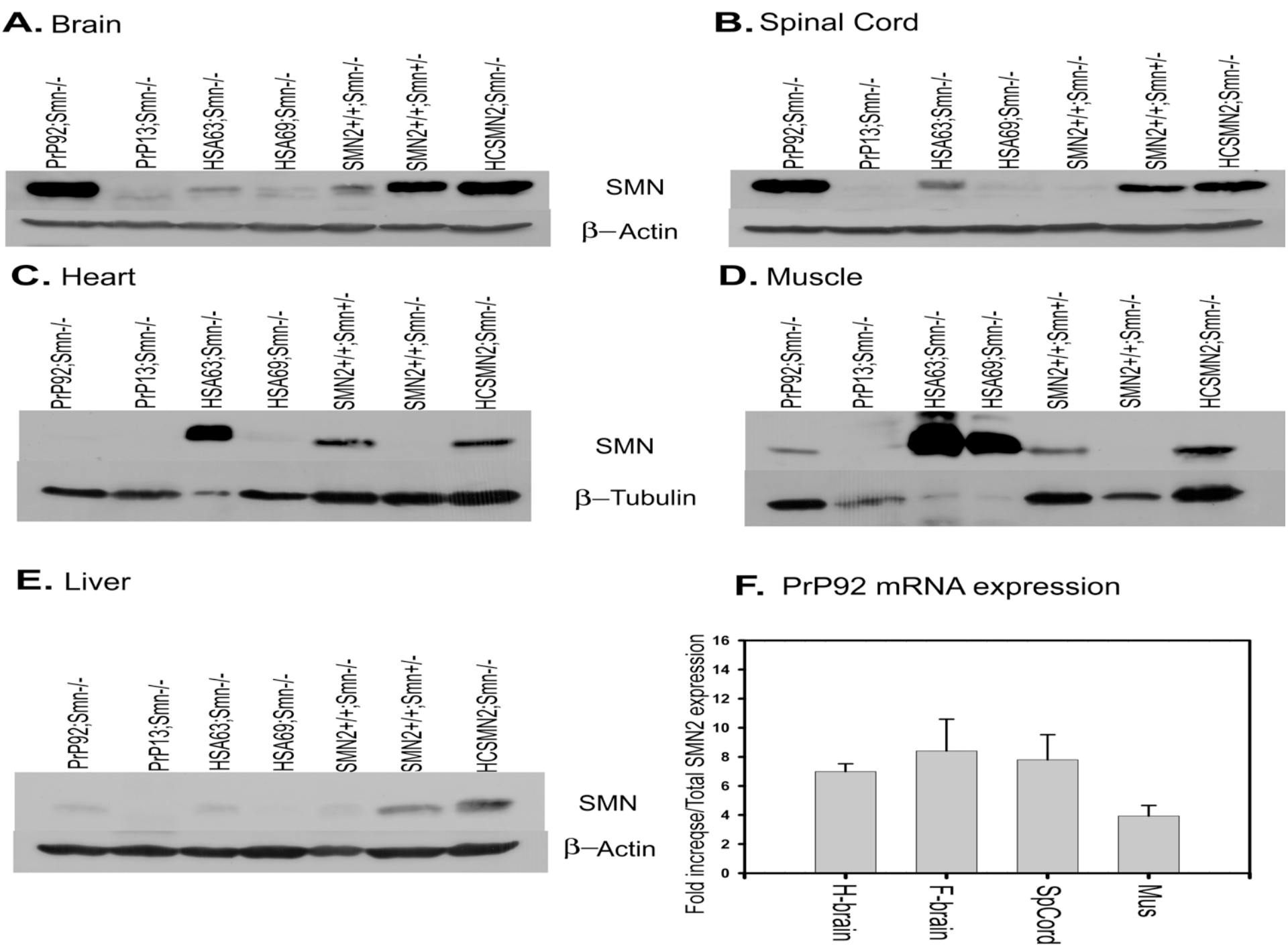
SMN is expressed in all cells and SMN2 can act as target for therapy. SMN functions in snRNP assembly.

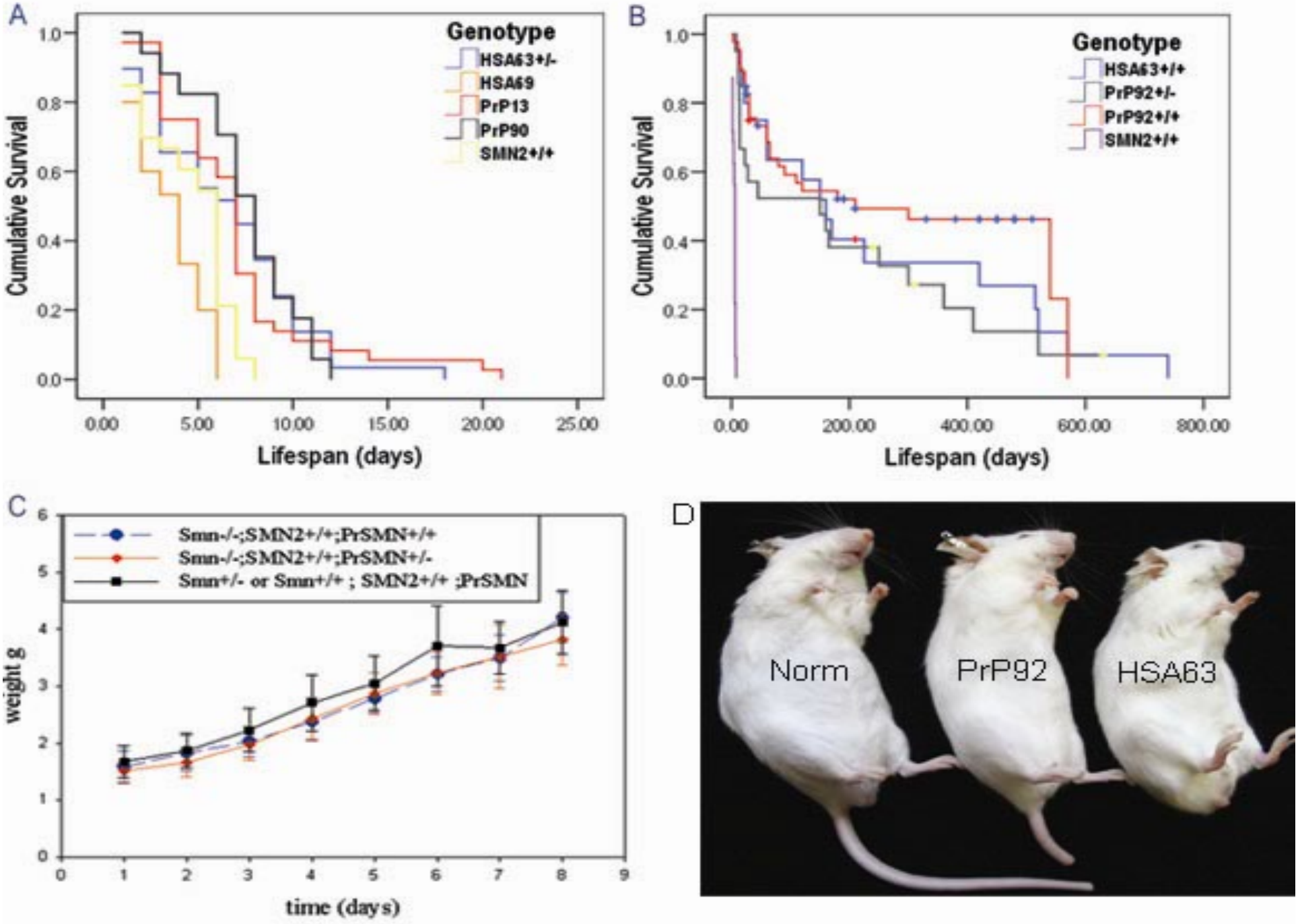
- Where and When are high levels of SMN required?
- Does 2 copies of SMN2 produce sufficient SMN for normal function of most tissues?
- What are the therapeutic approaches?

Approach express SMN in severe SMA mice with specific promoter



Gavrilina et al HMG 2008


A. Brain
PrP92;Smn-/-
PrP13;Smn-/-
HSA63;Smn-/-
HSA69;Smn-/-
SMN2+/+;Smn-/-
SMN2+/+;Smn+/-
HCSMN2;Smn-/-
SMN
β—Actin
B. Spinal Cord
PrP92;Smn-/-
PrP13;Smn-/-
HSA63;Smn-/-
HSA69;Smn-/-
SMN2+/+;Smn-/-
SMN2+/+;Smn+/-
HCSMN2;Smn-/-
C. Heart
PrP92;Smn-/-
PrP13;Smn-/-
HSA63;Smn-/-
HSA69;Smn-/-
SMN2+/+;Smn+/-
SMN2+/+;Smn-/-
HCSMN2;Smn-/-
SMN
β—Tubulin
D. Muscle
PrP92;Smn-/-
PrP13;Smn-/-
HSA63;Smn-/-
HSA69;Smn-/-
SMN2+/+;Smn+/-
SMN2+/+;Smn-/-
HCSMN2;Smn-/-
E. Liver
PrP92;Smn-/-
PrP13;Smn-/-
HSA63;Smn-/-
HSA69;Smn-/-
SMN2+/+;Smn-/-
SMN2+/+;Smn+/-
HCSMN2;Smn-/-
SMN
β—Actin
F. PrP92 mRNA expression
Fold increase/Total SMN2 expression
0
2
4
6
8
10
12
14
16
H-brain
F-brain
SpCord
Mus


A
Genotype
HSA63+/-
HSA69
PrP13
PrP90
SMN2+/+
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0.00
5.00
10.00
15.00
20.00
25.00
Lifespan (days)
B
Genotype
HSA63+/+
PrP92+/-
PrP92+/+
SMN2+/+
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0.00
200.00
400.00
600.00
800.00
Lifespan (days)
C
Smn-/-;SMN2+/+;PrSMN+/+
Smn-/-;SMN2+/+;PrSMN+/-
Smn+/- or Smn+/+ ; SMN2+/+ ;PrSMN
weight g
6
5
4
3
2
1
0
0
1
2
3
4
5
6
7
8
9
time (days)
D
Norm
PrP92
HSA63

1) Specific increase in muscle SMN minimal increase in survival with HSA-SMN or Myf 5 Cre drivers.

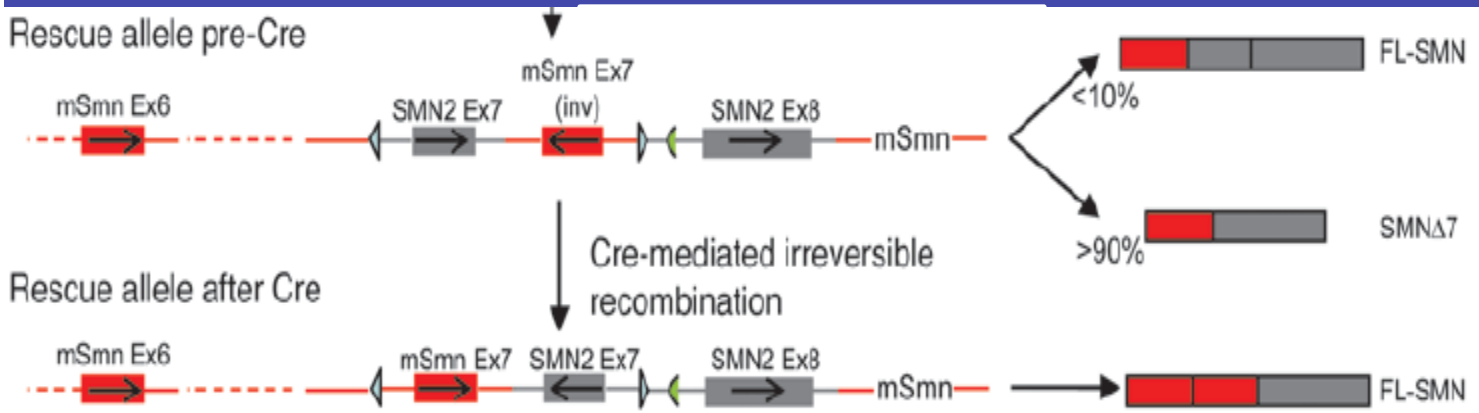
2) HSA 63 leaky heterozygote live 6.6 days vs 5.12 day with out transgene HSA 63 leaky Homozygous live 160 days. 3 fold increase in SMN from SMN2 major impact on SMA.

3) PrP 92 SMN 210 day survival. Not all tissues require high SMN levels. Two copies of SMN2 produce sufficient SMN for most tissues. Good neuronal drivers Do rescue with Cre or cause SMA with SMN removal.

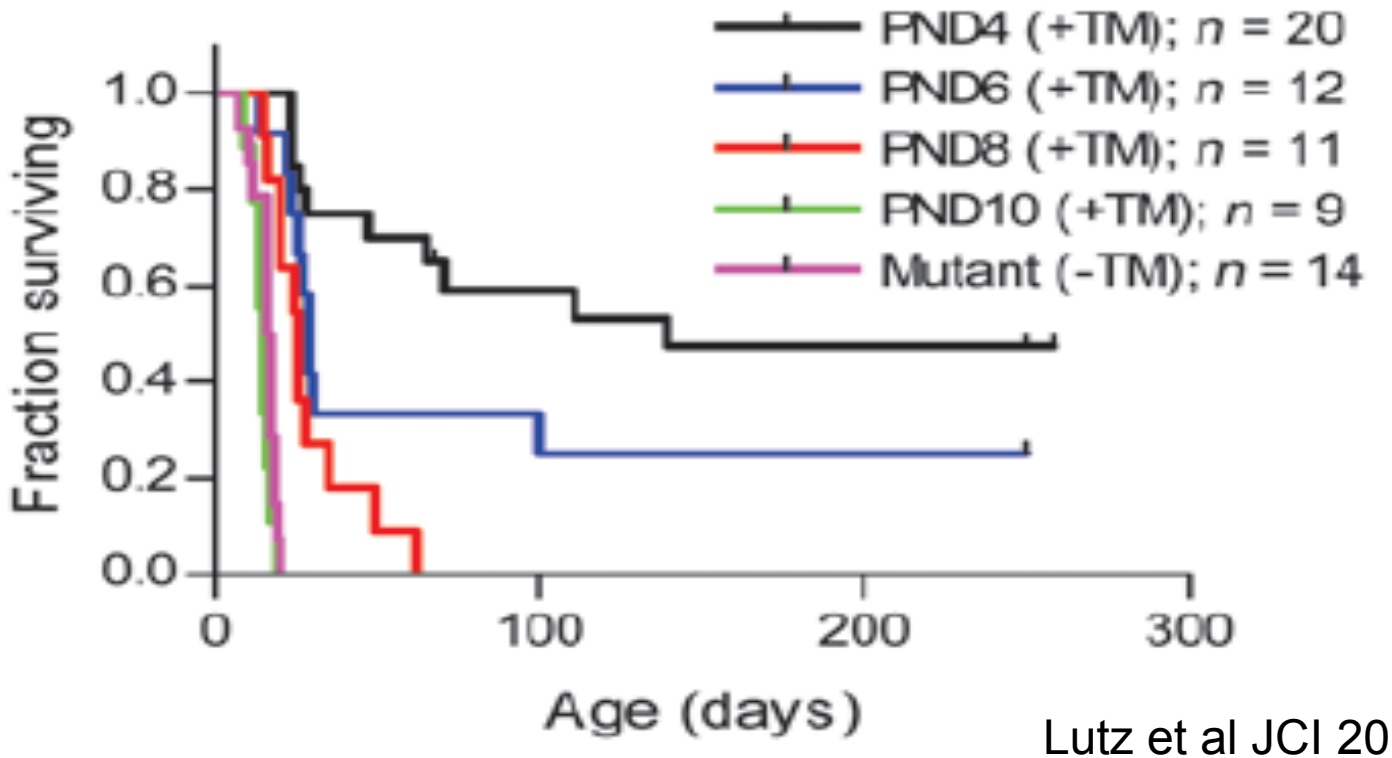
4)SMN2 provides sufficient SMN for normal function of muscle.

When do you need high SMN?

Rescue of SMA mice by inducible allele from postnatally



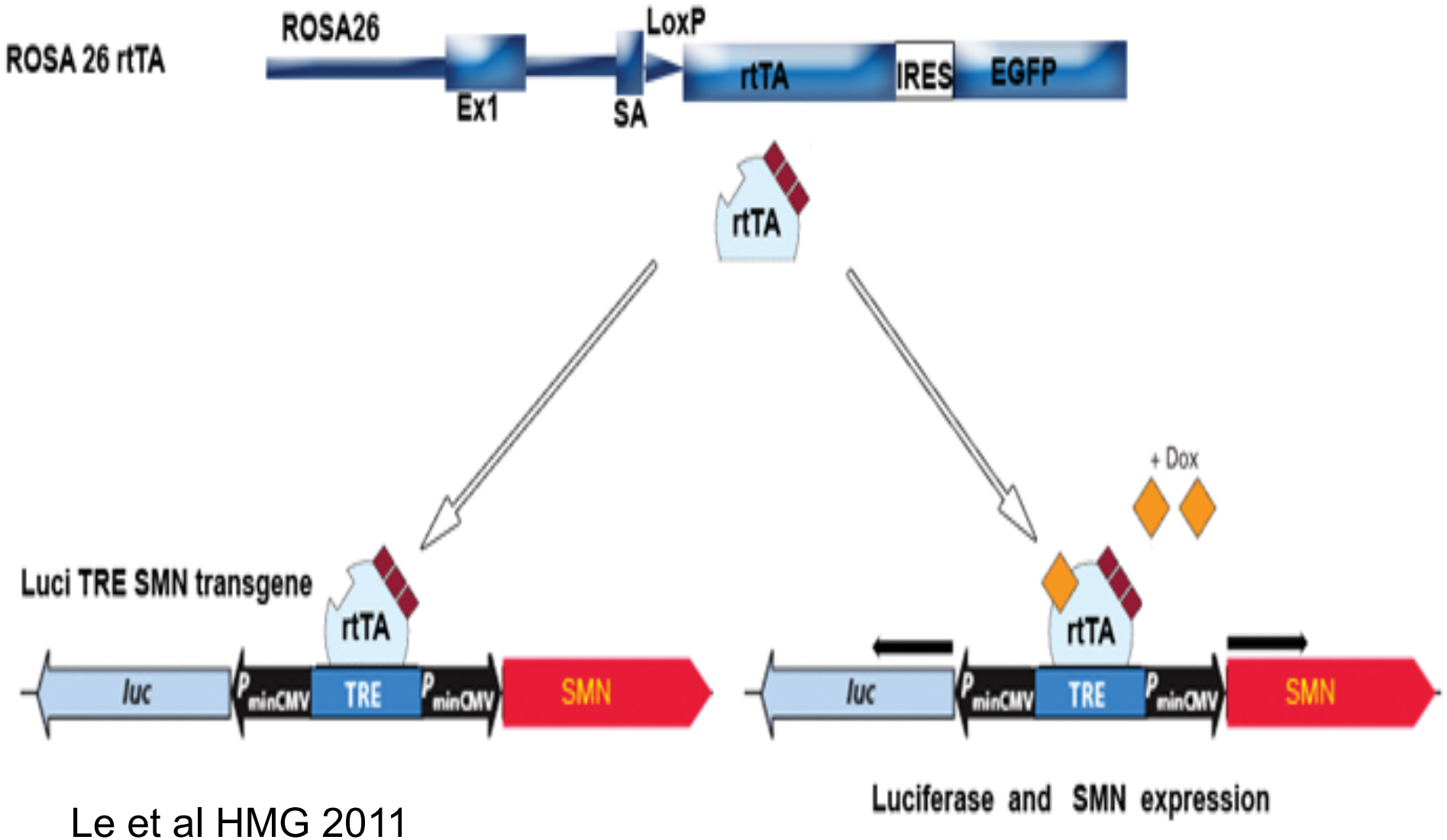
Tamoxifen Induces Reversion and restores SMN



Lutz et al JCI 2011

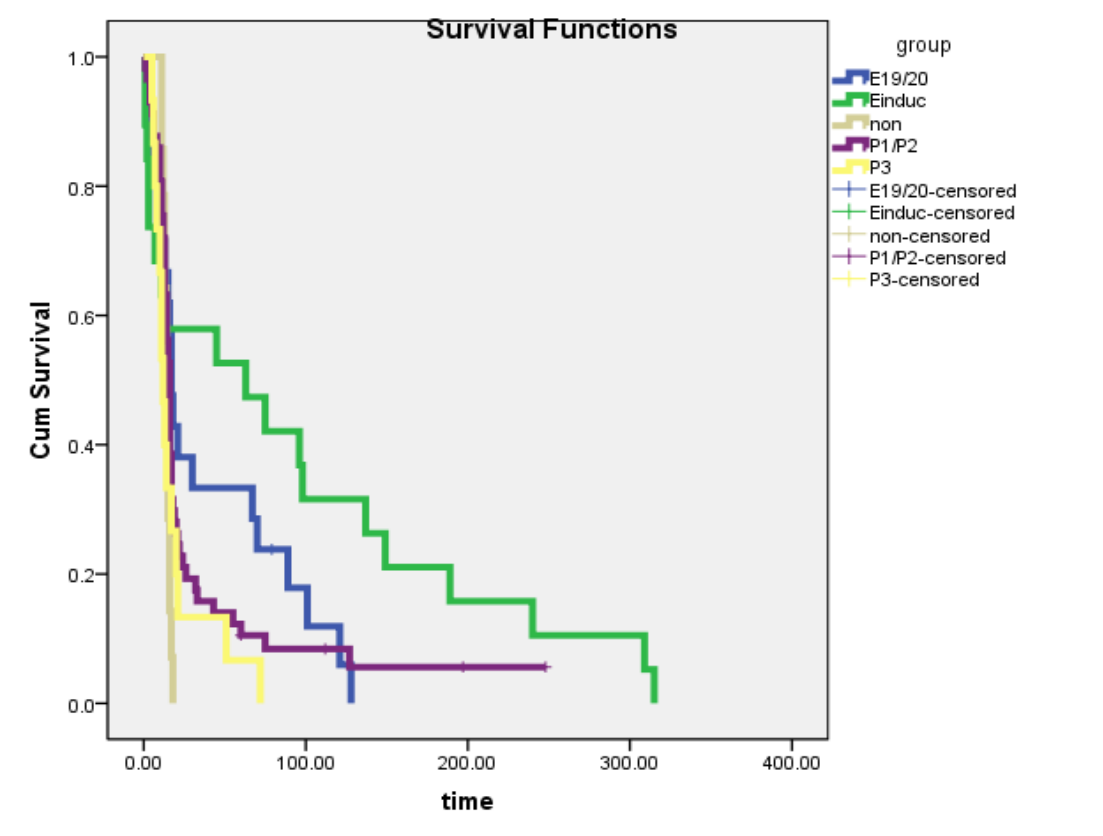
Rescue occurs Postnatally with Early SMA symptoms but the earlier the better.

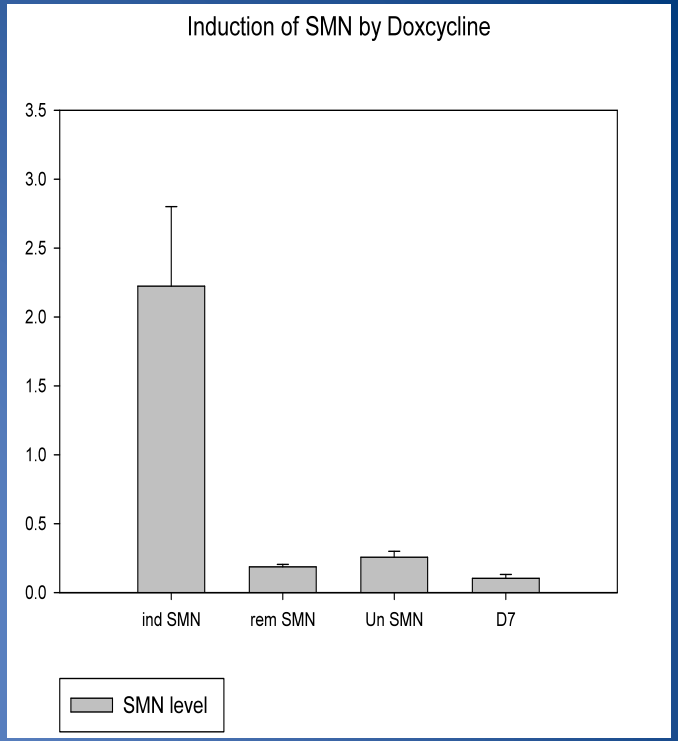
Inducible SMN in SMA mice using SMA delta7 mice.

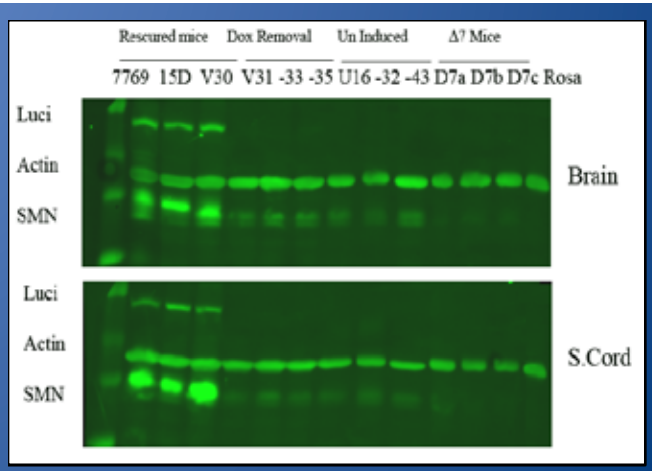


Le et al HMG 2011

Doxycycline inducible SMN mice show postnatal induction does rescue Survival.







SMN induction at day 3 or 4 results in mice that can live over 200 days.
Induction at day 6 reduces number of animals rescued and maximum live span is 72 Days. Removal of SMN induction no neuromuscular phenotype.

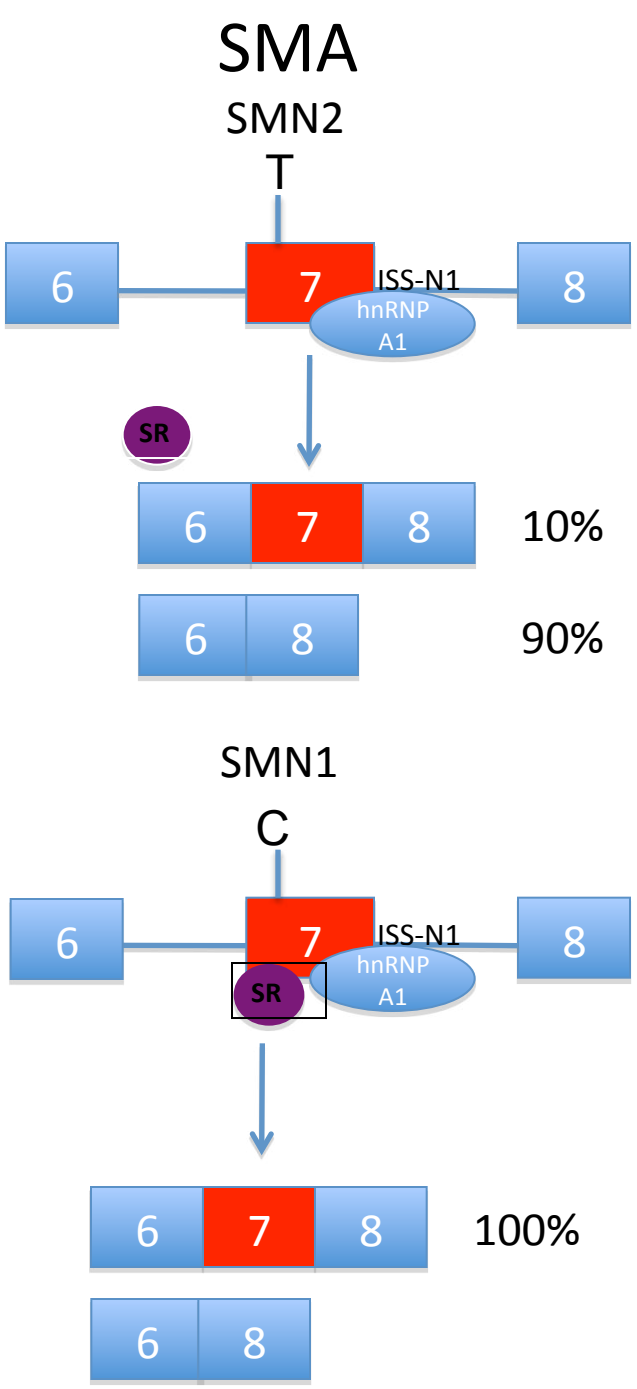
Summary

Postnatal induction of SMN start dox feed at day 1 or 2 get full induction 60hrs latter so day 3 or 4 get strong rescue some mice lived 200 days or more comparable to embryonic induction.

Start dox at day 3 so induction by day 5 some rescue but much reduced numbers of animals showing extension of life. Thus SMN induction is best early.

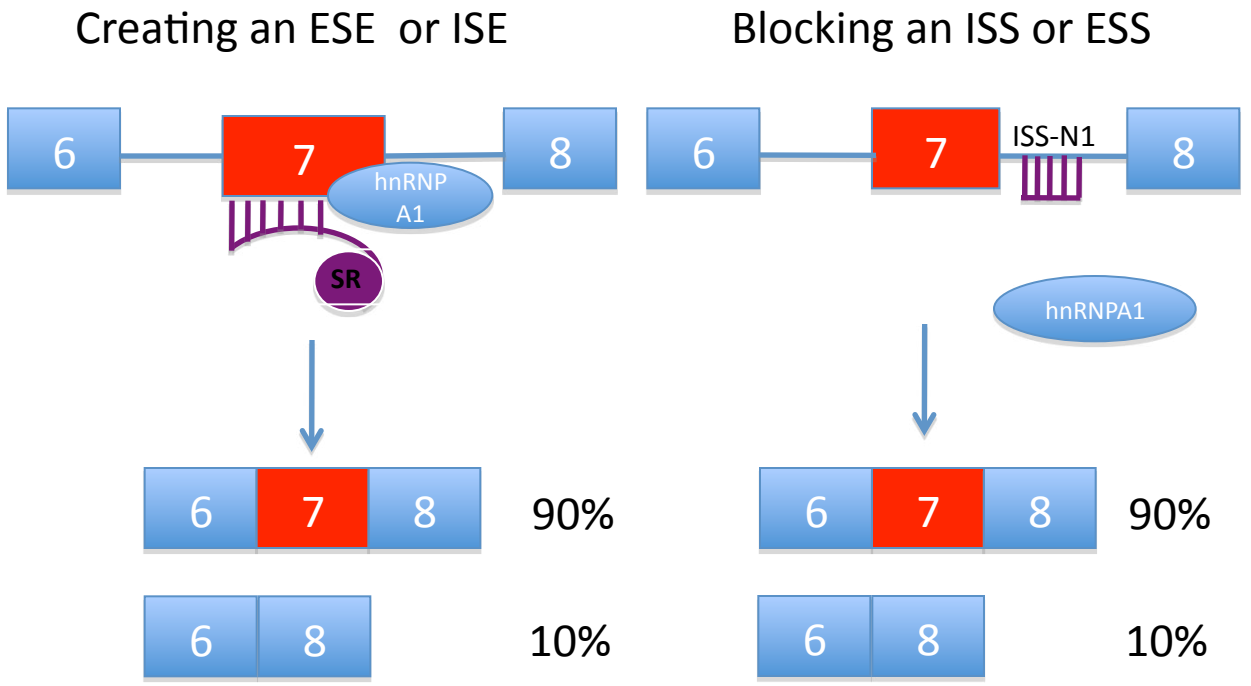
Rescued mice do not have completely restored weight and are smaller.

Removal of SMN mice to survive over a month and no motor neuron phenotype. Some animals are OK after dox removal.



Therapy for SMA

A. Make SMN2 produce more SMN : Drugs or Antisense oligos



B. Give SMN back

AAV-SMN gene therapy or SMN protein directly

Postnatal therapies for SMA

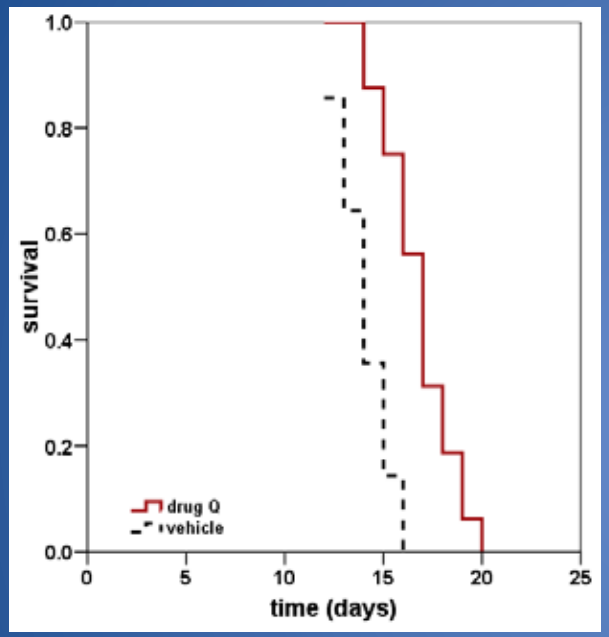
1) Drug compounds: That induce sufficient levels of SMN from SMN2. PTC have new compounds that are effective in mice.

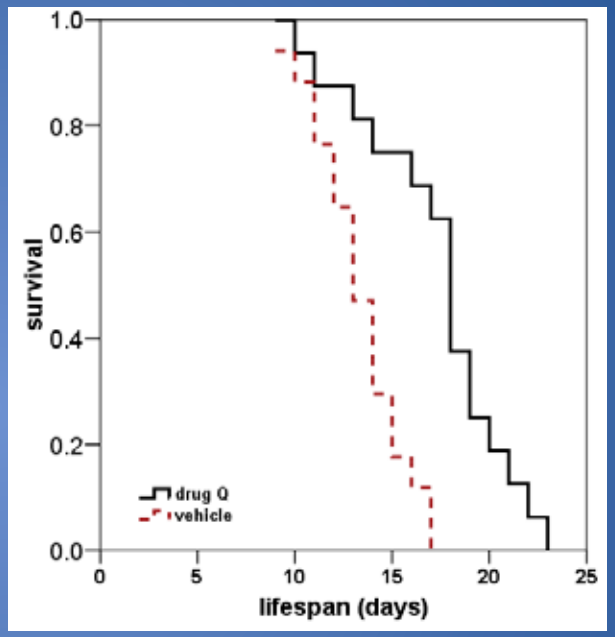
2) Anti sense Oligonucleotide that alter splicing of SMN2 by blocking splice inhibitors and inducing SMN. Either MOE (methoxyethyl) or MO (morpholinos)

3) Gene therapy AAV9 –SMN does cross the blood barrier
Kevin Foust and Brian Kaspar Nationwide Children's.
What happens with postnatal injections of scAAV9-SMN?.

Foust et al. (*Nat Biotechnol* **27**, 59-65 (2009) and
Duque et al. *Mol Ther* **17**, 1187-1196 (2009)

Oral Administration of D156844-04 to SMNΔ7 SMA Mice

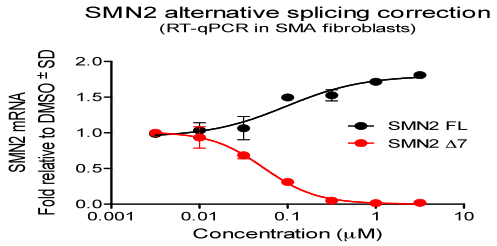


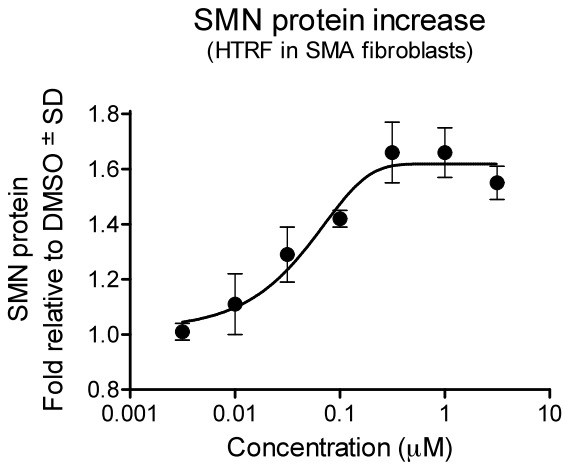


- SMND7 SMA mice receiving D156844-04 (3 mg/kg/day) at PND04 had a significant (~21%) increase in mean lifespan (17.0±0.5 days vs. 14.0±0.4 days; C^2=16.7)
- prenatal treatment (starting at E11) increased lifespan by ~38% (18.0±0.5 days vs. 13.0±0.7 days; C^2=15.0) Butchbach et al HMG2010

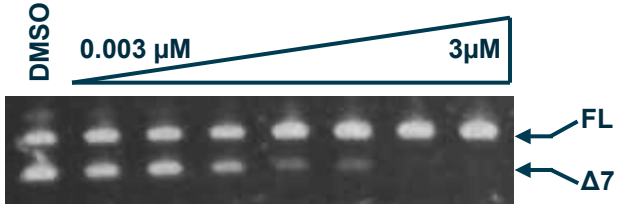
Need drugs that give better induction of SMN from SMN2

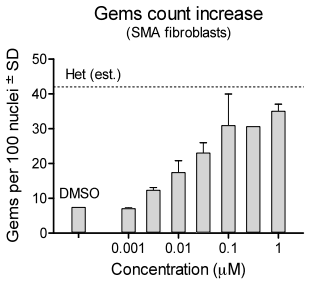
PTC-SSN corrects SMN2 alternative splicing and increases SMN protein and gems count in vitro in SMA patient cells





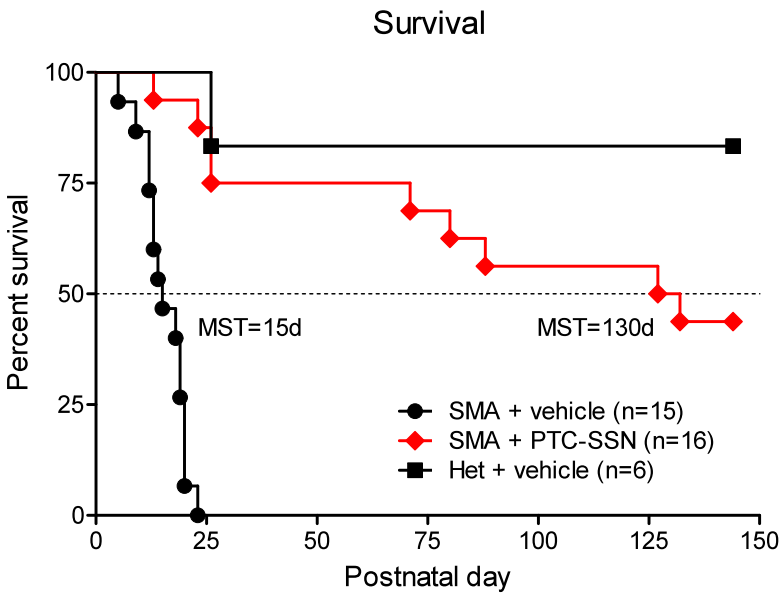
SMN2 alternative splicing correction
(end-point RT-PCR in SMA fibroblasts)

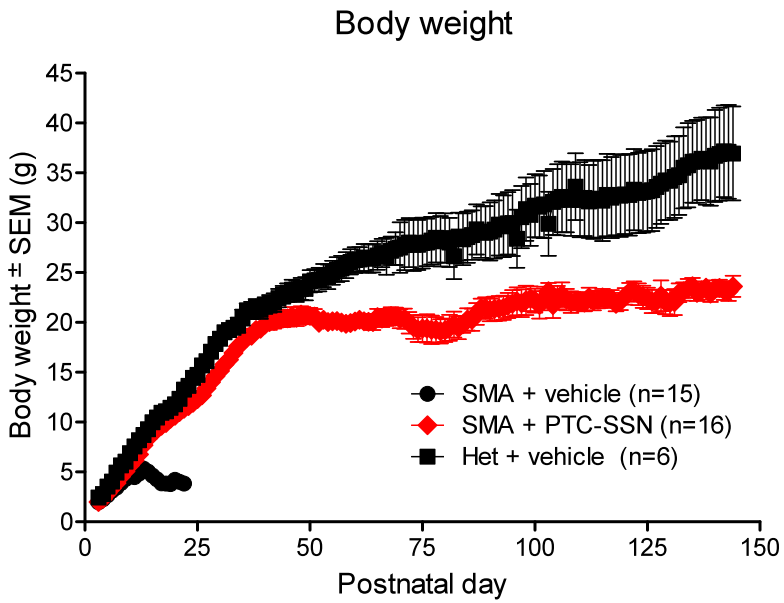




PTC-SSN significantly improves body weight and motor function in Δ7 SMA mice

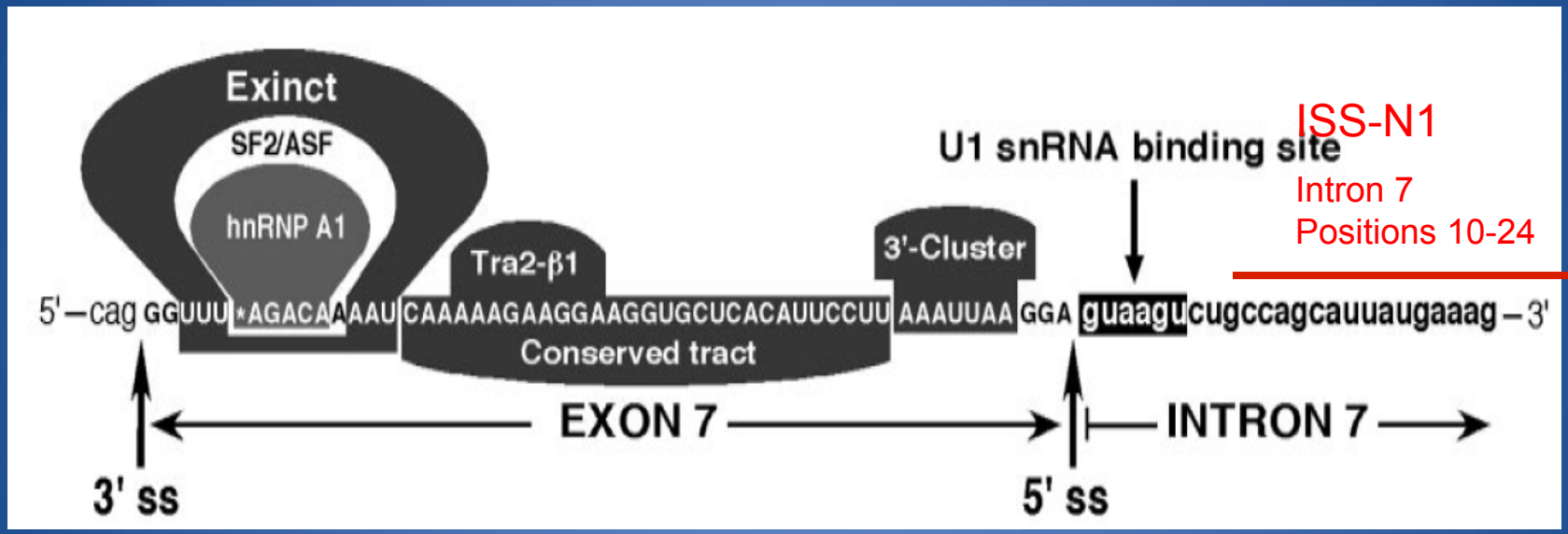
- IP dosing 10 mg/kg once a day P3 – P23, oral dosing 30 mg/kg twice a day P24 – P144





- Mice do not have necrosis, exhibit normalized phenotype

Cis-acting splice modifiers increase exon 7 incorporation



Singh, Nk *et al*. Splicing of a Critical Exon of Human *Survival Motor Neuron* Is Regulated by a Unique Silencer Element Located in the Last Intron. Molecular and Cellular Biolog; Feb. 2006, p. 1333–1346 Vol. 26, No. 4

Hua Y, *et al.* Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am Hum Genet*. 82:834-848, 2008.

Methods

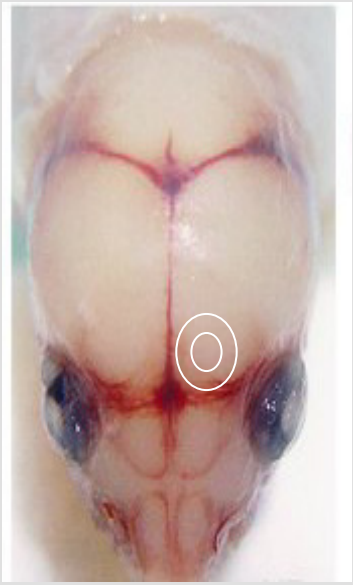
- Breeders: Smn+/-, SMN2+/+, Δ7+/+

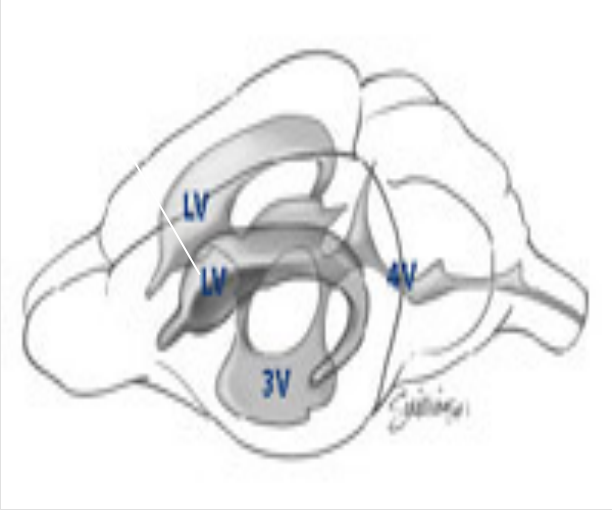
	2µL Morpholino Injection
Smn +/+	Scramble (control)
Smn +/-	ISS-N1 (27ug, 40.5ug, 81ug)
Smn -/-	ISS-N1 (27ug, 40.5ug, 81ug)

Average newborn mass: 1.5g

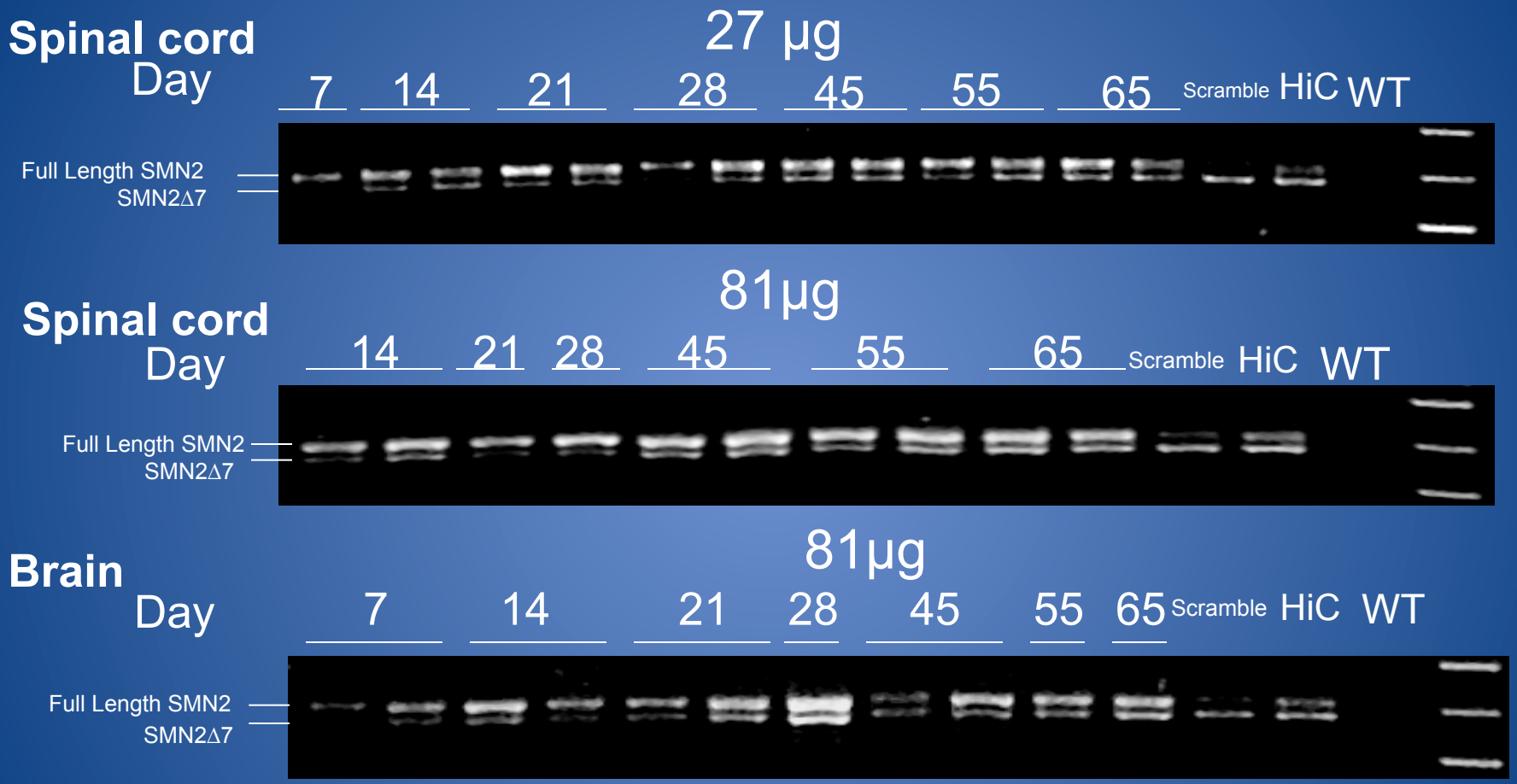
Average Smn -/- survival: 14.6 days

- Outcomes:
  - –Survival, mass
  - –Protein
  - –RNA quantification



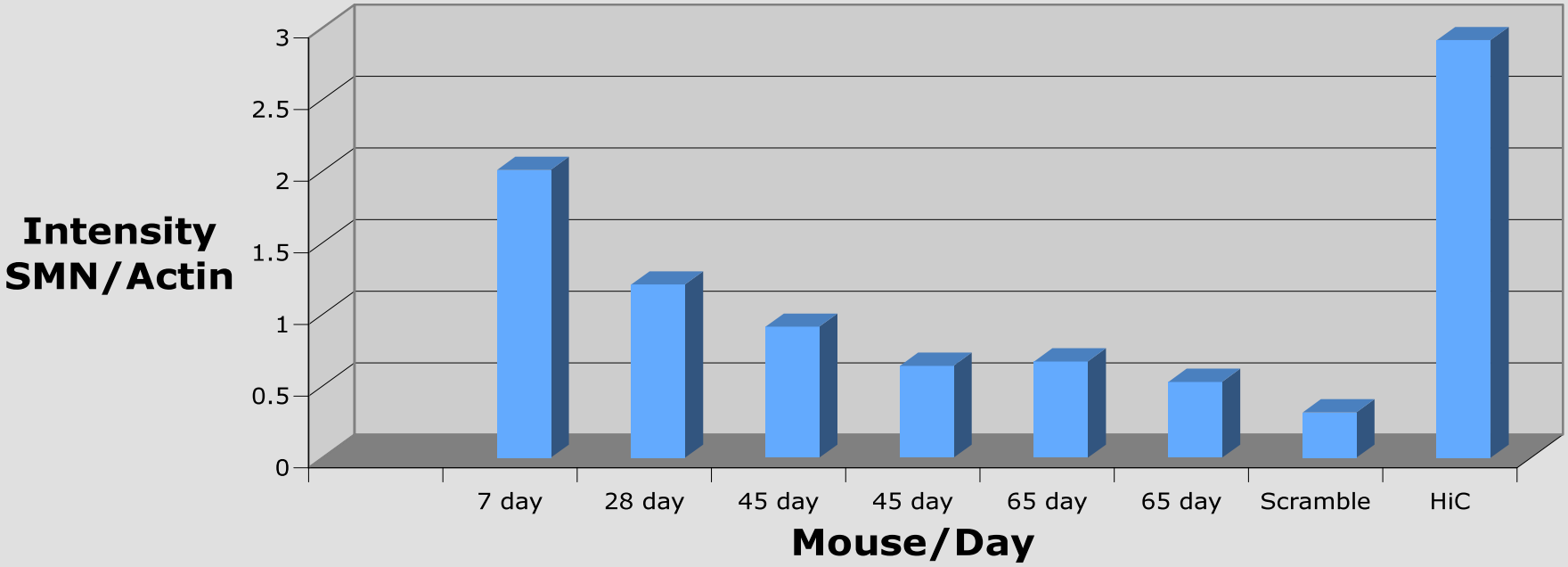


RT-PCR after ASO injection

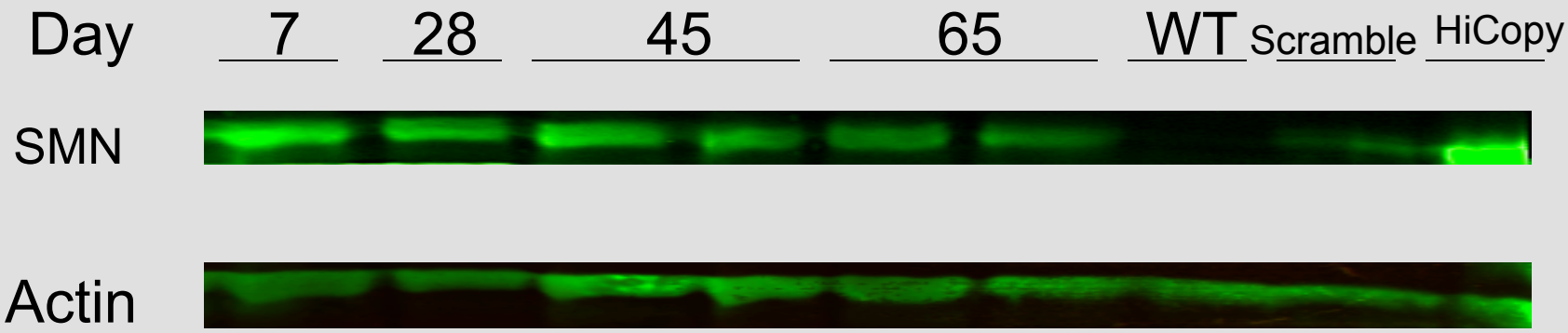


-primer specific to SMN2 (does not amplify Δ7 or Smn)
-HiC= 16 copies SMN2, Smn -/-

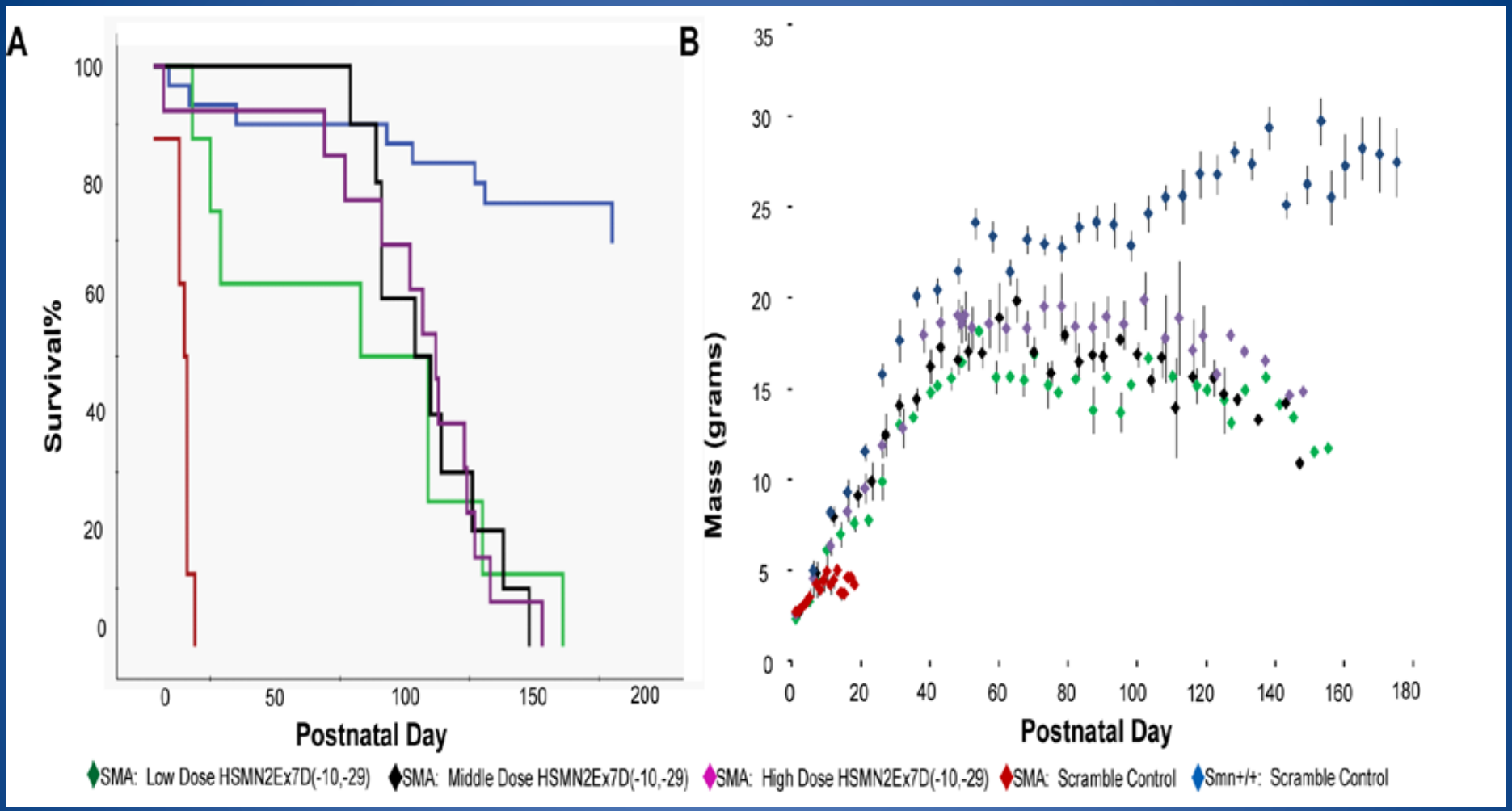
SMN increased after ASO delivery



27 ug ISS-N1



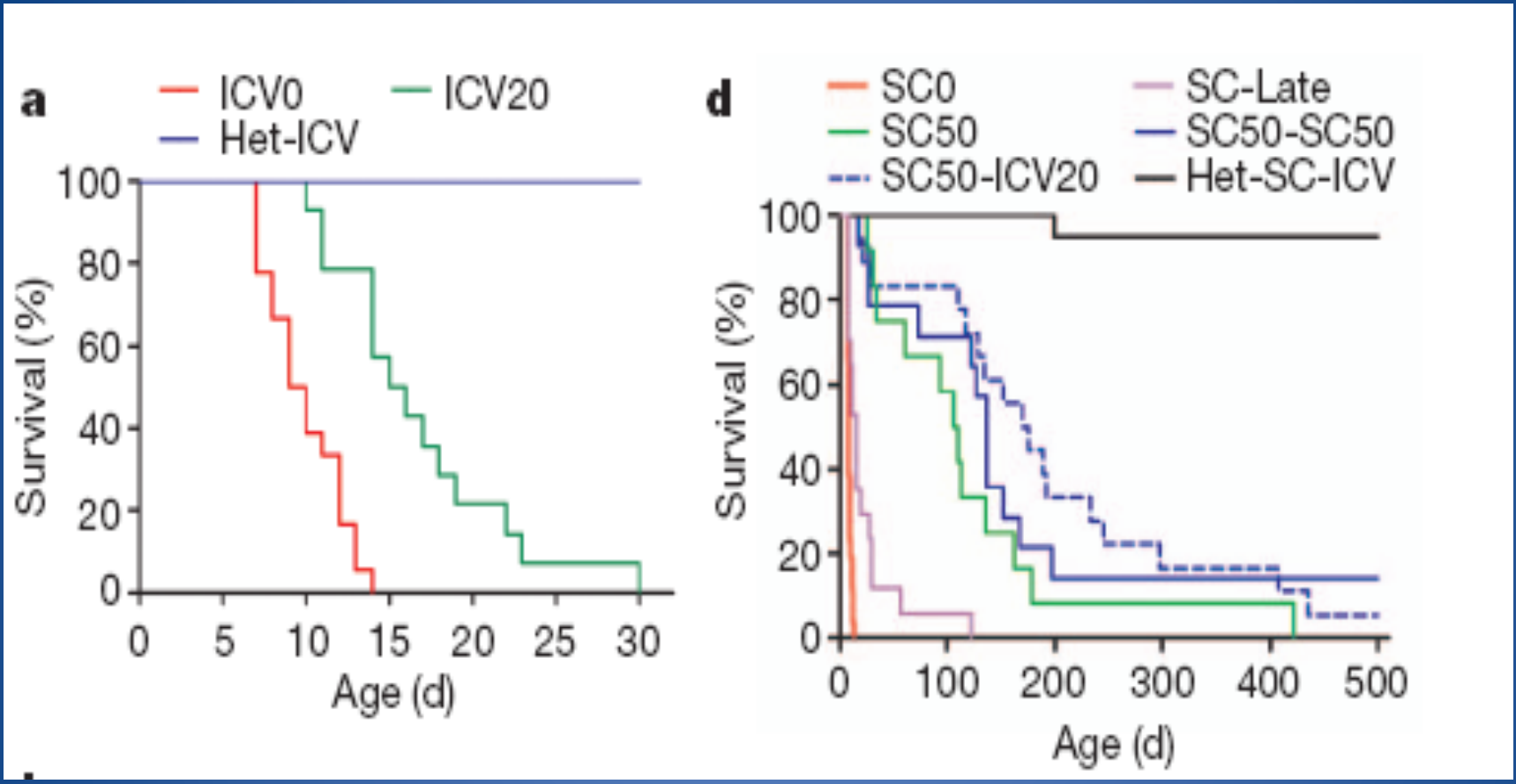
ICV (Neuronal Only) ASO result in long term survival of SMA mice



SMA Delta 7 mice with a one time dose (27-81ug) of Antisense mopholino mice survive over 100 days as opposed to 14 days.

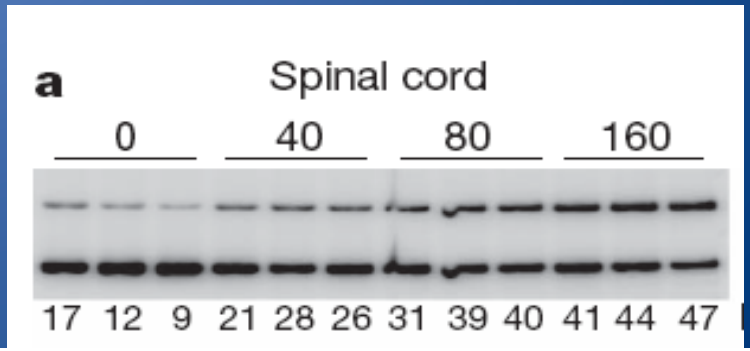
Porensky et al HMG

Peripheral SMN restoration (with MOE ASOs) is essential for long-term Rescue Hua et al Nature 2011

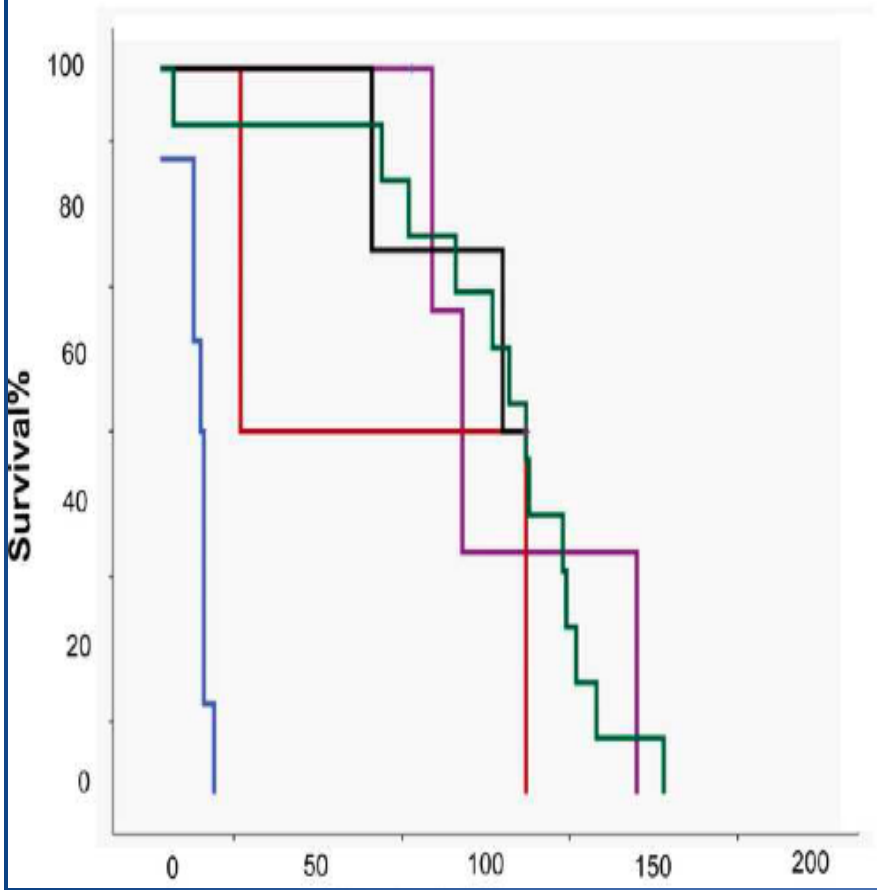


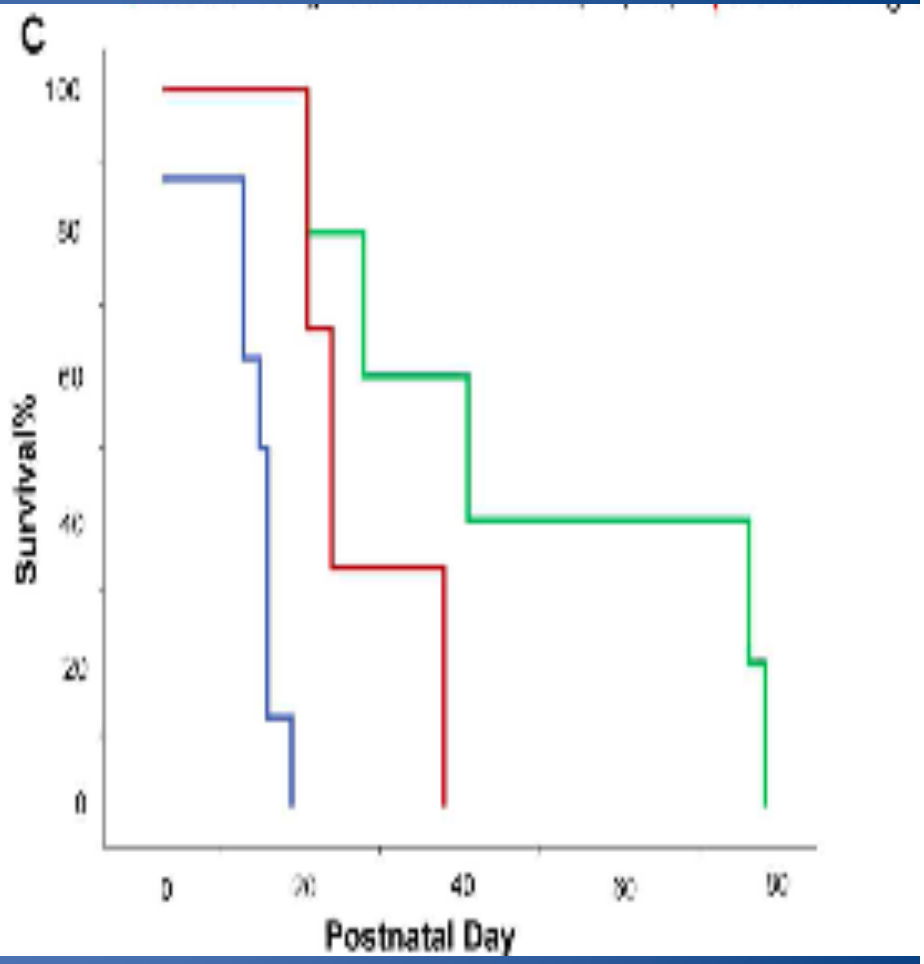
But Morpholinos ?

SMN levels are Changed in neurons



ASO- Morpholino peripheral delivery at P0 is the same as ICV but at 4 days ICV better than Peripheral must get neurons (BBB penetration low at 4 days)





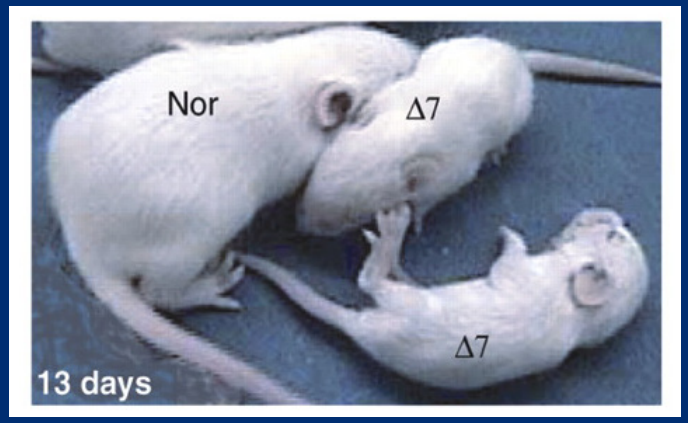
Postnatal Day

- SMA: P0 ICV Scramble
- SMA: P0 Facial Vein HSMN2Ex7D(-10,-29)
- SMA: P0 ICV HSMN2Ex7D(-10,-29)
- SMA: P0 Facial Vein & ICV HSMN2Ex7D(-10,-29)
- SMA: P0 & P30 ICV HSMN2Ex7D(-10,-29)

Gene Delivery to Treat SMA
Pre-Clinical Studies with the SMNΔ7 SMA Mouse Model

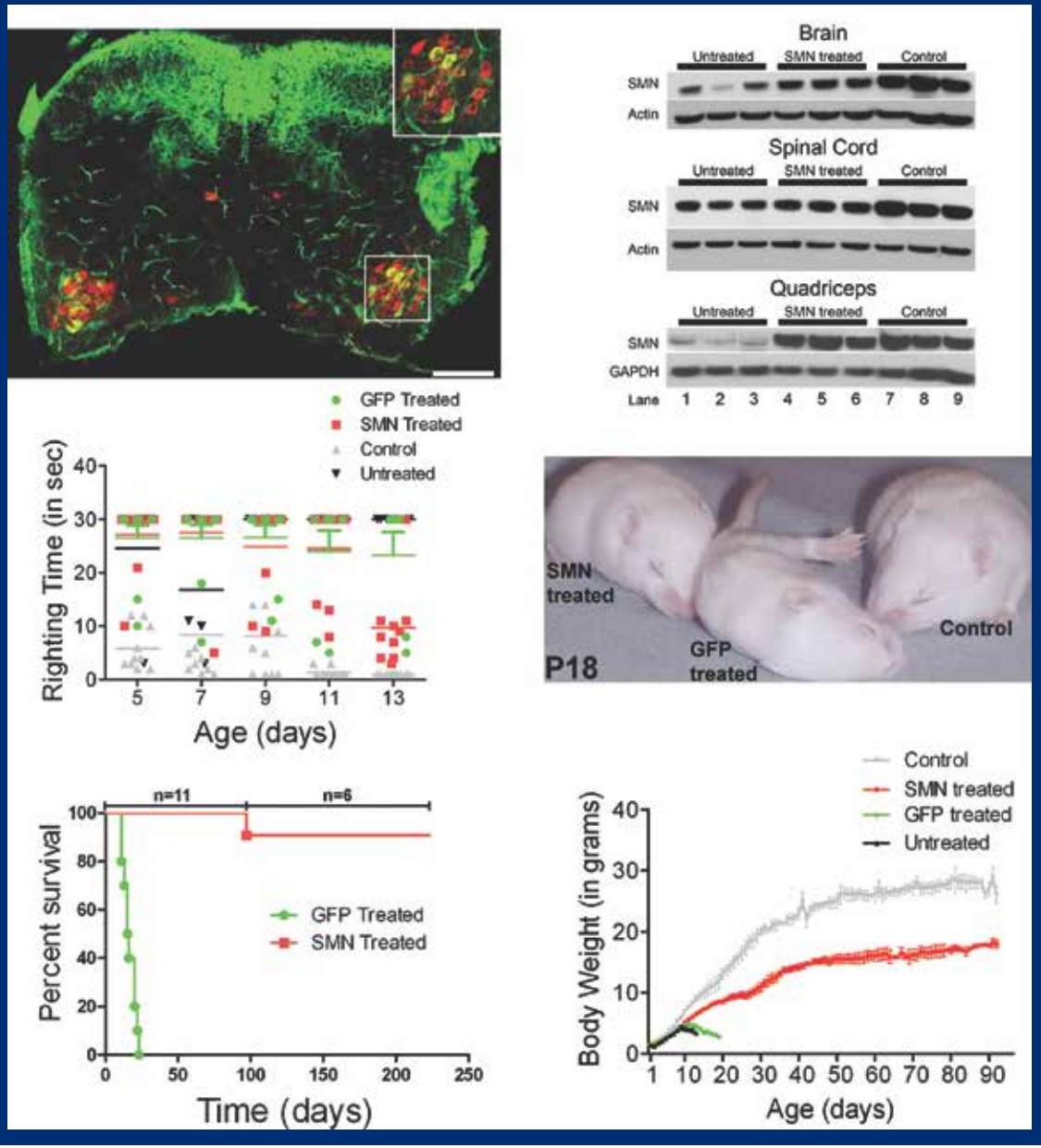
- scAAV9-SMN construct

- SMNΔ7 SMA Mouse Model
  - Molecularly mimics disease
  - Severe model
    - Severe atrophy
    - End-stage ~16 days of age

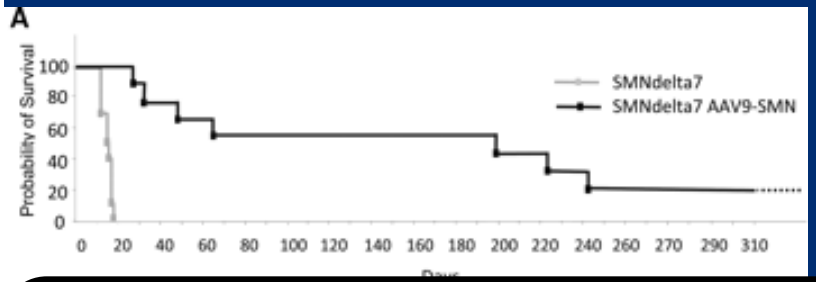


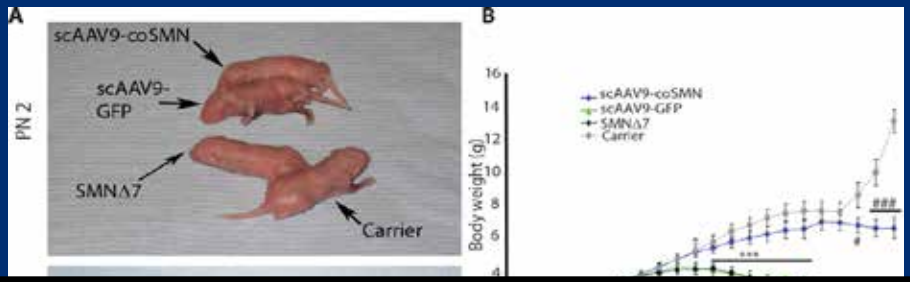
Le, et al. *Human Mol Gen 14*(6) 845-857

One-time gene delivery of scAAV9-SMN Rescues Severe Mouse Model of SMA



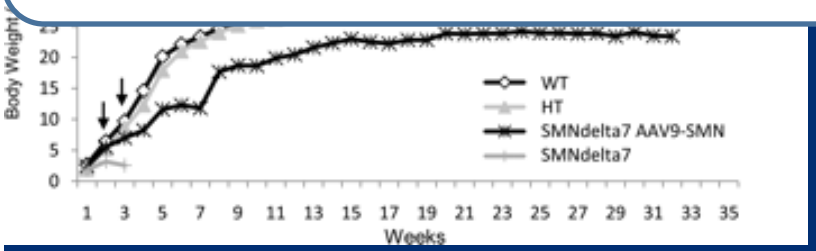
Intravenous scAAV9 delivery of a codon-optimized *SMN1* sequence rescues SMA mice

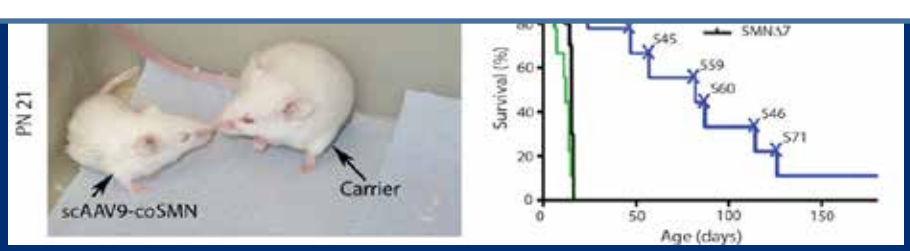




4 Independent Laboratories Demonstrate Remarkable Success in Increasing Lifespan and Function in Severe Model of SMA

Can this be Translated to a Larger Species?



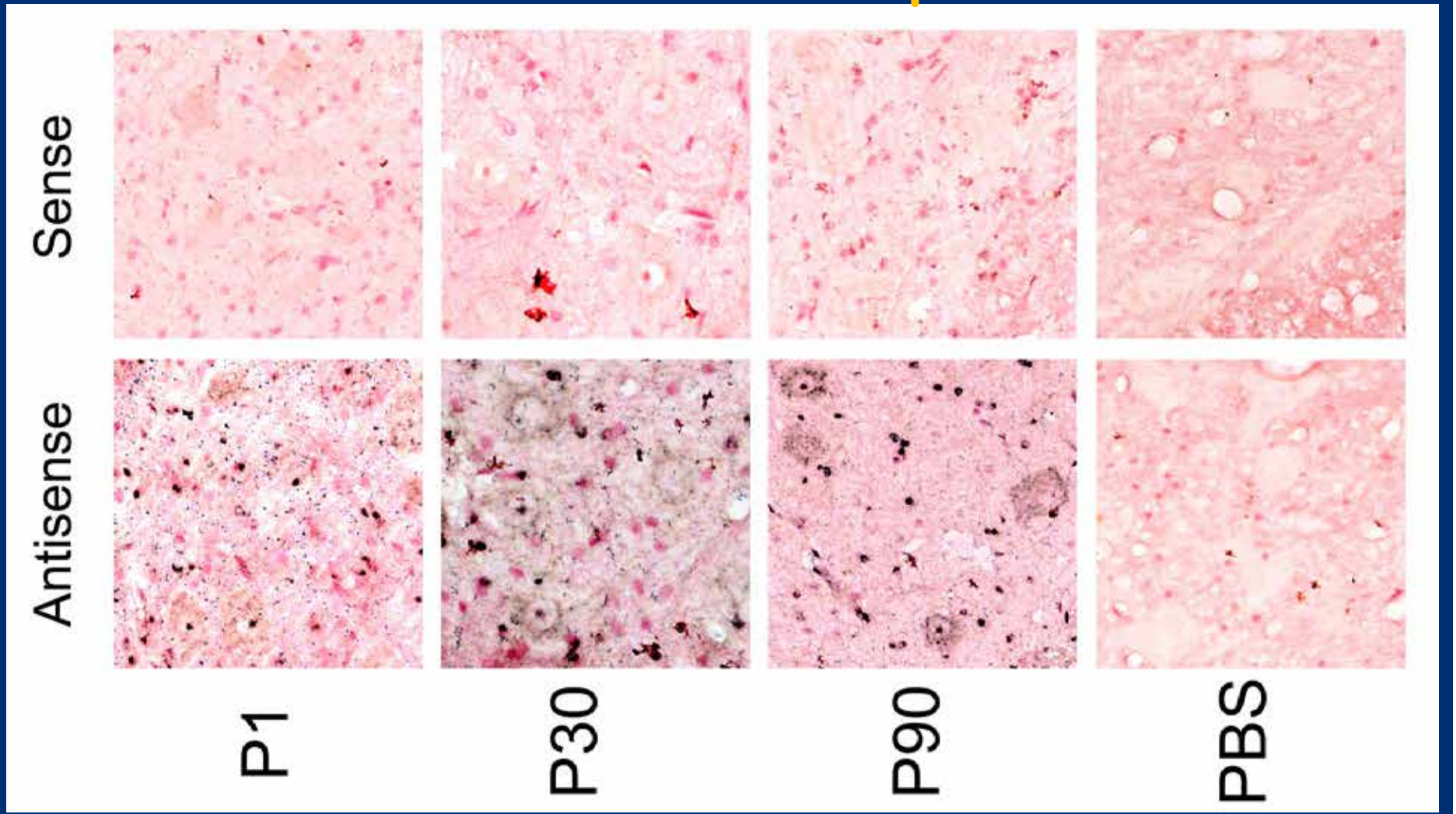


Dominguez…Barkats,
2011 Human Molecular Genetics

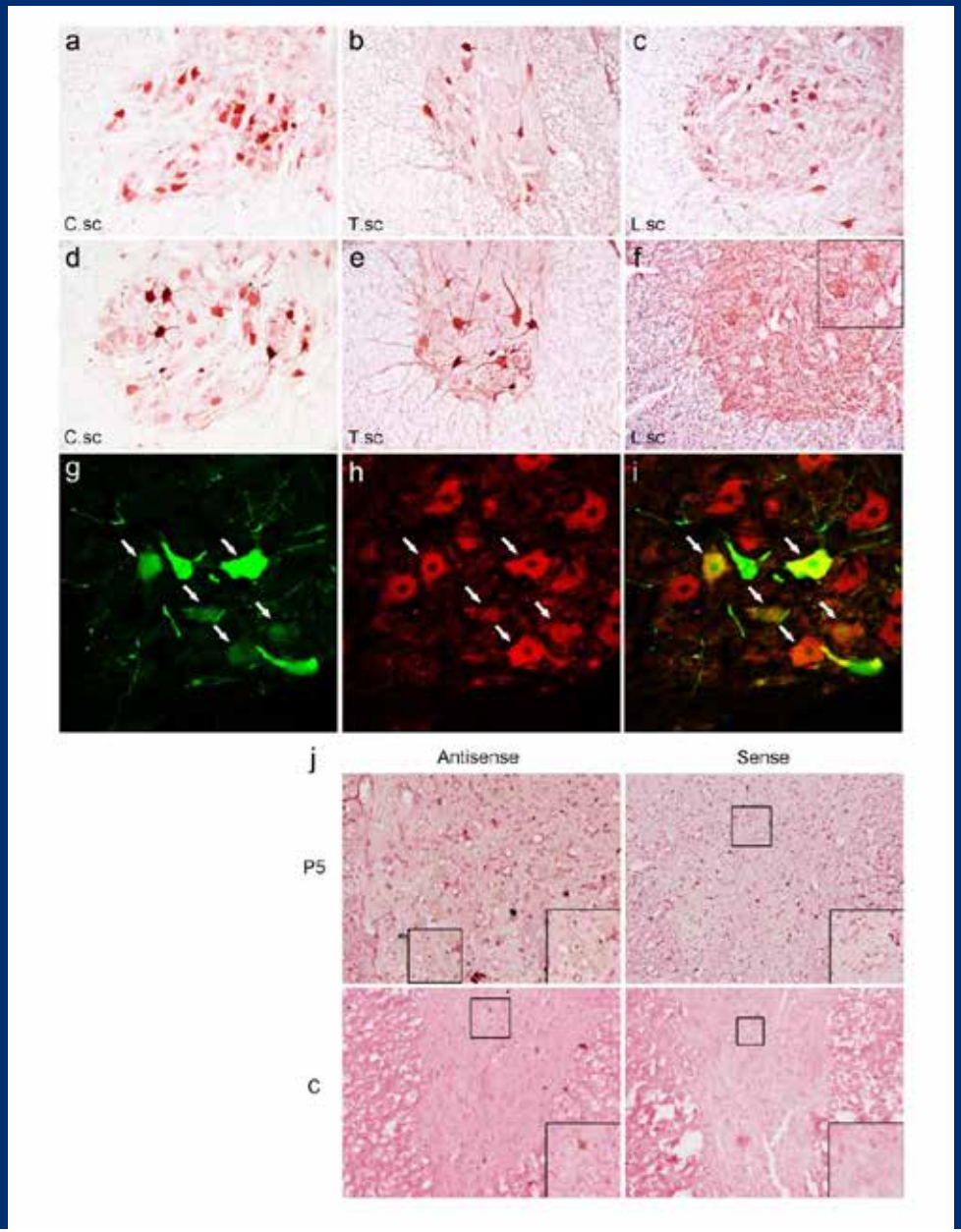
Sci Transl Med 9 June 2010:
Vol. 2, Issue 35, 35ra42
Azzouz and colleagues

Evaluating scAAV9-GFP in Non-Human Primates

In Situ Hybridization Reveals Robust Transduction in Ventral Spinal Cord



Intrathecal and Intracisternal Delivery of scAAV9 leads to MN transduction



Summary

scAAV9-SMN results in over 400 days of SMA mice survival. scAAV9 is even more effective in larger animals (primates) with a larger window to target motor neurons. Studies to enable IND under way. Intravenous and intrathecal delivery can be considered both target motor neurons.

Antisense oligonucleotide (ASOs) that block ISS-N1 increase SMN and and extends survival of SMA animals to over 100 days with a single ICV Injection when using morpholinos. Repeated early doses of MOE to give high MOE in a wide range of neurons and other tissues gives long survival (over 100 days).

Drugs that induce SMN well do have a major impact on SMA mouse survival.

Thus three therapeutic strategies work in mice when given early.

What about human trials will it be critical to deliver the treatment early Presymtomatic or early in the disease?

Amytrophic lateral sclerosis (Lou Gehrig disease)

1/10,000 Onset about age 56

90% sporadic, 10% Genetic

Genetic forms are caused by missense mutations in
Superoxide Dismutase 1 SOD1, TAR-DNA biding protein 43 (TDP43)
and fused in sarcoma/translated in liposarcoma (FUS/TLS)

Both TDP43 and FUS/TLS bind RNA and are important for pre-mRNAsplicing of certain genes)

C9ORF72 Hexanucleotide repeat GGGGCC expanssion
? Bind splicing factors and thus disrupt correct splicing.

Dominant mutation but how does it work ???? Expressed every where but affects Motor neurons

ALS: Pharmacologic Treatment

- Riluzole (Rilutek®) is the only currently available medications for the treatment of ALS.
- Glutamate Inhibitor although precise mechanism of action in ALS is unclear.
- Clinical trials have shown prolonged survival of approximately 2-3 months.

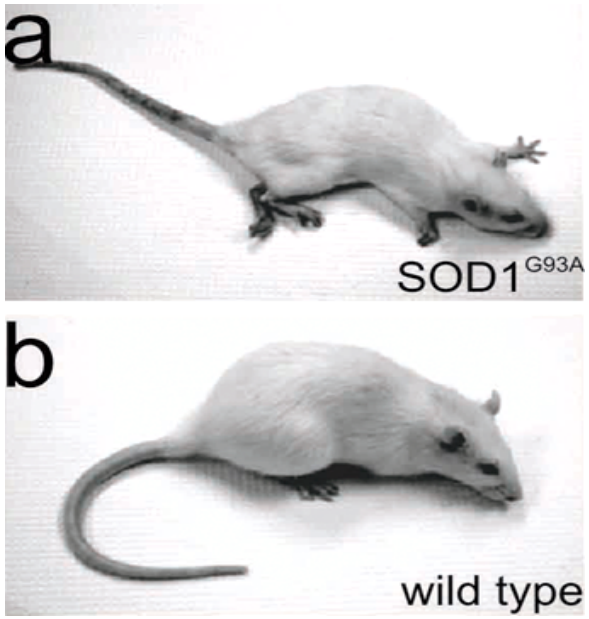
Multiple therapies have been tested
5 with some positive phase II human data

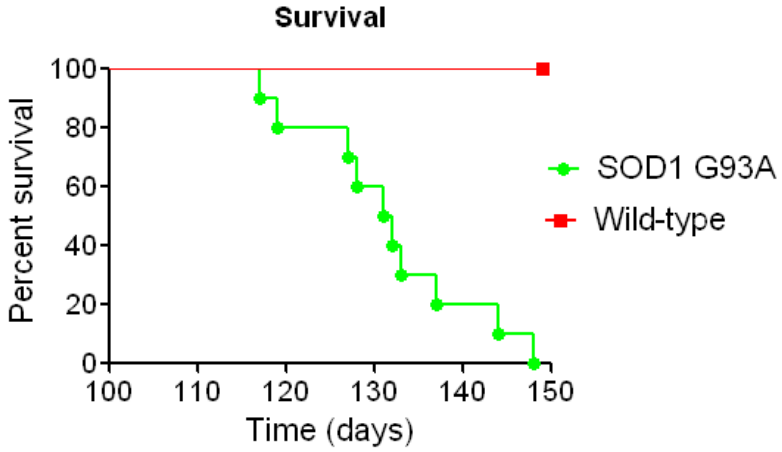
- Anti-glutamate
  - **Riluzole**
  - Gabapentin
  - **Topiramate**
  - Dextromethorphan
  - ONO-2506
  - **Talampanel**
- Growth Factors
  - BDNF-IT and SC
  - CNTF
  - IGF-1
  - Xaliproden
- Anti-inflammatory
  - Celebrex
  - Minocycline
- Antioxidants/Bioenergetics
  - Creatine (5 and 10 g)
  - Vitamin E
  - Acetylcysteine
  - Coenzyme Q
  - **R+ pramipexole (KNS-760704)**
  - **Tamoxifen**
- Anti-apoptotic
  - TCH386
  - Pentoxyfilline
- Protein aggregation
  - Arimoclomol
  - **Lithium**

ALS Rodent Model

Mouse model of ALS

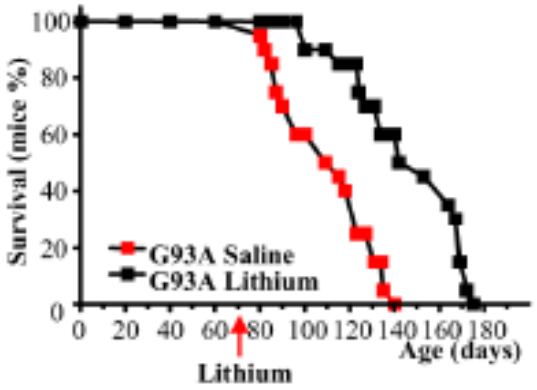
- **Transgenic mouse that carries human mutant SOD1^{G93A} gene (gain of function mutation)**
- **Ubiquitous SOD1^{G93A} expression**
- **Animals closely mimic human disease**
- **Clinical onset at 90 days of age**
- **Progressive motor neuron degeneration resulting in death after ~4 months**





Lithium in ALS- transgenic mouse data

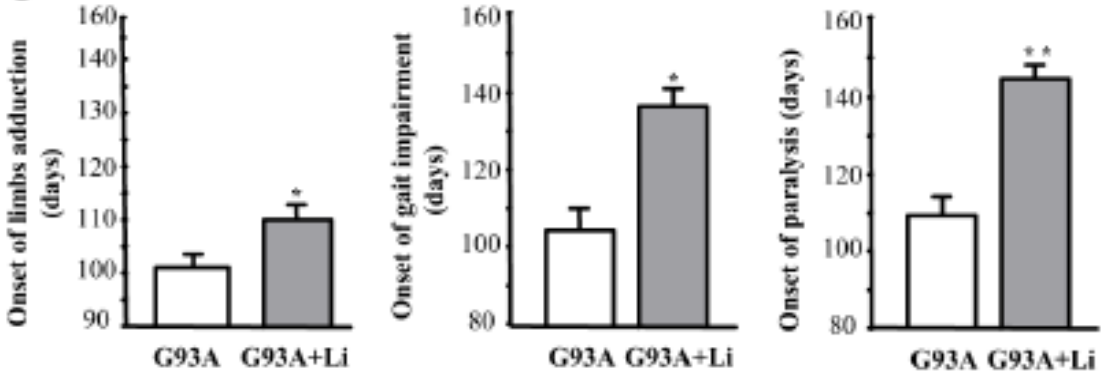
a



b

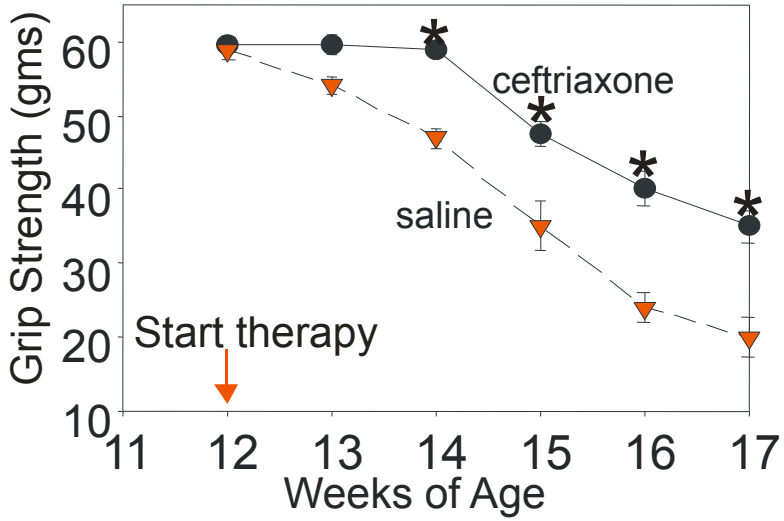
	G93A Saline (age days)	G93A Li (days)
Onset of Limbs adduction	101.0±2.5	109.2±3.2*
Onset of Gait impairment	105.0±5.6	137.5±4.2*
Onset of Paralysis	109.8±5.4	145.1±4.1**
Life-span	110.8±5.0	148±4.3**

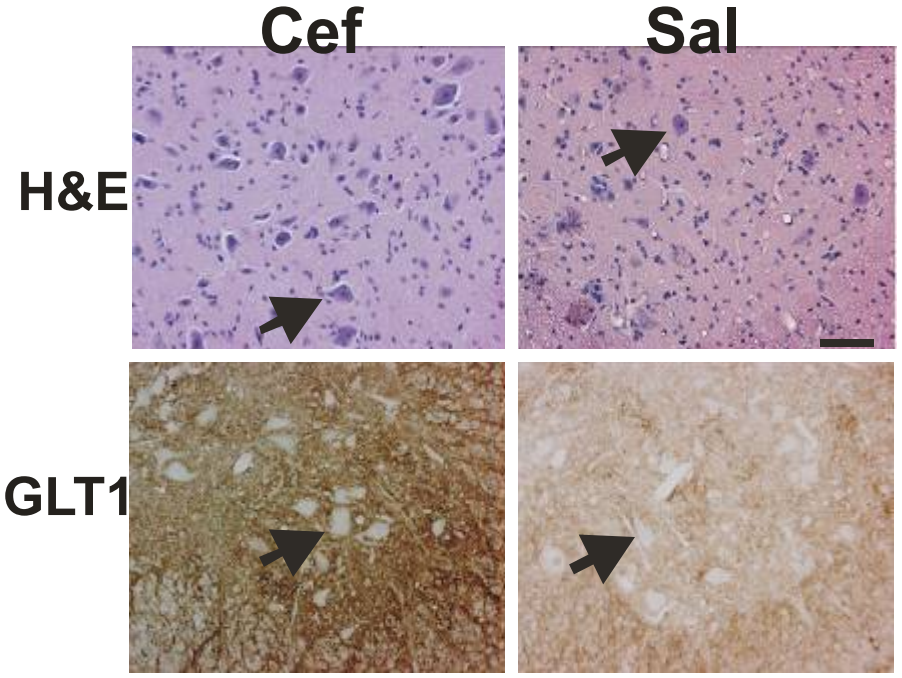
c



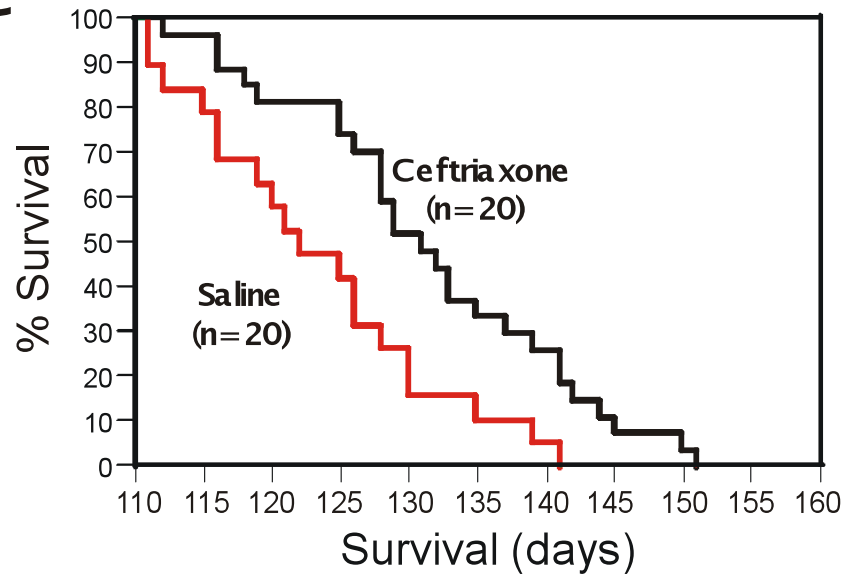
Fornai et al., PNAS 2008

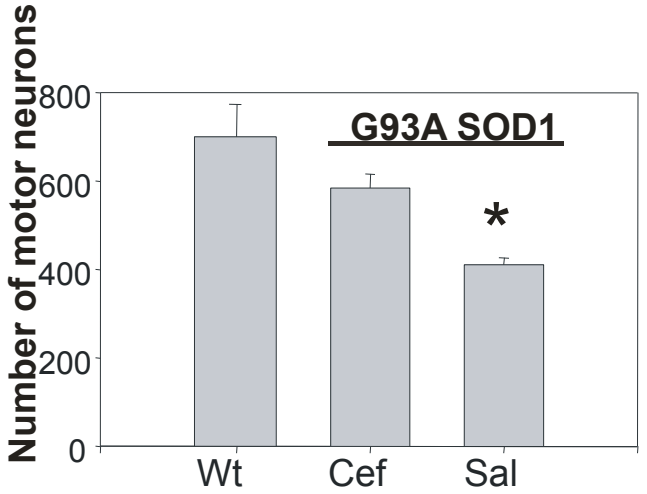
Late ceftriaxone treatment increases survival of G93A SOD1 mice, delays loss of MN and increases GLT1





e





(ceftriaxone, 200 mg/kg ip x 5-7 days; Start Rx: 90 days age)

Boilee et al Science 312, 1389

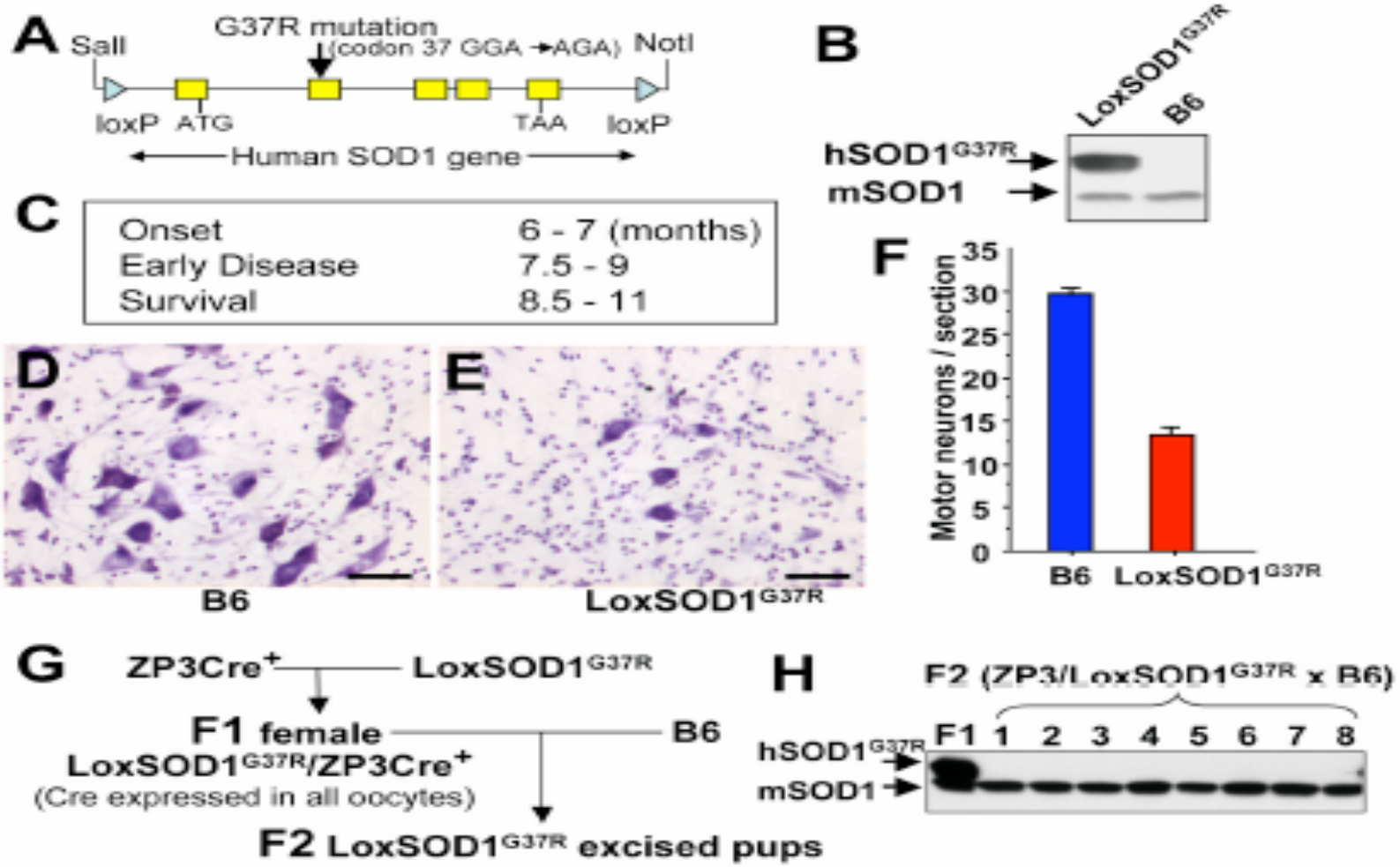
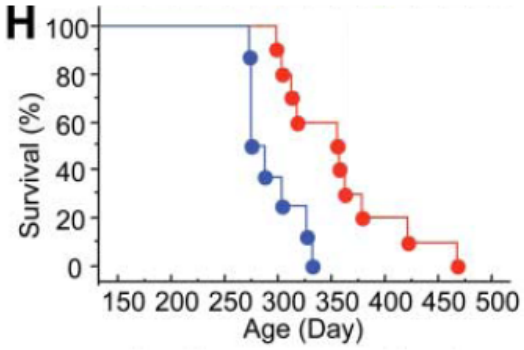


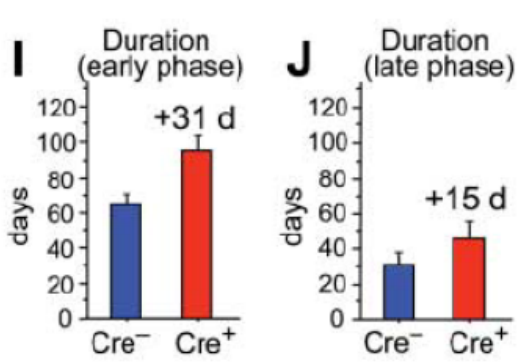
Figure S1. LoxSOD1^{G37R} transgenic mice develop motor neuron disease from an SOD1 transgene that can be removed by Cre-mediated recombination.
A. Schematic drawing of the LoxSOD1^{G37R} transgene. The human SOD1 gene with mutation G37R was flanked by loxP sequences. **B.** Spinal cord extract from a LoxSOD1^{G37R} mouse

Transgenic Studies to Support The Hypothesis of Non-Cell Autonomous Aspect of ALS

- Removal of mtSOD1 from different CNS cell types

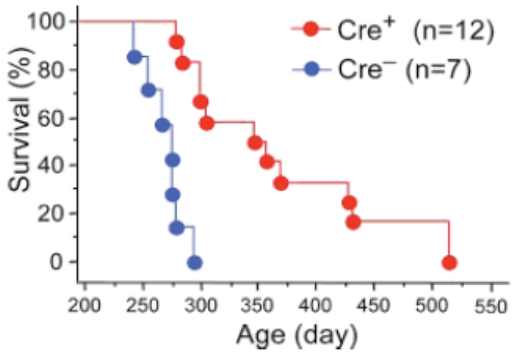
Islet1-Cre

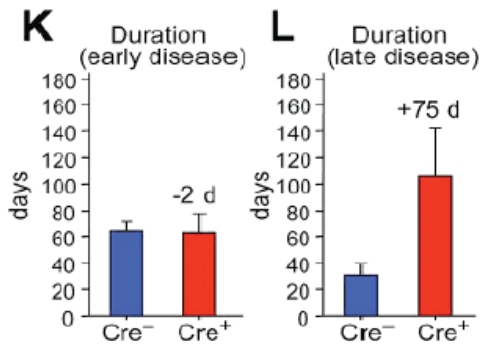




Motor neurons
Delays onset and progression of disease

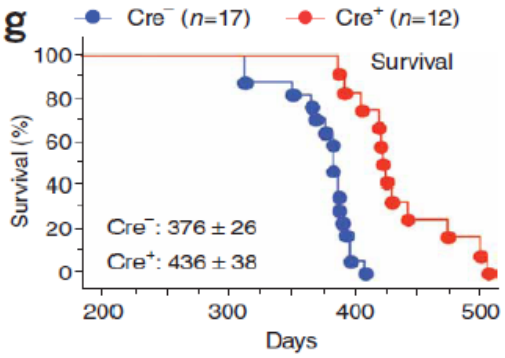
Cd11b-Cre

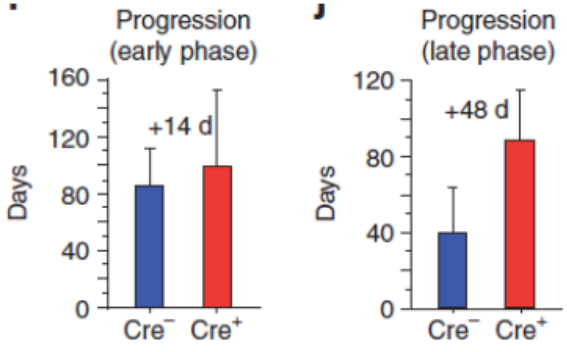




Microglia
Extend progression of disease

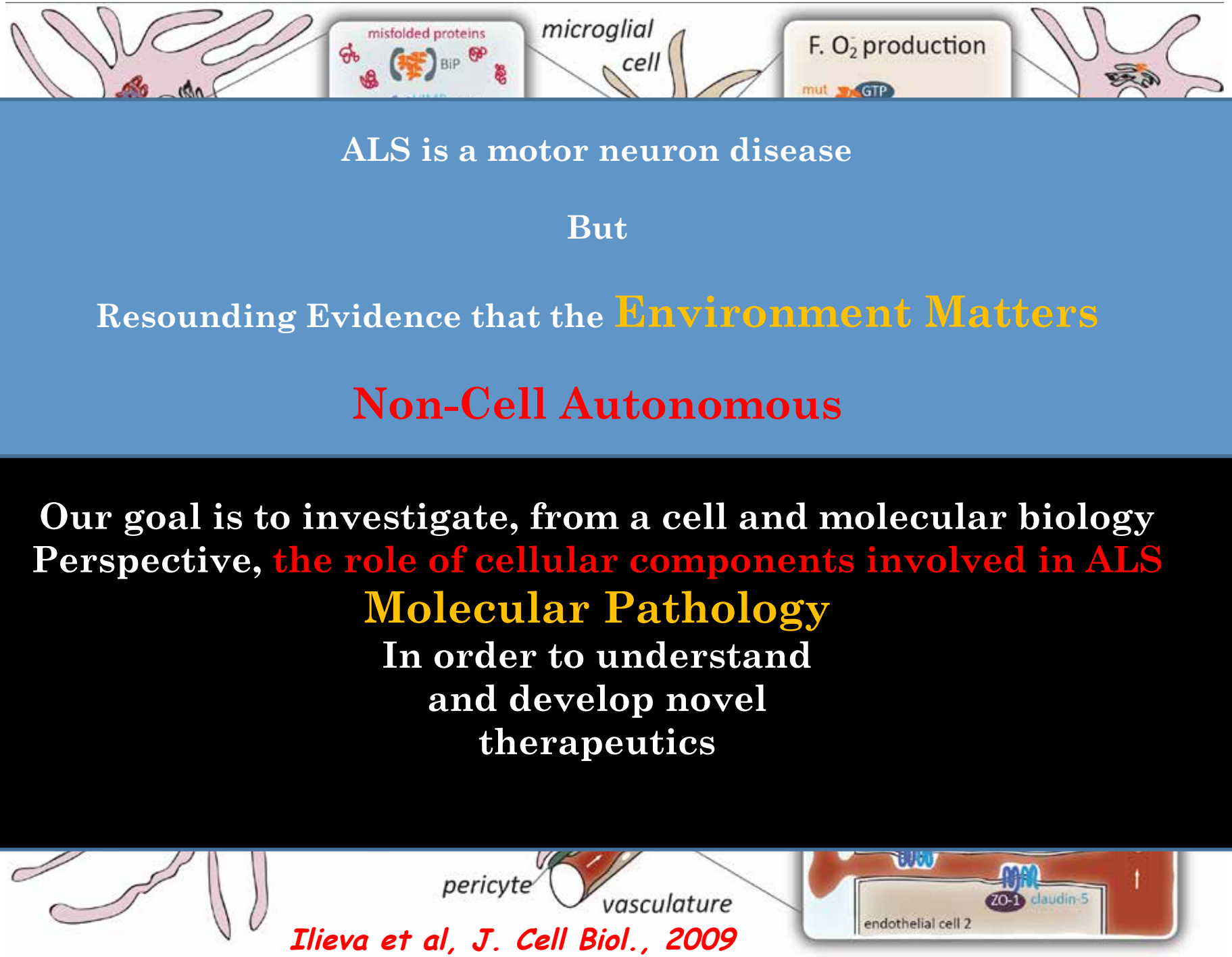
GFAP-Cre





Astrocytes
Extend progression of disease

Boillee *at al*, 2006; Yamanaka *et al* 2008



ALS is a motor neuron disease

But

Resounding Evidence that the Environment Matters

Non-Cell Autonomous

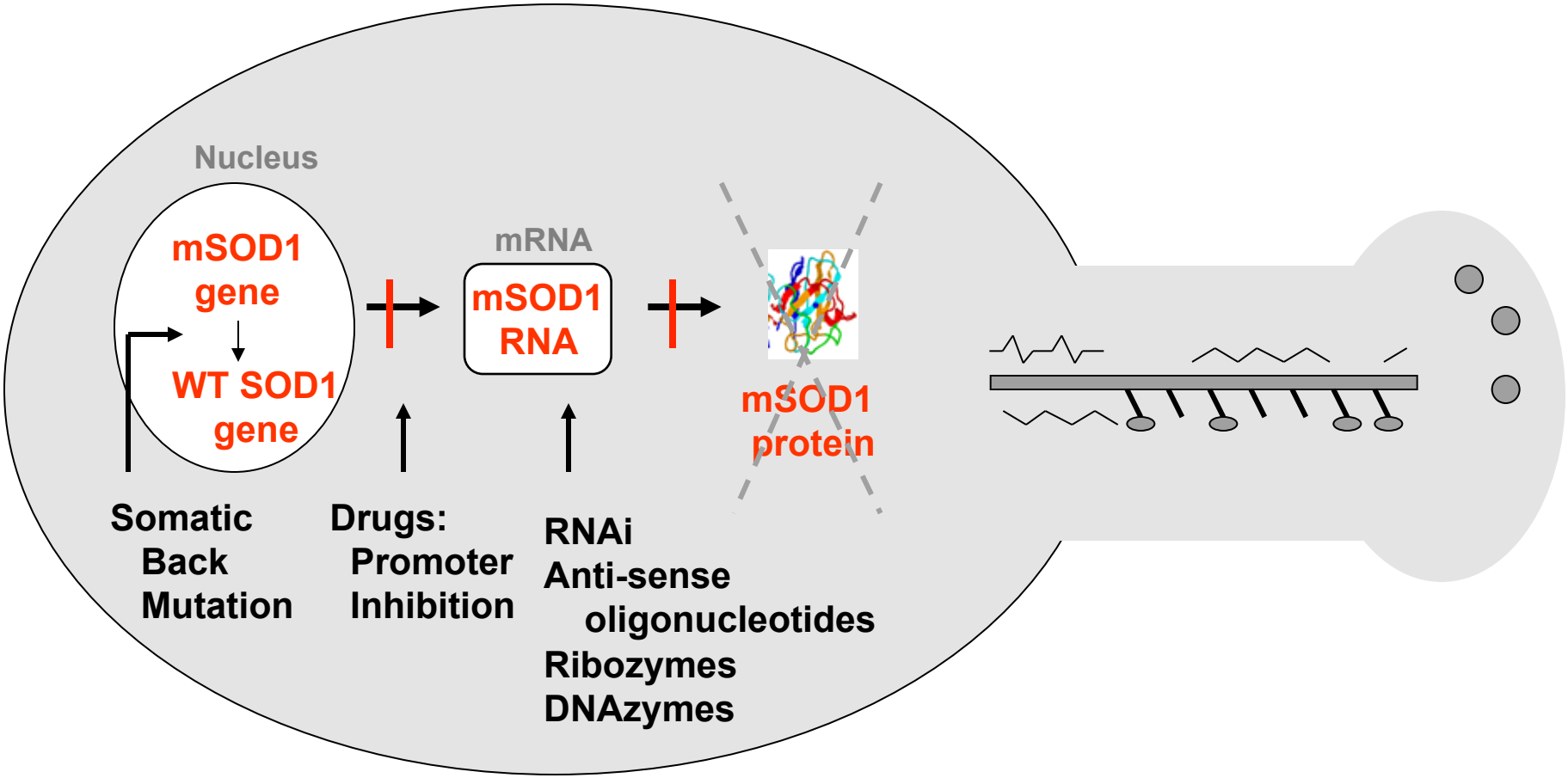
Our goal is to investigate, from a cell and molecular biology Perspective, the role of cellular components involved in ALS

Molecular Pathology

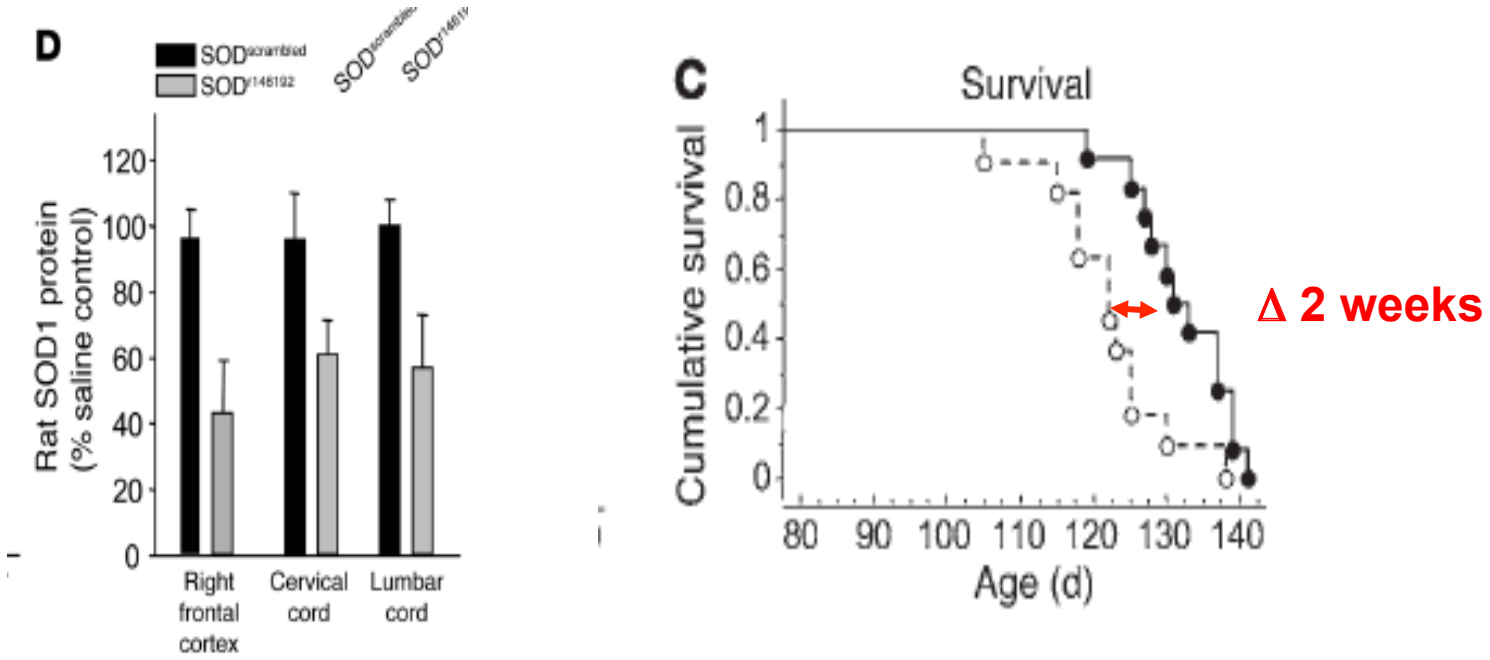
In order to understand and develop novel therapeutics

Ilieva et al, J. Cell Biol., 2009

The most proximal treatment for SOD1-related ALS is to turn off the sick gene.



Trials of allele inactivation therapy for SOD1-mediated ALS are planned (Isis)



Intraventricular infusion of anti-SOD1 anti-sense oligonucleotides prolongs survival in transgenic $SOD1^{G93A}$ rats.

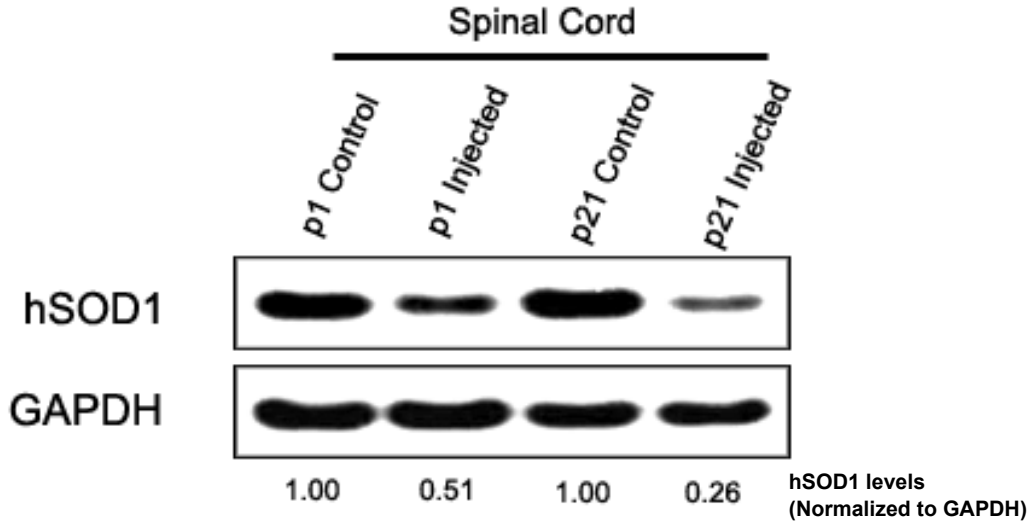
Smith RA, Mller T, Bennett CF, Cleveland DW et al.
J. Clin. Inv. 116(8): 2290-2296, 2006.

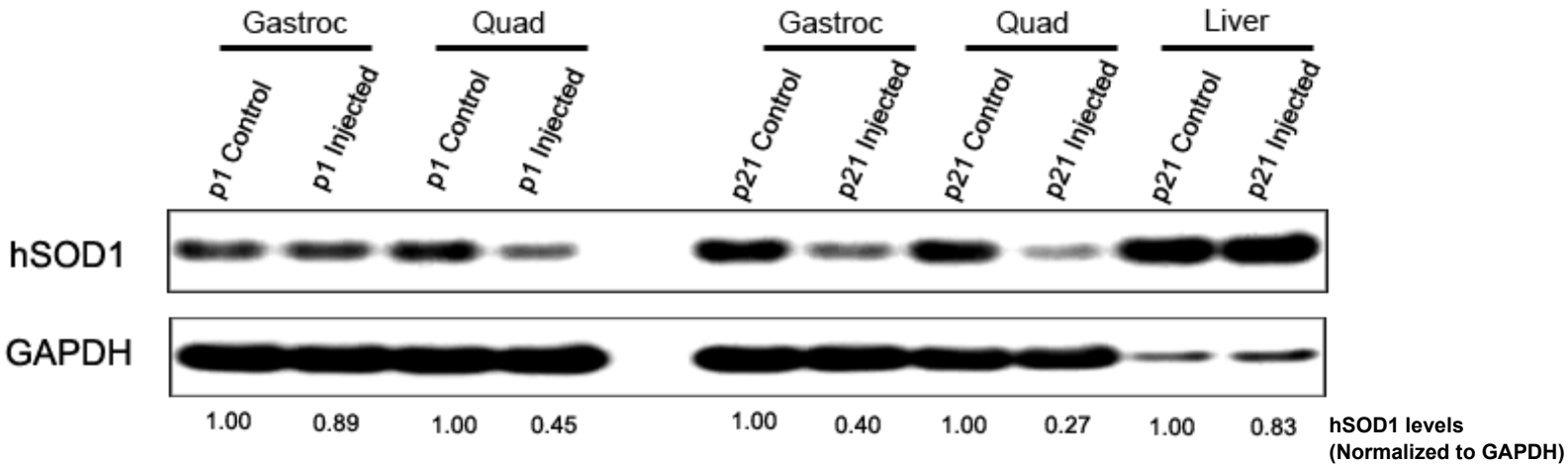
Phase 1 Clinical Trial

- Intrathecal
- Toxicology in rats and rhesus monkey
- Trial design (Phase I)
  - Single dose infusion
  - 32 familial SOD1 patients (target A4V)
  - 4 Dosages

AAV9-shRNA SOD1

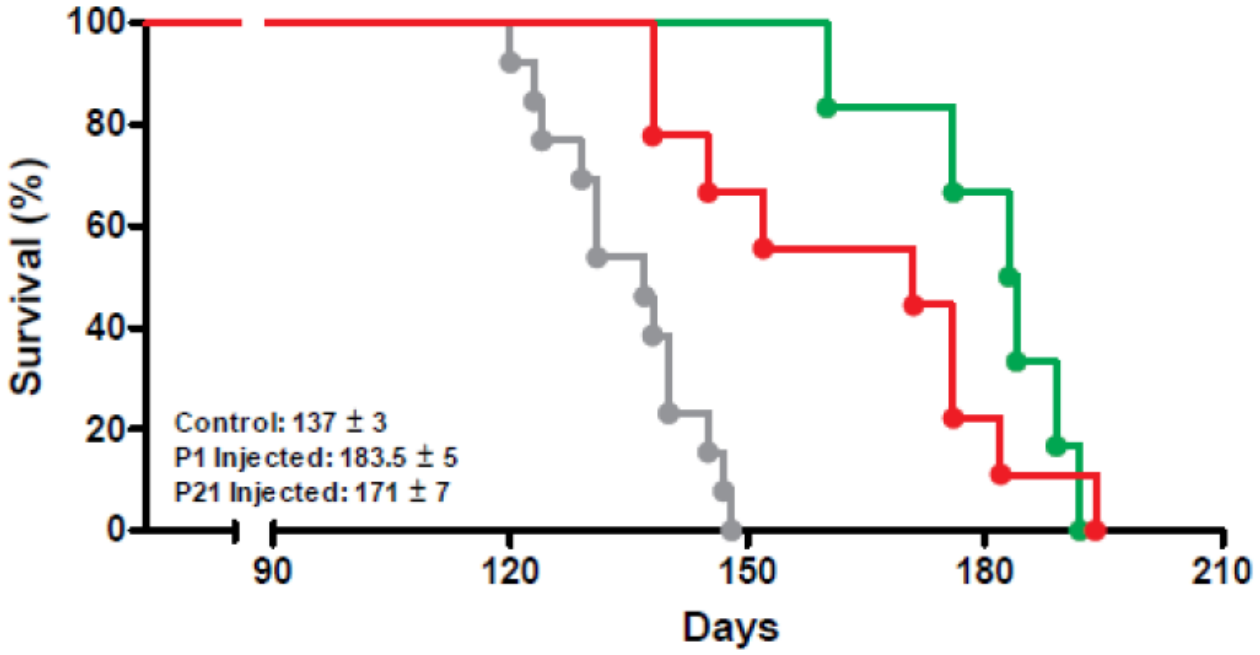
Delivered Systemically at P1 or P21 in *G93A SOD1 animals*

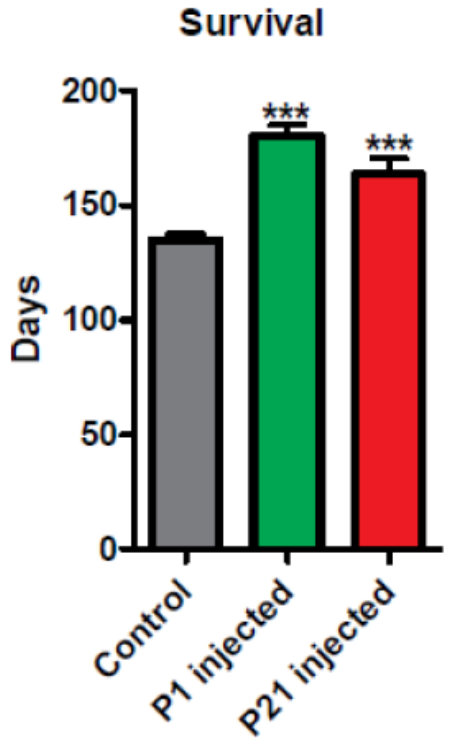




Survival increase by delivery of AAV9-shRNA against SOD1

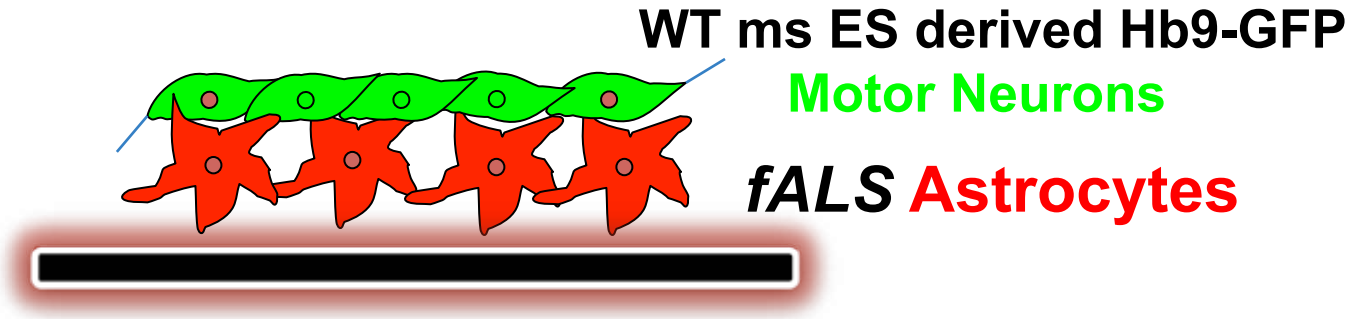
Demonstrates remarkable life extension in ALS mice





% increase in survival: P1 Injected- 35% P21 Injected-25%

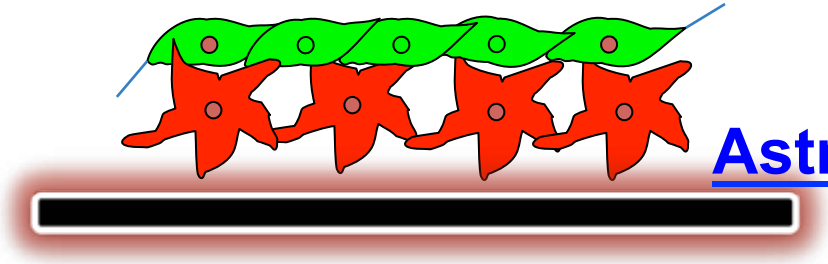
Co-culture based models of *fALS* demonstrates Astrocytes Convey Toxicity to Motor Neurons



Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons.
Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S.
Nat Neurosci. 2007 May;10(5):615-22.

Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model.
Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K.
Nat Neurosci. 2007 May;10(5):608-14.

Co-culture based models of familial ALS and sporadic ALS



WT ms ES derived Hb9-GFP

Motor Neurons

Astrocytes

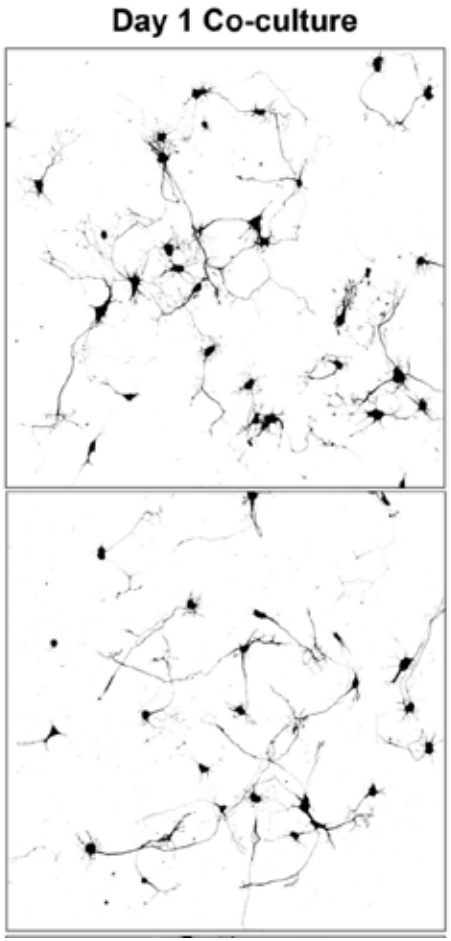
- NON-ALS
- Familial ALS
- Sporadic ALS

ALL Astrocytes have been derived from post-mortem derived NPCs

G93A SOD1 Mouse NPC-derived astrocytes convey toxicity to embryonic derived motor neurons in an *in vitro* co-culture model

Non-ALS Astrocytes

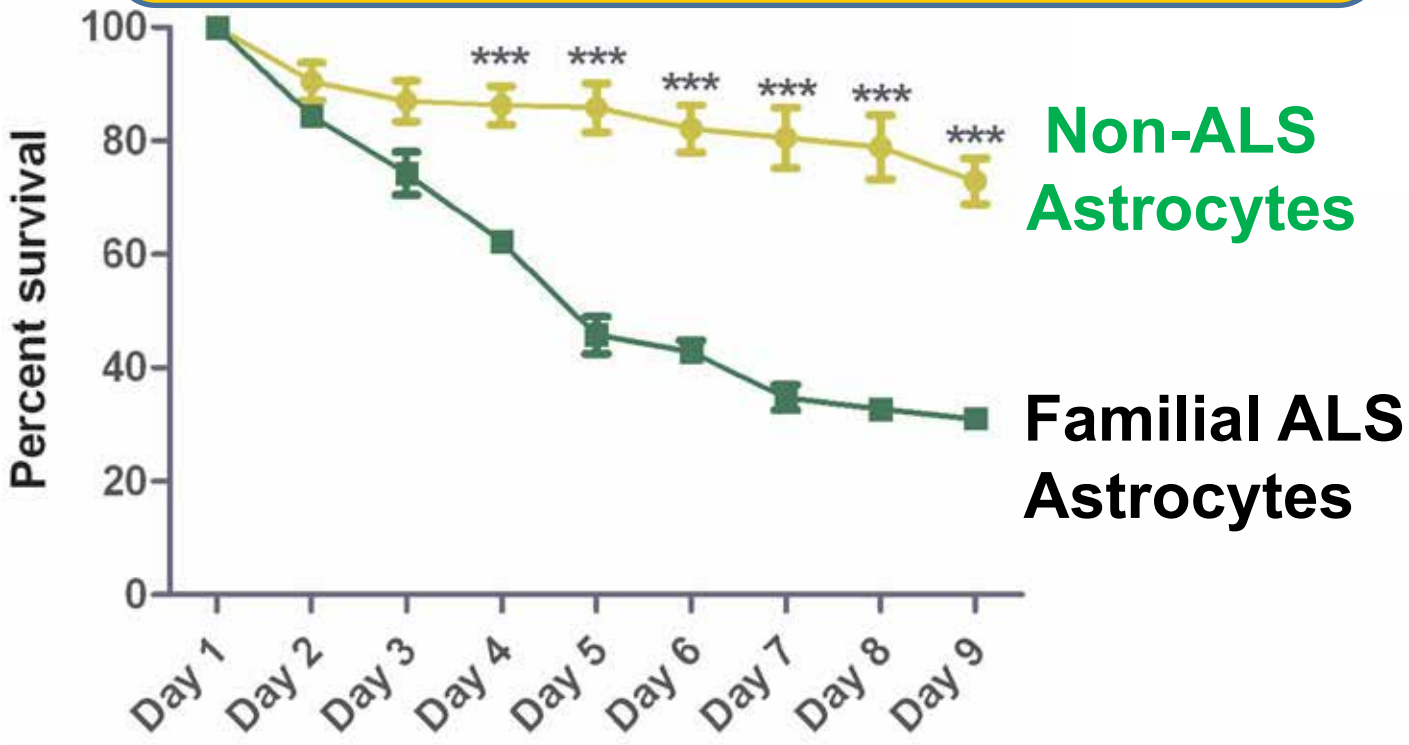
Familial ALS Astrocytes



24 Hours → 72 Hours → 120 Hours

G93A SOD1 Mouse NPC-derived astrocytes convey toxicity to embryonic derived motor neurons in an *in vitro* co-culture model

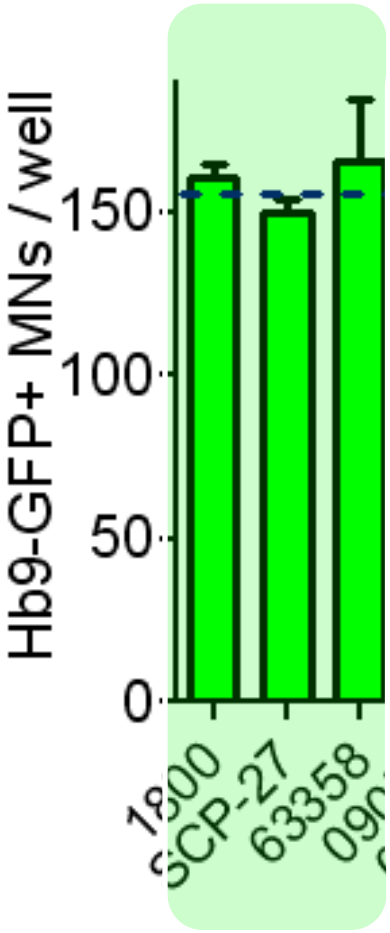
Robust, Highly Repeatable Co-culture Model where Familial ALS astrocytes kill Motor Neurons



But, These Models Only Recapitulate familial SOD1 cases…

What about the other (sporadic) cases? iPS technology/Other Methods

Co-Culture Evaluation of Motor Neurons with Non-ALS-, fALS- and sALS-Astrocytes



NON-ALS **Sporadic ALS** **Familial ALS**

Summary

AAV9 results have been replicated by Don Cleveland's laboratory

G37R SOD1 suppression in progress and very promising.

In vitro rodent based models of familial ALS are robust to model Astrocyte Mediated Motor Neuron Toxicity

We (Kaspar Lab) isolated Neural Progenitors from familial ALS, Sporadic ALS and Non-ALS post mortem spinal cords which can differentiate into Astrocyte-like cells

Both familial ALS and sporadic ALS derived astrocytes retained intrinsic toxicity to kill motor neurons and toxicity is secreted. Microglia are another

Oligonucleotide-based, Gene Delivery, Antibodies and Drugs to Regulate Glial Toxicity appear to be a viable therapeutic approach for both familial and sporadic ALS.

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  - Miracles for Madison
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  - SMA Angels

PACKARD CENTER
P2ALS Project A.L.S.
NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

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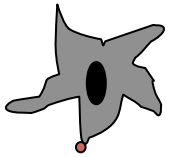
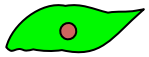
Ashley Frakes, Shibi Likhite, Leah Schmelzer

Next Weeks class is at 2.30pm in room
Room WA1525 at Nationwide Children's Hosiptal
 Wexner Research Institute

You should be able to get from the Moobury side

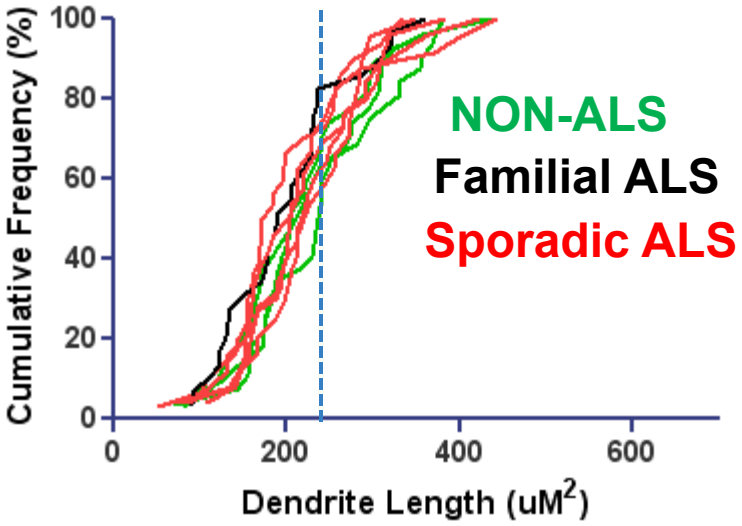
Motor Neuron Neurite length over time following co-culture

Astrocytes  NON-ALS
Familial ALS
Sporadic ALS

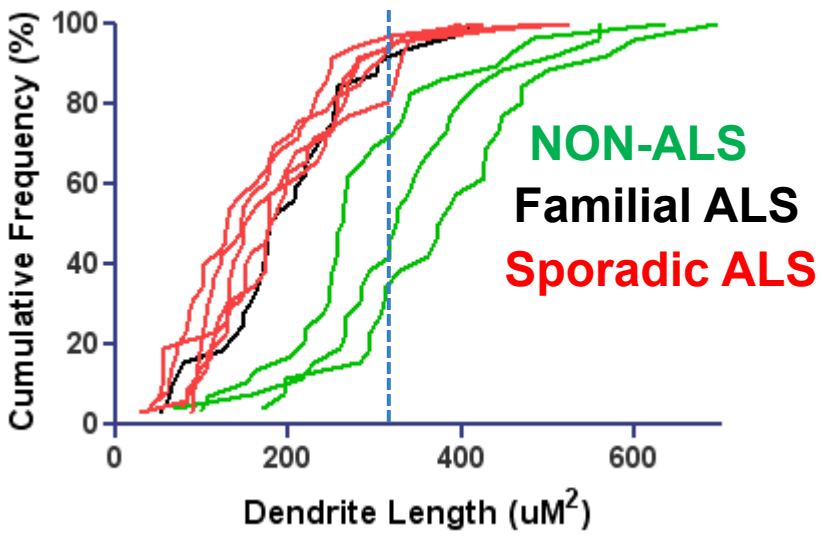
+ Motor Neurons 

Readout =
Motor Neuron
Neurite Length

DAY 2 in Culture



DAY 4 in Culture



By Day 4 of co-culture, Motor Neuron Neurite Length on fALS and sALS Astrocytes are significantly smaller than Non-ALS Astrocytes

But do Motor Neurons die?

Clinical Applicability

CNS Access: for diagnostic or therapeutic interventions

- Pre-term and newborn infants:
  1) Lumbar puncture:
  2) Percutaneous fontanelle puncture
  3) External Ventricular Drain (EVD)
  4) Indwelling subcutaneous ventricular reservoir

- Adolescent and Adult
  1) Lumbar Puncture
  2) External Ventricular Drain
  3) Ommaya indwelling reservoir
  4) Lumbar Drain
  5) Lumbar shunt connected to indwelling pump