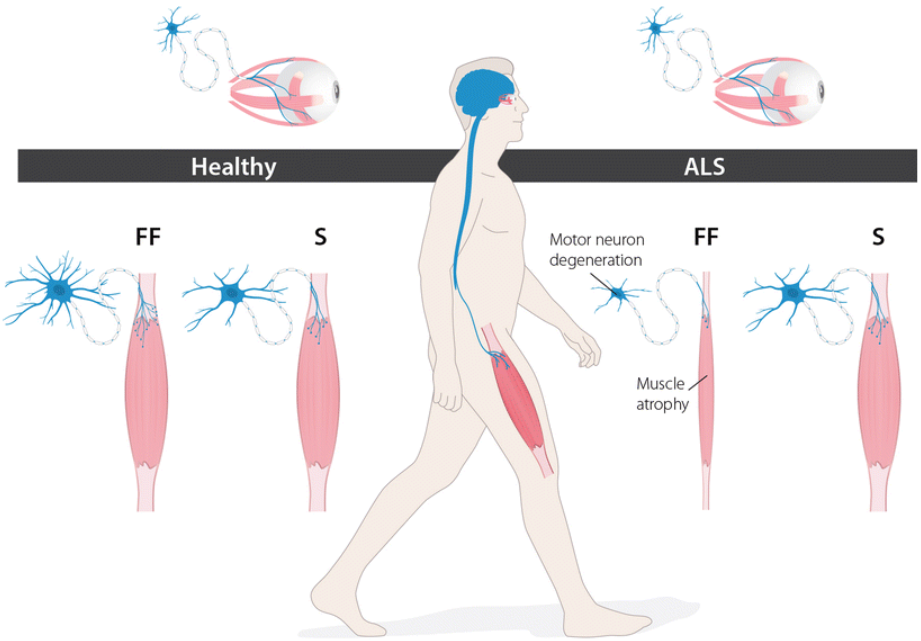
Exercise: design a vector-based gene therapy for SOD1 ALS

Amyotrophic lateral sclerosis (ALS)

- motor neuron degeneration leading to skeletal muscle paralysis
- fatal within 2-5 yrs, locked-in syndrome, respiratory failure
- no effective treatment available



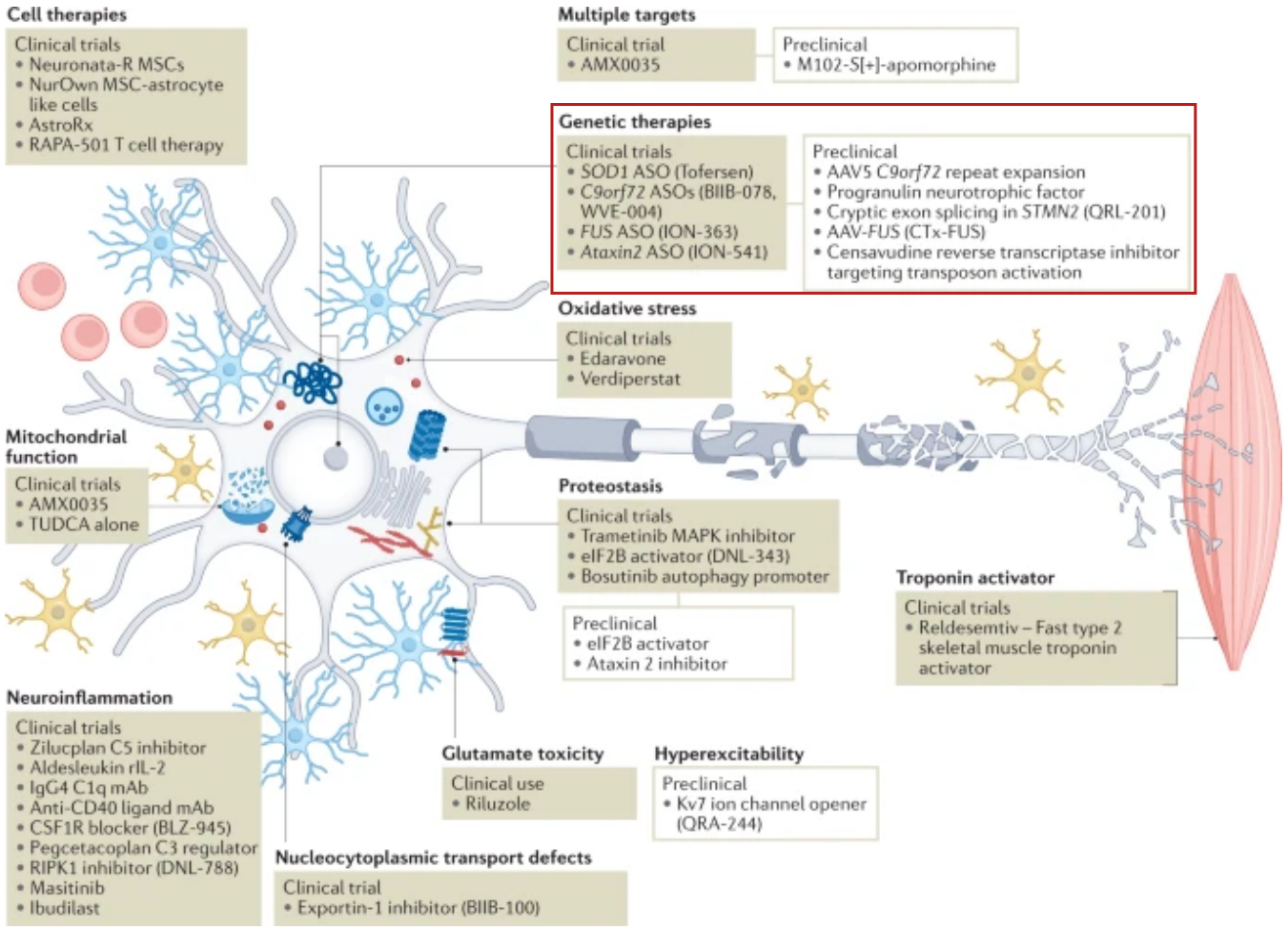
Therapeutic strategy: reduce the level of toxic factors (mutated SOD1 protein, mutated C9ORF72 RNA)

Current approaches:

- antisense oligonucleotides (tofersen: phase I → III trials)

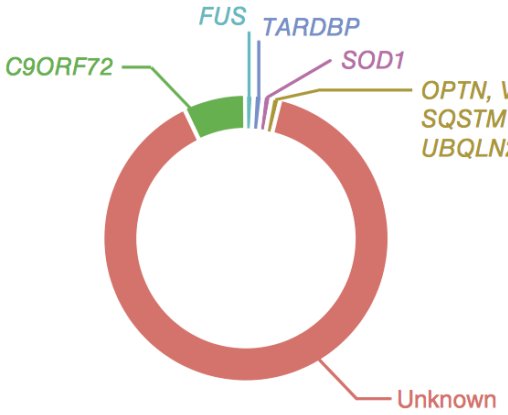
Nijssen, J. et al, Acta Neuropathol (2017) 133: 863.

Amyotrophic lateral sclerosis (ALS)



Nat Rev Drug Discov 2023;22(3):185-212. doi: 10.1038/s41573-022-00612-2

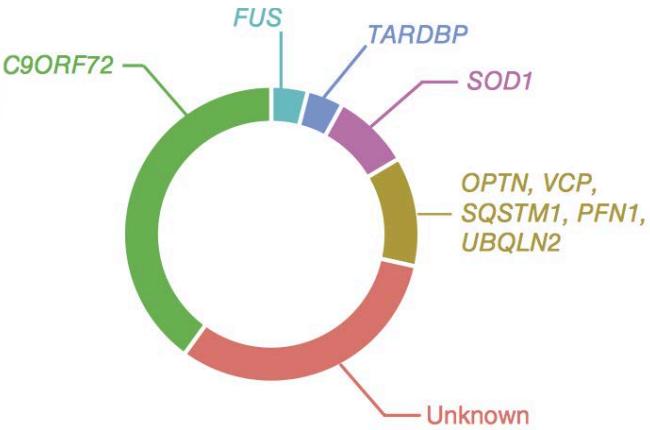
ALS is linked to genetic causes and SOD1 pathology



Amyotrophic Lateral Sclerosis

85-90% sporadic

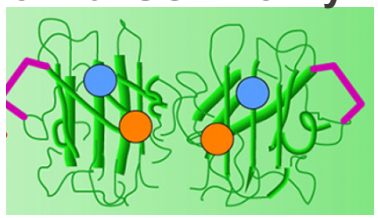
10-15 % familial



Misfolded SOD1

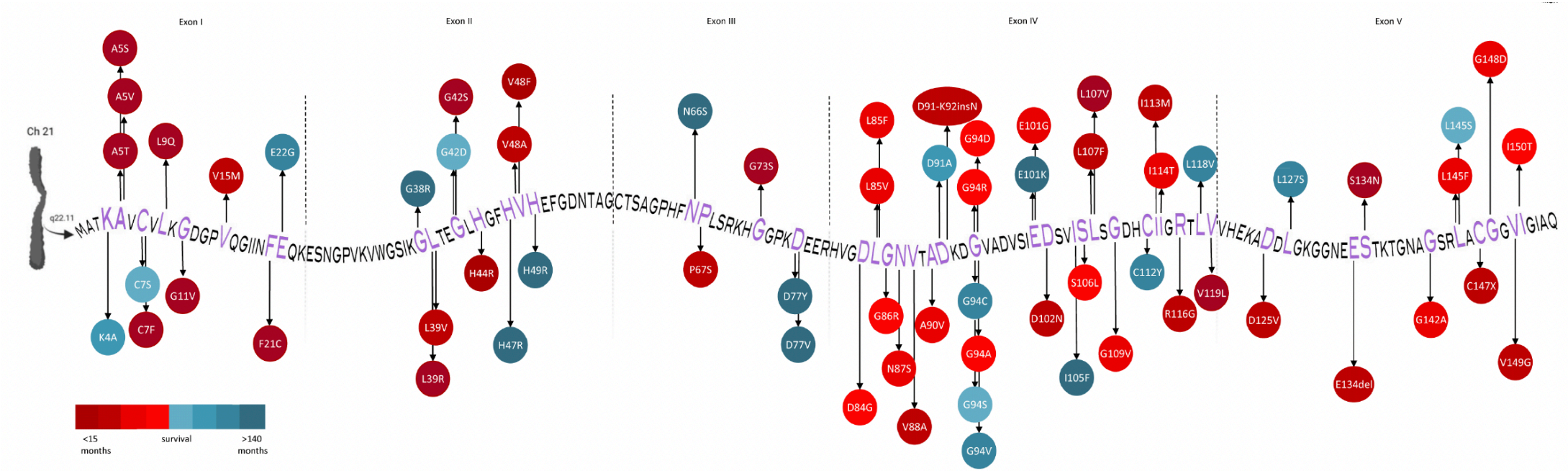
Mutations, oxidation, Zn depletion

Normal SOD1 enzyme



"Aggregation - Gain of toxic function" → degeneration of motoneurons

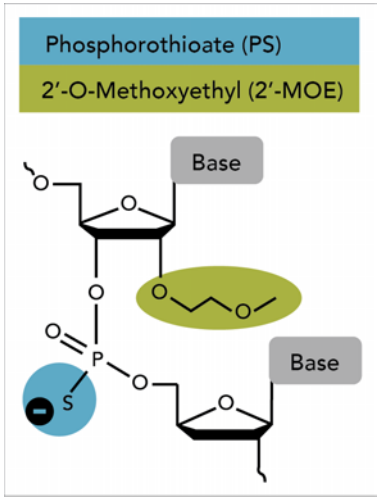
SOD1 mutations and Amyotrophic Lateral Sclerosis

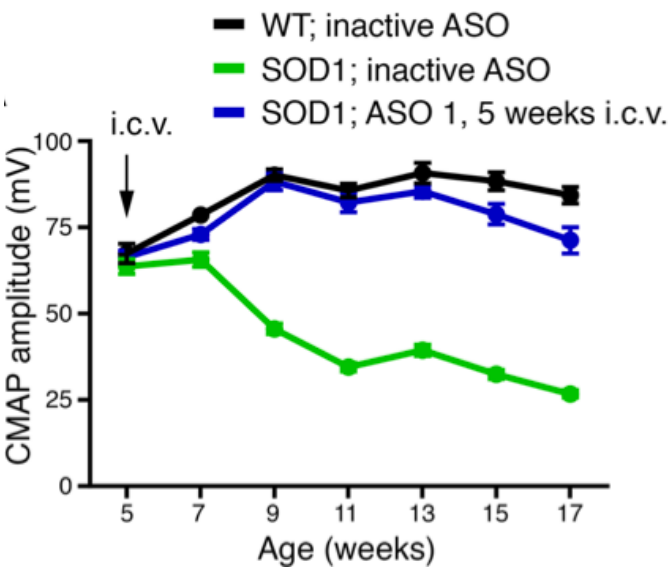


Neural Regeneration Research 18(7):p 1427-1433, July 2023. | DOI: 10.4103/1673-5374.361535

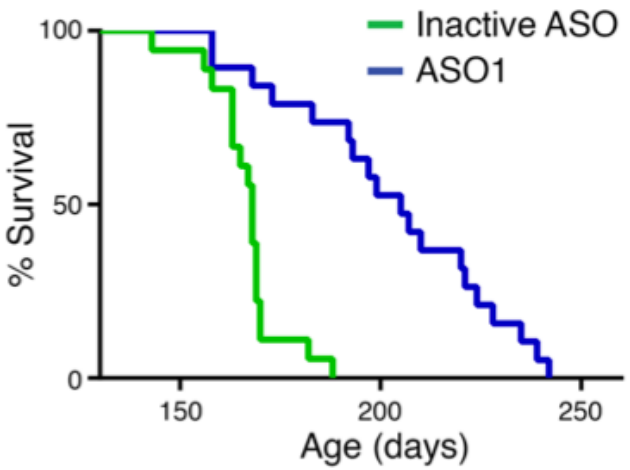
Targeting human SOD1 with antisense oligonucleotides

Proof-of-concept in high-copy SOD1 G93A mice





i.c.v. ASO administration
at day 50 and day 94 (300 µg)



Phase I/II → Phase III clinical trials with Tofersen (20 → 100 mg)

- McCampbell A et al, JCI 2018

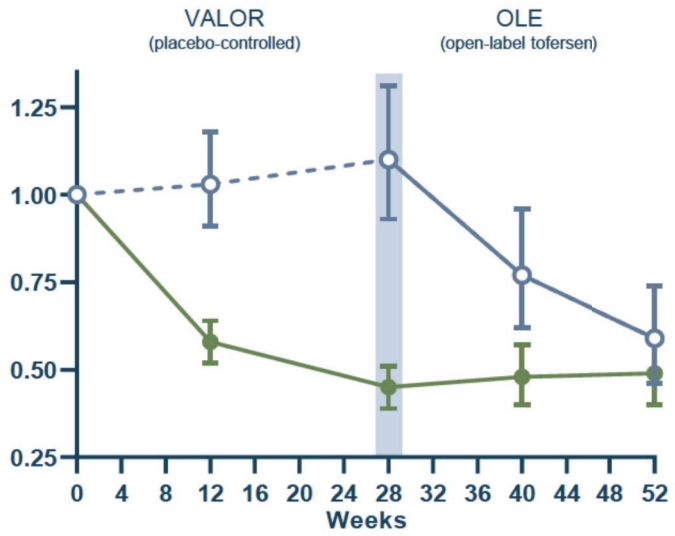
Antisense oligonucleotide to reduce SOD1 expression: phase III trial

Tofersen

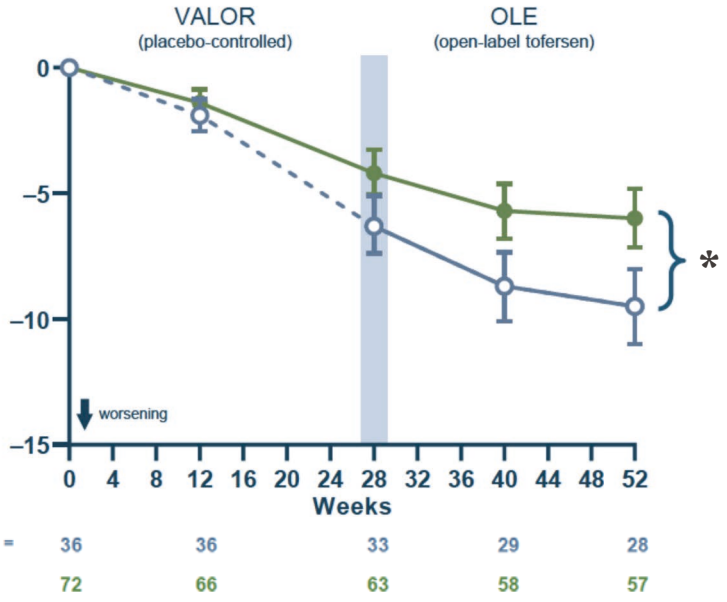
(modified ASO against hSOD1 mRNA)

Repeated intrathecal injections

Plasma Nfl level



ALSFRS



Placebo → Delayed-start tofersen, n =	36	36	33	29	28
Early-start tofersen, n =	72	66	63	58	57

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Miller T, ANAAM 2021
Miller T et al, NEJM 2020
Miller T et al, ENCALS 2022

Objective:

Develop an alternative gene therapy approach using a viral vector for single time administration.

Gene therapy: question 3

One consider a gene therapy approach against SOD1 as a treatment for SOD1-related familial ALS.

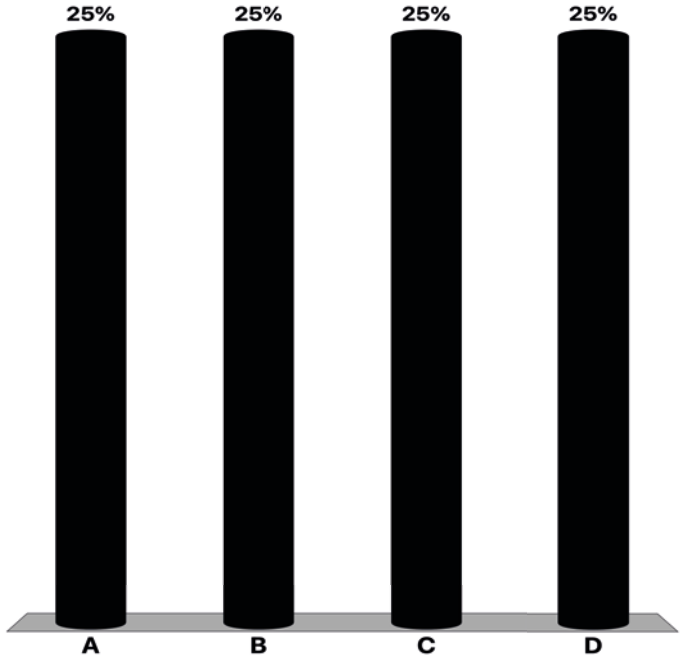
What would you consider as a realistic strategy? (consider gain- or loss-of-function as a cause of SOD1 toxicity as well as the number of mutations)

A. Silence selectively the mutated SOD1 protein by RNA interference.

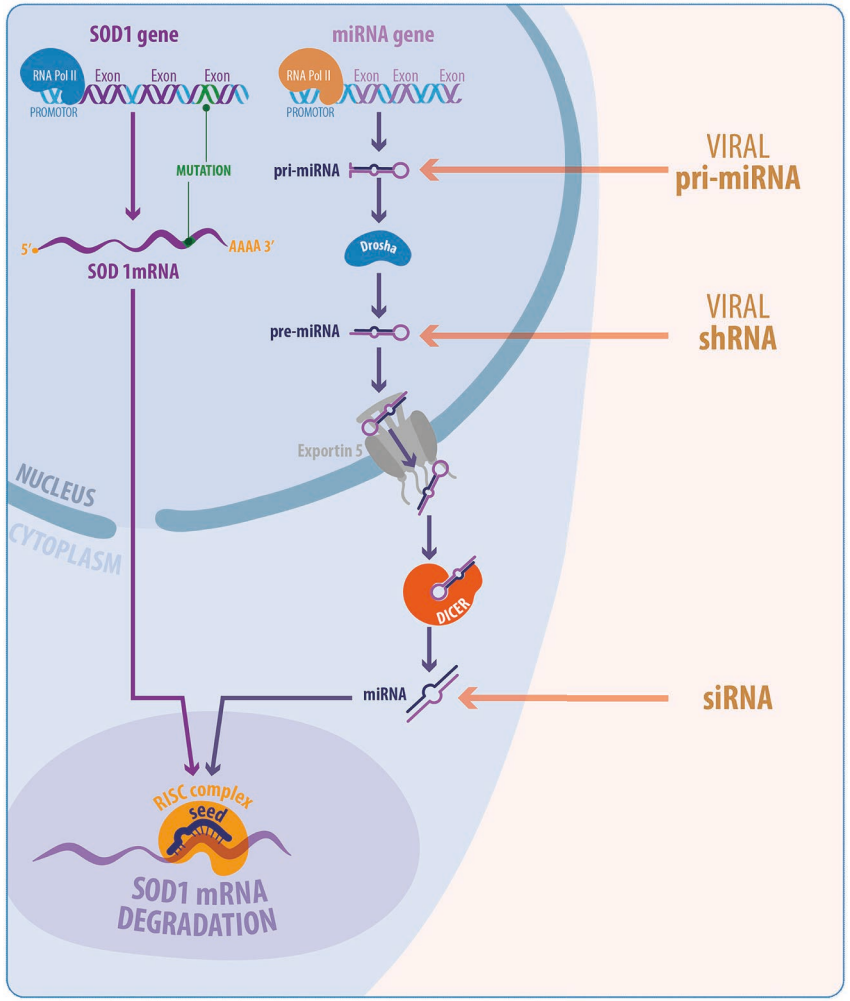
B. Gene edit the mutated SOD1 allele.

C. Knock-out the expression of SOD1 using CRISPR/Cas9.

D. Silencing all forms of the SOD1 protein to reduce overall SOD1 level.



Vector-based RNA interference against SOD1



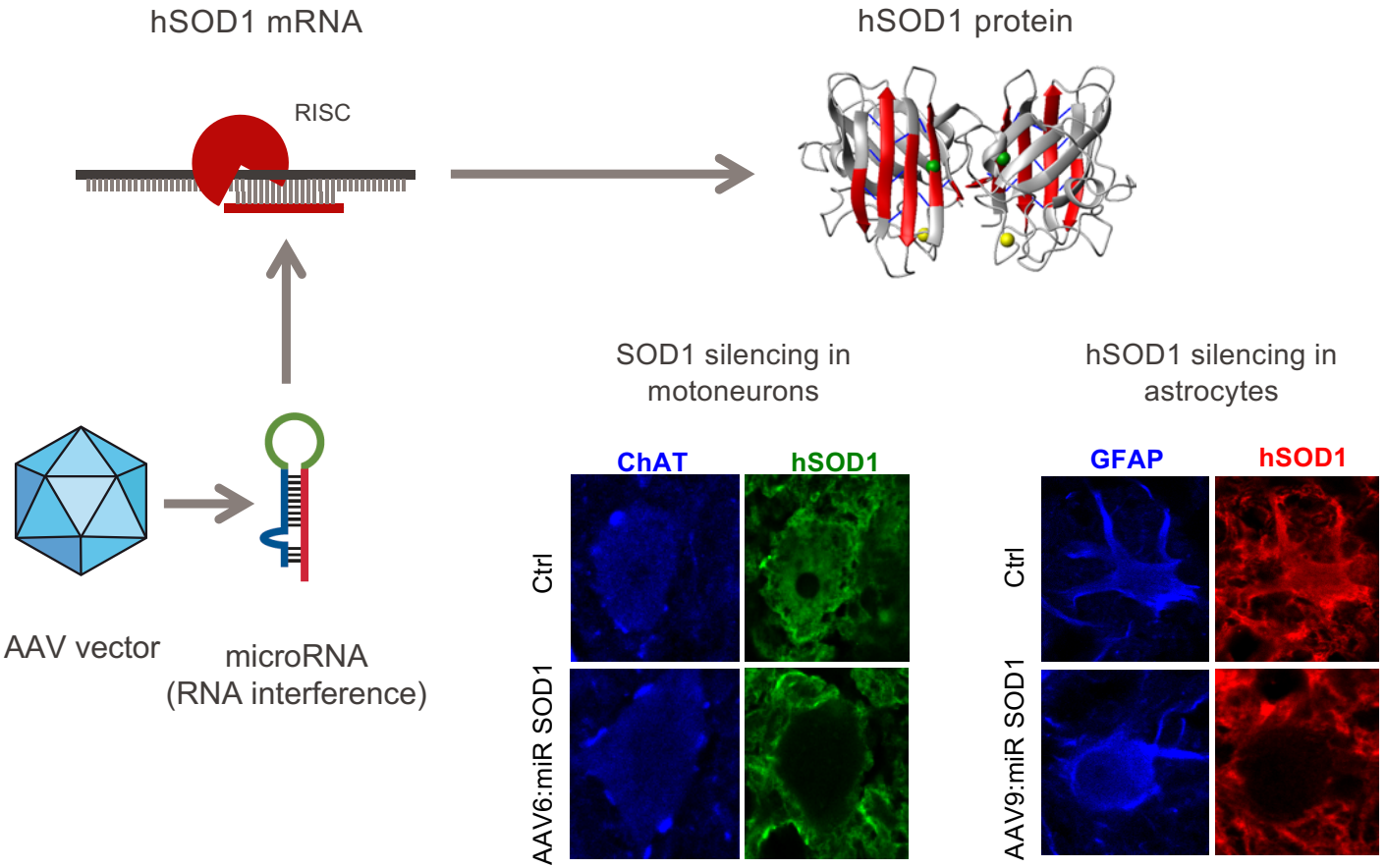
Therapeutic approach:

- AAV-mediated **RNA interference** against SOD1 mRNA: **expression of an artificial micro RNA**

Van Zundert et al, Neurosci Lett 2016

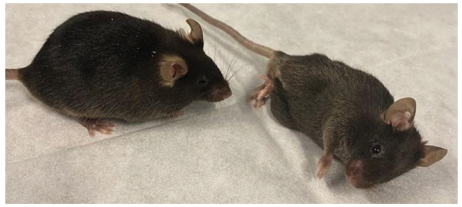
Gene therapy: neuromuscular system

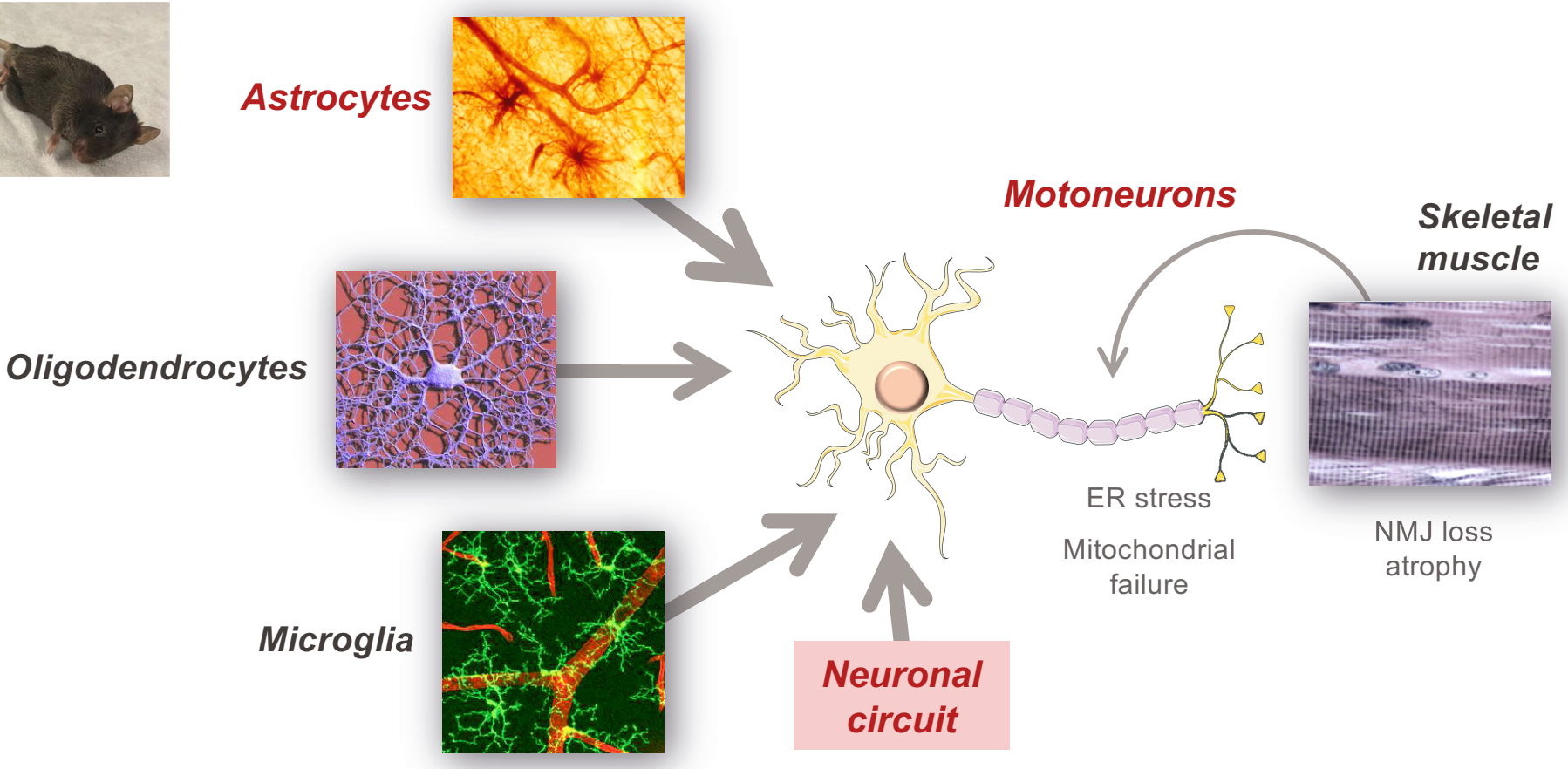
miRNA silencing of human SOD1 expression in the spinal cord



Multiple cell types contribute to ALS pathogenesis

B6.Cg mutated SOD1
transgenic mice



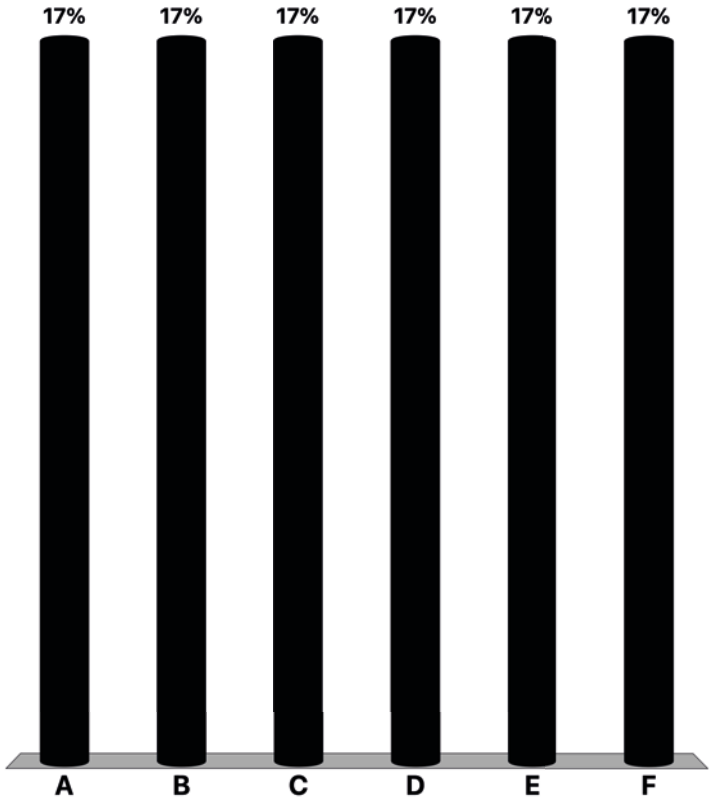


Gene therapy: question 4

For efficacy:

For gene therapy against mutated SOD1 to be successful, assuming that you have an effective vector for each of them, which cell type(s) would you target in priority? (multiple answers are possible)

A. Astrocytes
B. Neurons including motoneurons
C. Skeletal muscle
D. Microglia
E. Oligodendrocytes
F. Schwann cells



Gene therapy: question 5

Which vector system would you consider?

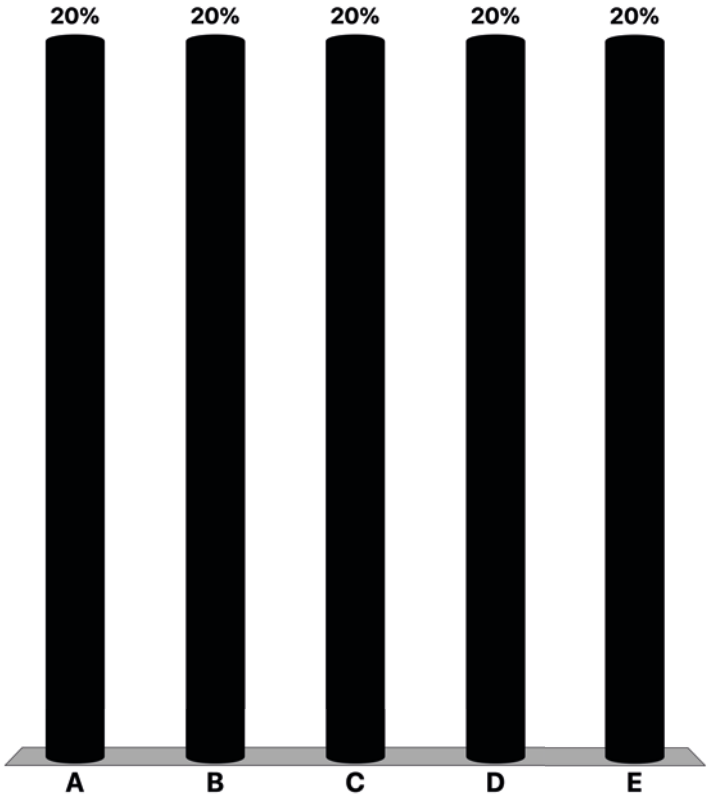
A. AAV

B. Lipid nanoparticles

C. Lentivirus

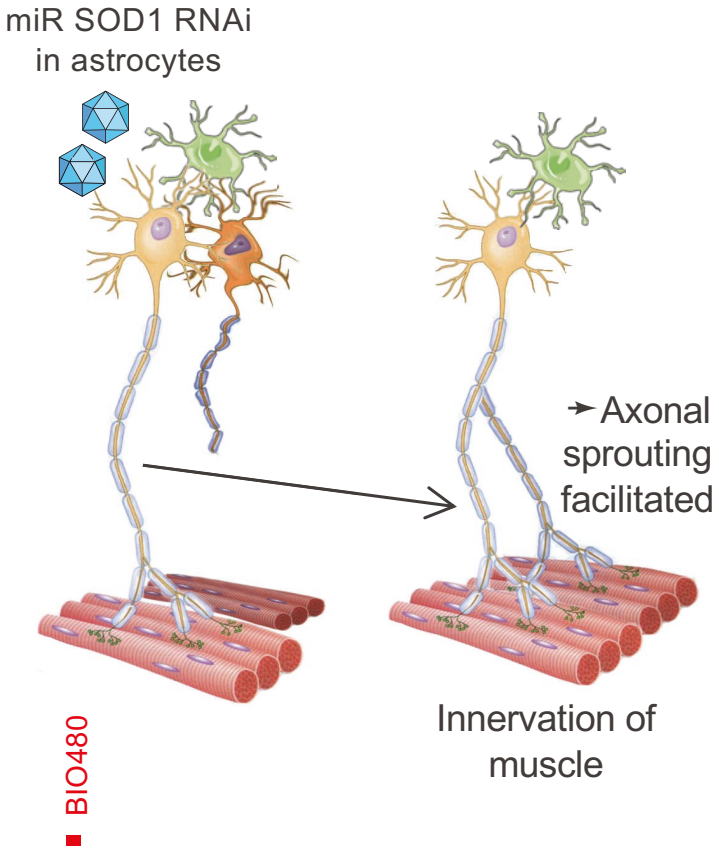
D. Adenovirus

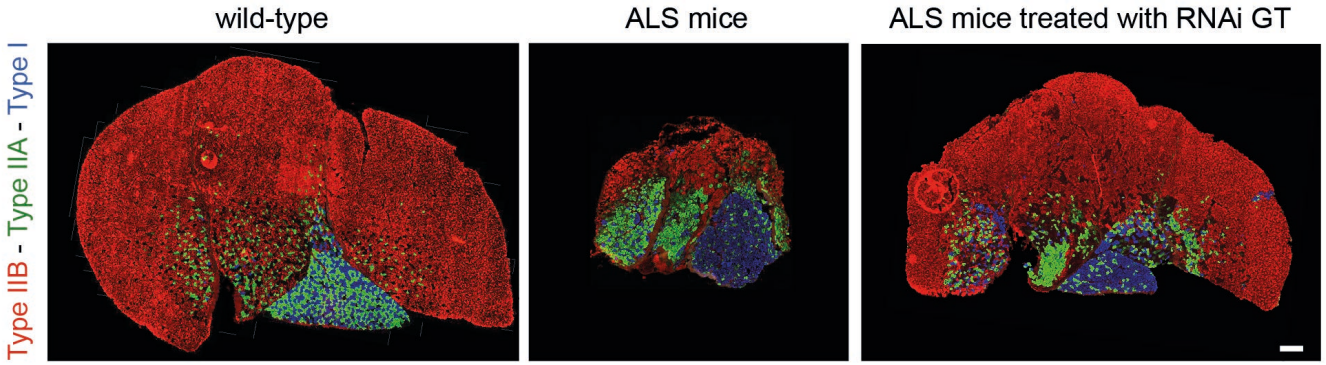
E. Exosomes



Gene therapy: SOD1 ALS

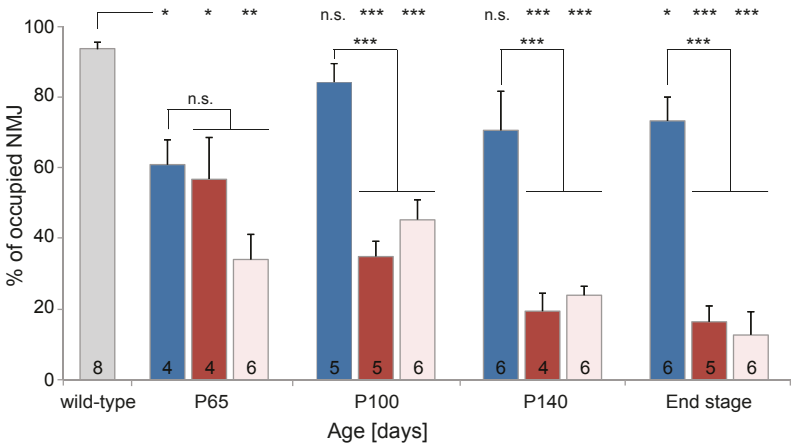
In vivo silencing of SOD1 in astrocytes protects neuromuscular junction innervation



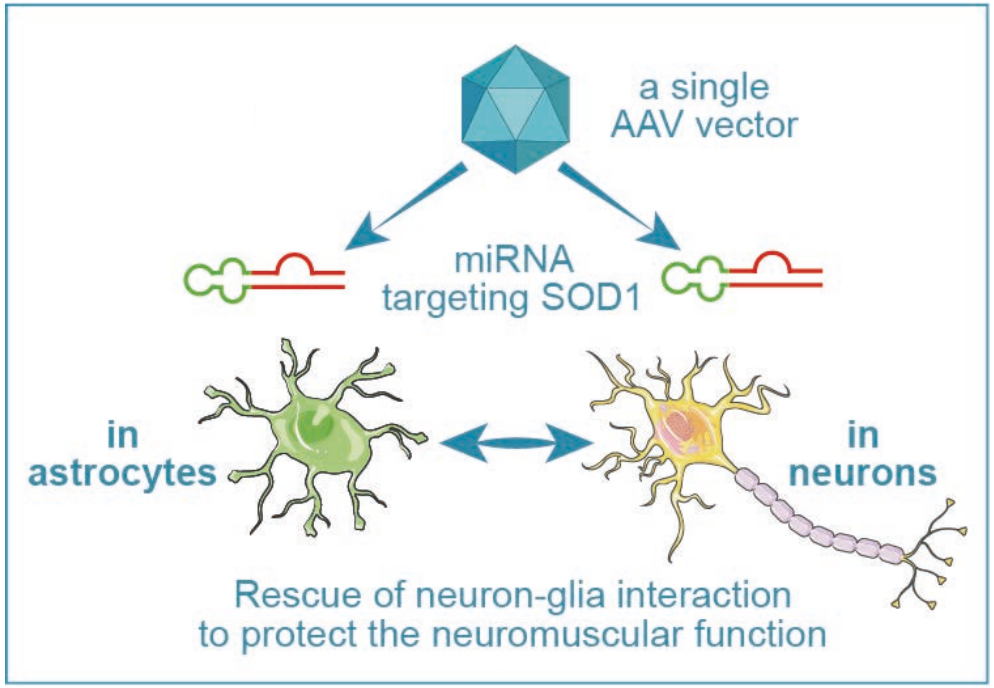


Occupancy of neuromuscular junctions

- wild-type
- ALS mice - RNAi ctrl
- ALS mice - RNAi GT
- ALS mice



Targeting SOD1 in neurons & astrocytes to synergize therapeutic efficacy



- hSOD1 RNAi in neurons
  to optimize **neuroprotection**
- hSOD1 RNAi in astrocytes
  to promote **muscle innervation**

→ **Synergistic effects ?**

Gene therapy: question 6

To achieve targeting of SOD1 in the key cell types, what you consider as the most important element(s):
(Rank from the most relevant to the least relevant)

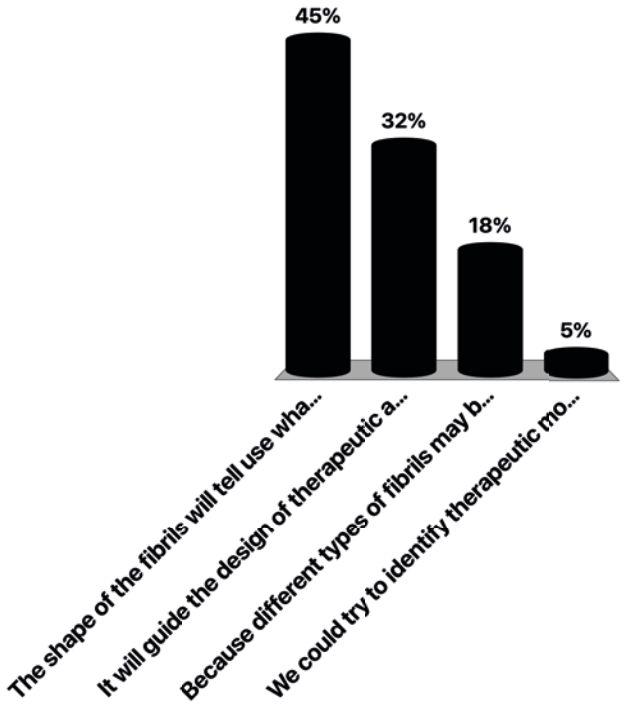
A. Expression system (promoter/enhancer)

B. Vector type

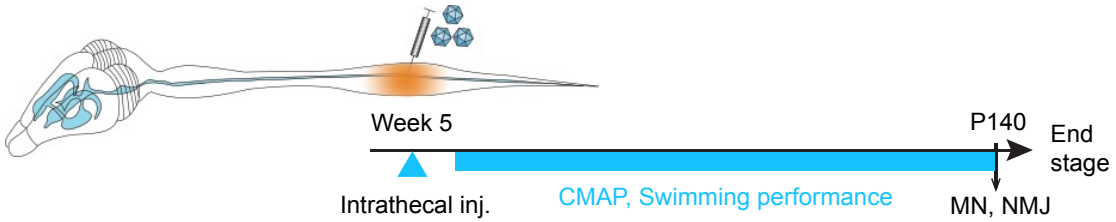
C. RNAi sequence

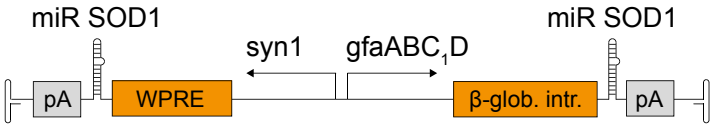
D. Route of administration

E. Vector dose

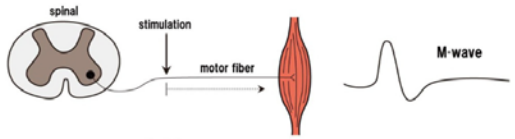


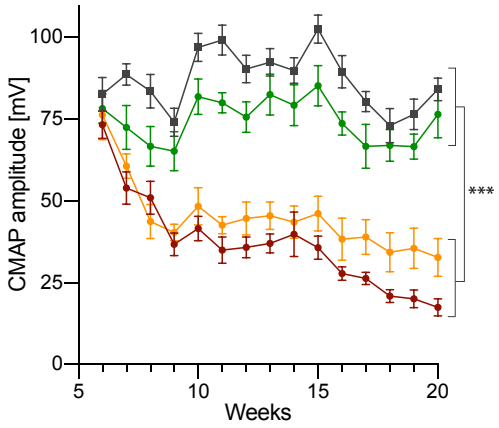
Targeting SOD1 in neurons & astrocytes with a single AAV vector provides maximized therapeutic efficacy



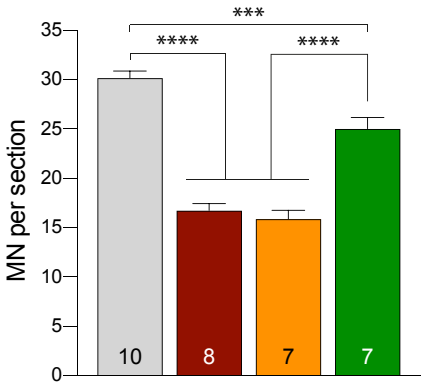


- wild-type mice
- SOD1 mice + PBS
- SOD1 mice + AAV-syn1-RFP-miR SOD1
- SOD1 mice + AAV-syn1/gfaABC$_1$D-miR SOD1

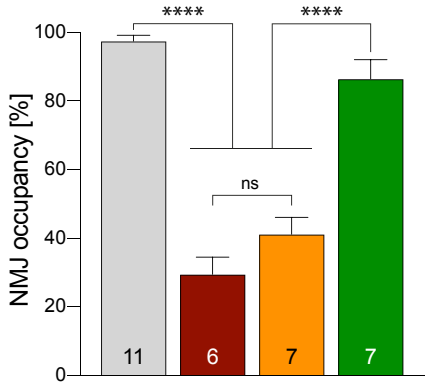




Motoneuron survival at end stage



NMJ occupancy at end stage



Gene therapy: question 7

In which model would you preferably test the therapeutic efficacy of your gene therapy:

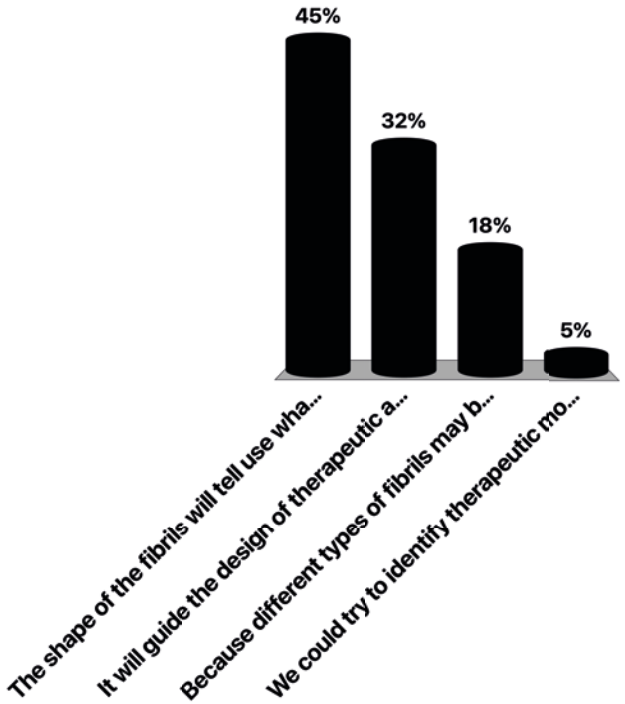
(Rank from the most relevant to the least relevant)

A. A cell line overexpressing hSOD1

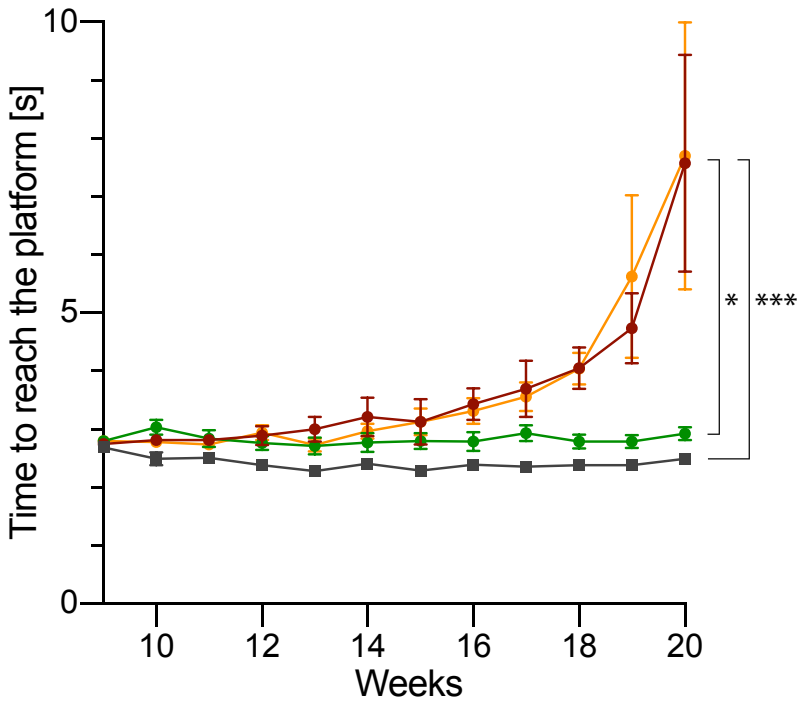
B. iPS-derived motoneurons carrying a SOD1 mutation

C. ALS mouse model overexpressing mutated SOD1

D. A co-culture glia-neurons



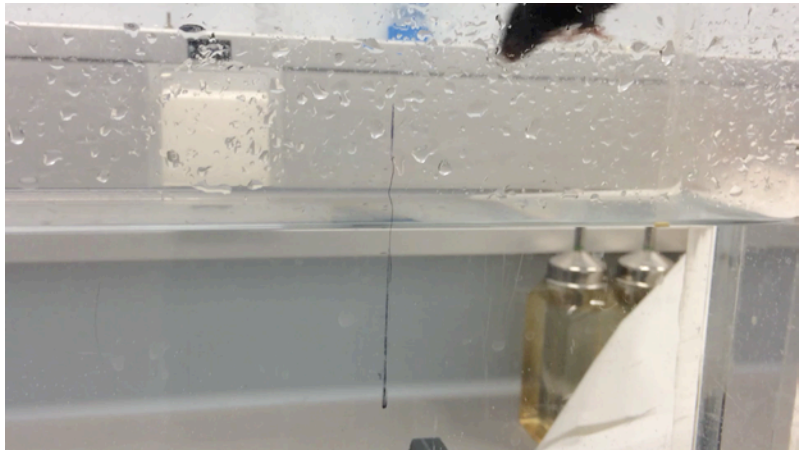
Targeting astrocytes in addition to neurons for SOD1 RNAi has potent effects on the motor function



- wild-type mice
- SOD1 mice + PBS
- SOD1 mice + AAV-syn1-RFP-miR SOD1
- SOD1 mice + AAV-syn1/gfaABC$_1$D-miR SOD1

ALS SOD1^{G93A} **PBS injected**

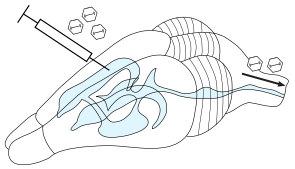
ALS SOD1^{G93A} **+ AAV9-syn1/gfaABC$_1$D-miR SOD1**



Bicistronic vector for miR SOD1 expression in neurons and glia has potent neuroprotective effects on SOD1 ALS mice

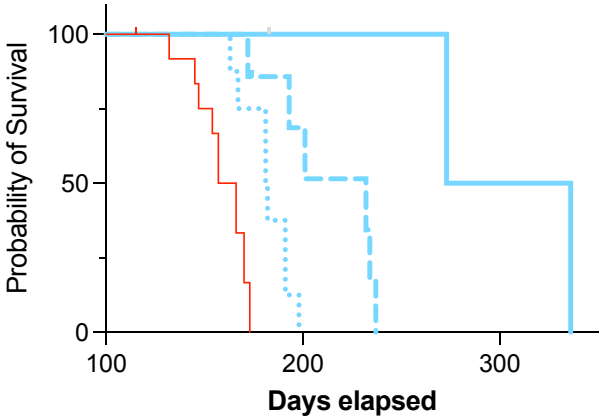
AAV9

ICV injection in neonatal SOD1^{G93A} mice (P1-3)

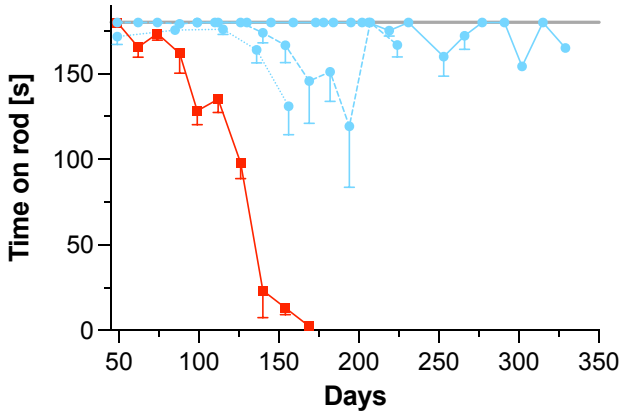


SOD1 G93A non-treated
SOD1 G93A + AAV9-miR SOD1 [4^{E}10 VG]
SOD1 G93A + AAV9-miR SOD1 [3^{E}11 VG]
SOD1 G93A + AAV9-miR SOD1 [2.4^{E}11 VG]

Survival proportions



Rotarod



Gene therapy: question 8

For safety:

Considering possible off-target effects of your therapy, in which tissue(s) would you try to spare SOD1 expression in priority ?

(Rank from the most relevant to the least relevant)

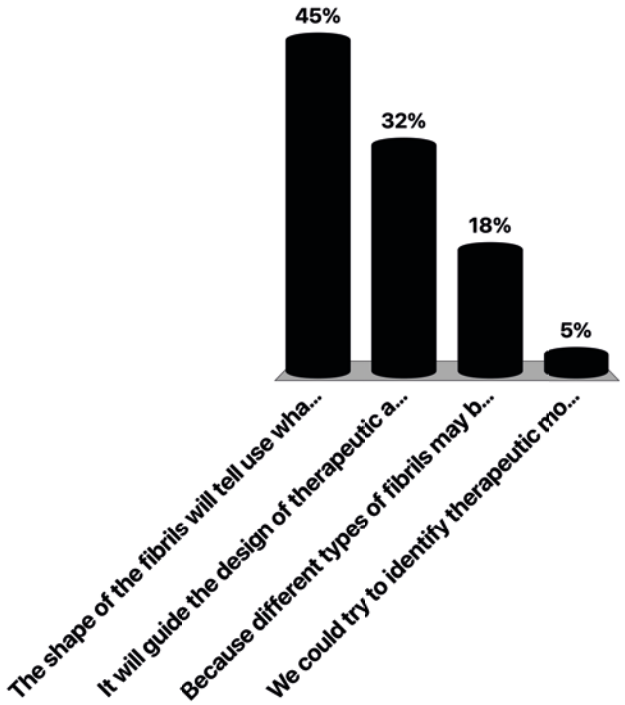
A. Skeletal muscle

B. Heart

C. Brain

D. Peripheral nervous system

E. Liver



Gene therapy: question 9

To limit the off-target effects of SOD1 silencing, what you consider as the most important element(s): (Rank from the most relevant to the least relevant)

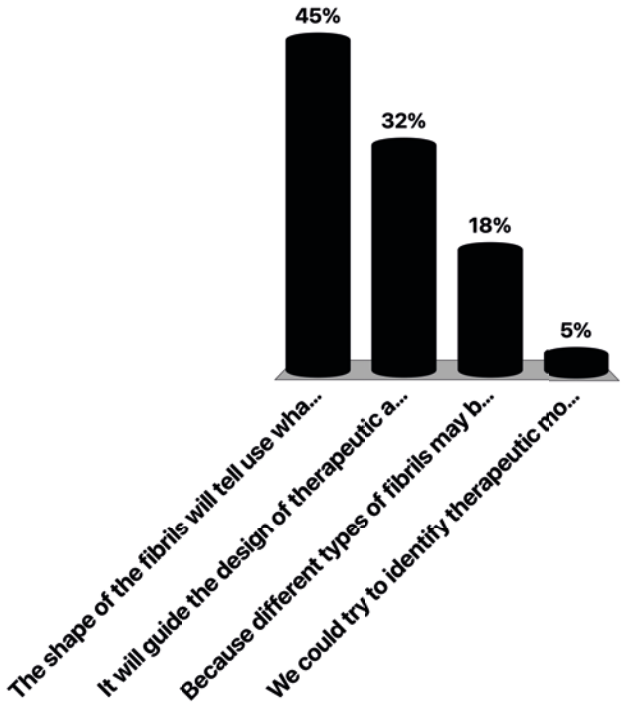
A. Expression system (promoter/enhancer)

B. Vector type

C. RNAi sequence

D. Route of administration

E. Vector dose



Gene therapy: question 10

In which model would you preferably test the toxicity of your gene therapy:

(Rank from the most relevant to the least relevant)

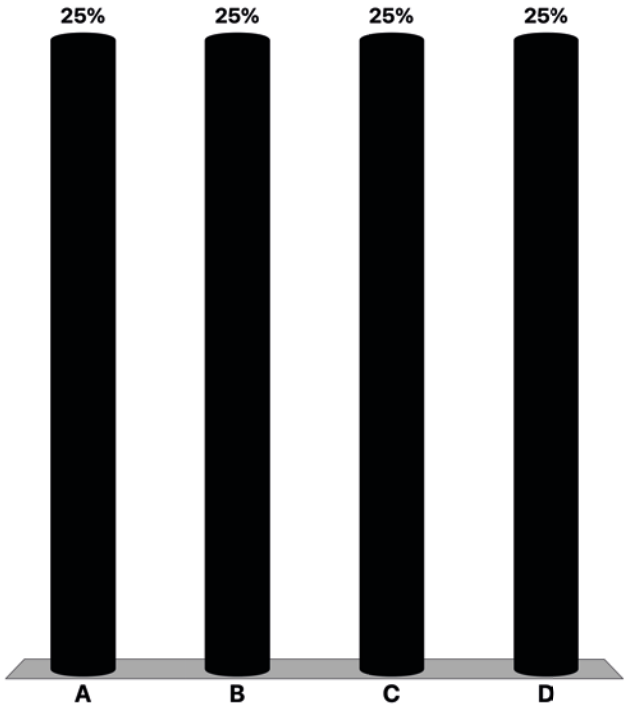
A. iPS-derived motoneurons

B. A cell line overexpressing hSOD1

C. ALS mouse model overexpressing mutated SOD1

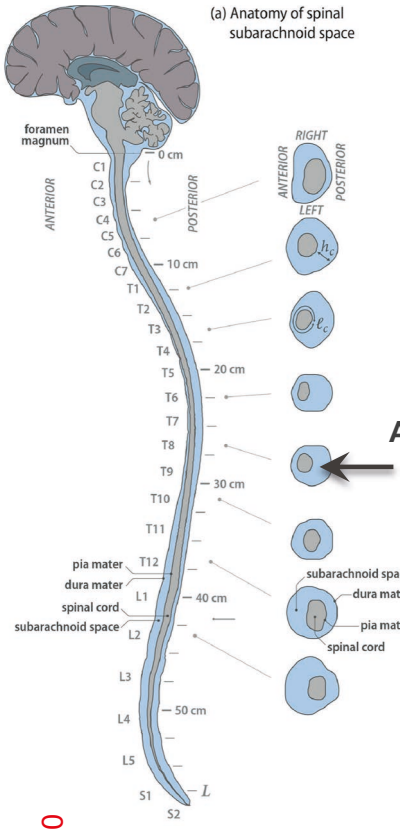
D. Non-human primate

E. iPS-derived glial cells



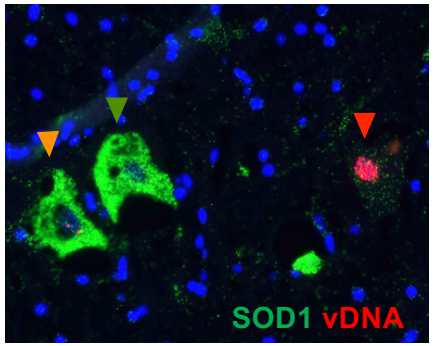
CNS cell type-specific promoters limit transgene expression in peripheral tissues

Macaca fascicularis

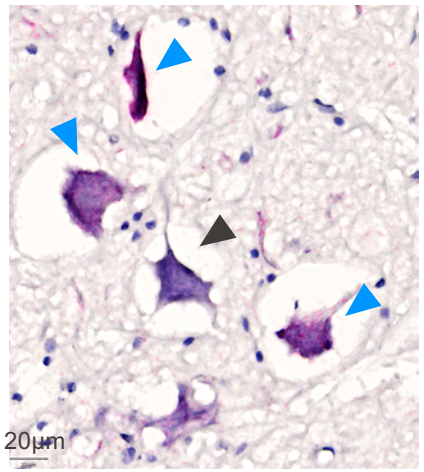


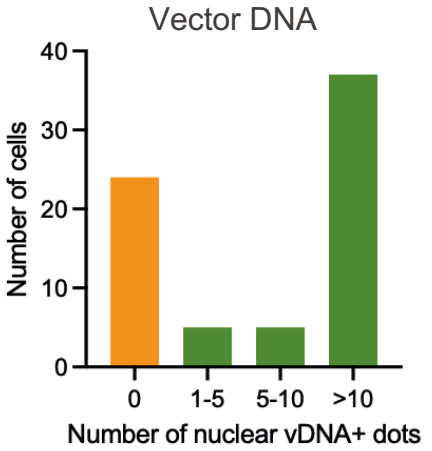
IT AAV9-miR SOD1 injection
$5^{E}13$ VG

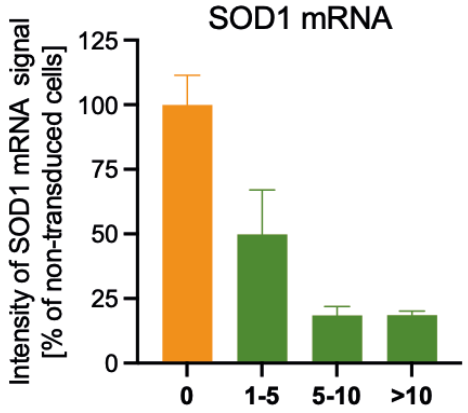
Anterior horn of the spinal cord [RNA/miRNAscope]

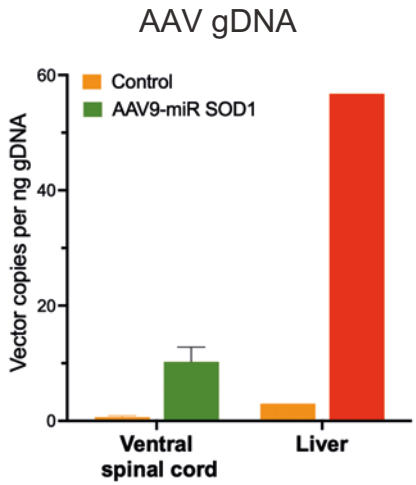


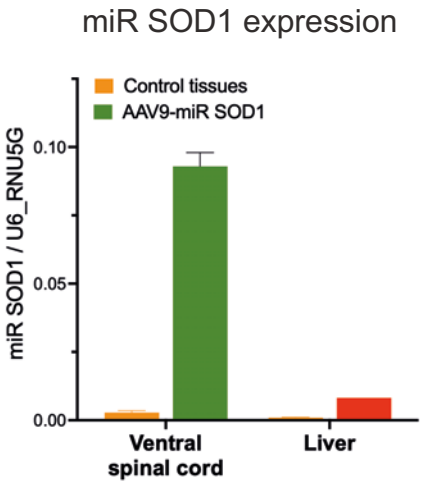
miR SOD1 (FastRed)











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Gutierrez-Montes C et al, 2021
Valdés P et al, unpublished