European Reference Network
for rare or low prevalence complex diseases

Network Neuromuscular Diseases (ERN EURO-NMD)

Present and future of gene therapy in Neuromuscular Diseases

Satellite Scientific Symposium endorsed by ERN EURO-NMD

February, 22nd 2024

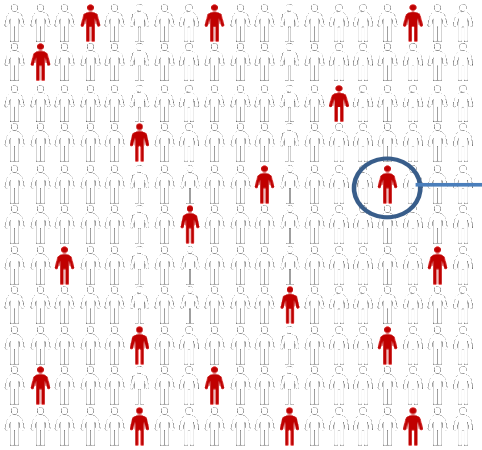
ALS gene therapy

Vincenzo Silani, FEAN, FAAN

IRCCS Istituto Auxologico Italiano, Milano

ALS genetics

familial ALS (fALS) 5%
sporadic ALS (sALS) 95%



monogenic ALS
10%-15% of all ALS cases

c9orf72 (40%)

TARDBP (5%)

SOD1 (20%)

FUS (5%)
minor genes (~30, <5%)

UNKNOWN

ALS HERITABILITY COEFFICIENT (h^2): 0.54

ASOs

Key figure

Schematic representation of the effect of antisense oligonucleotides (ASOs) on different targets in amyotrophic lateral sclerosis (ALS)

Van Daele et al., Trend Mol Med 2024

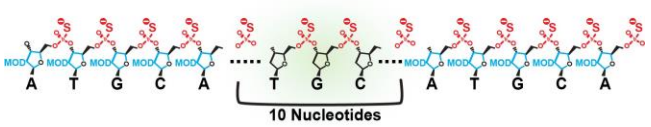
ASOs

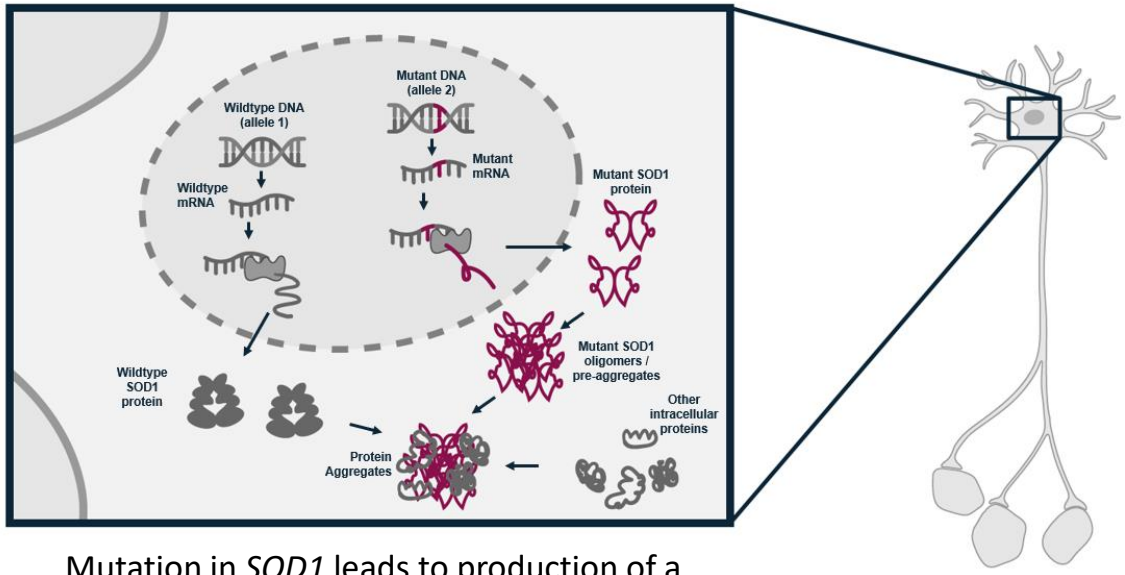
Table 1. Overview of the different ASOs developed for ALS

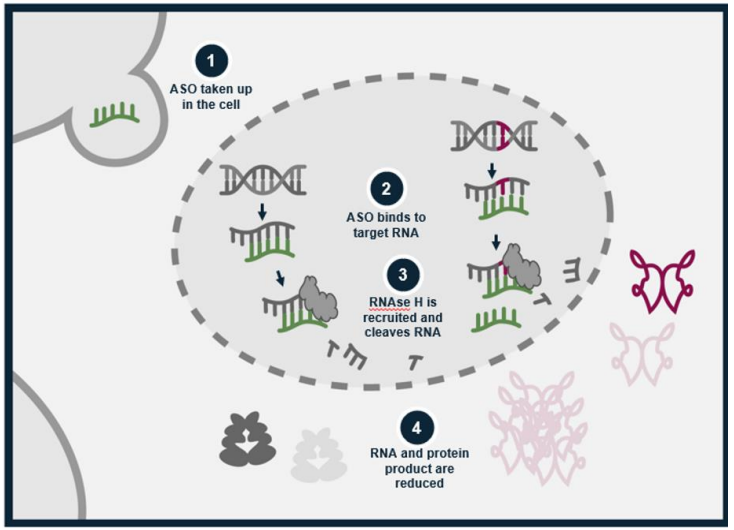
Target	Name of ASO	RNase H	Clinical studies	Status	Trial registration no.
SOD1	BIIB067=tofersen= Qalsody	Yes	Phase 3	Finished [9]	NCT02623699[v]
			Open label study	Ongoing	NCT03070119[xiv]
C9orf72	BIIB078	Yes	Phase 1/2	Stopped	NCT03626012[vii]
	WVE-004	Yes	Phase 1/2	Stopped	NCT04931862[vi]
	ASO5-2 = afinersen	Yes	Individual patient	Finished [22]	NA[a]
FUS	ION363 = jacifusen	Yes	Individual patients	One patient finished [26] and ongoing	NA
			Phase 1–3	Ongoing	NCT04768972[ix]
Ataxin-2	BIIB105	Yes	Phase 1/2	Ongoing	NCT04494256[xiii]
Stathmin-2	QRL-201	No	Phase 1	Ongoing	NCT05633459[xi]
UNC13A	NA	No	No, only preclinical	NA	NA
SYF2	NA	Yes	No, only preclinical	NA	NA
PIKFYVE	AS-202	Yes	No, only preclinical	NA	NA

Van Daele et al., Trend Mol Med 2024

RNA-based therapies: the case of *SOD1*
RNase-H-mediated ASO







Mutation in *SOD1* leads to production of a toxic specie of SOD1 protein

Tofersen is an ASO that targets the *SOD1* mRNA

courtesy of Biogen

Preclinical antisense strategy

= Completed

Determine Antisense oligo Distribution in CNS Following ICV Administration

Rat

Identification of Rat SOD-1 ASOs

Demonstrate SOD-1 Inhibition in Liver Following Systemic Administration

Demonstrate SOD-1 Inhibition in CNS Following ICV Administration

Examine Dose Schedule Requirements, PK & Histopathology

ASO Medicinal Chemistry

Richard A. Smith, M.D.
Center for Neurologic Study
Scripps Research Institute

Human

Identification of Human SOD-1 ASO Candidates

Test in Human Fibroblasts from A4V Patients

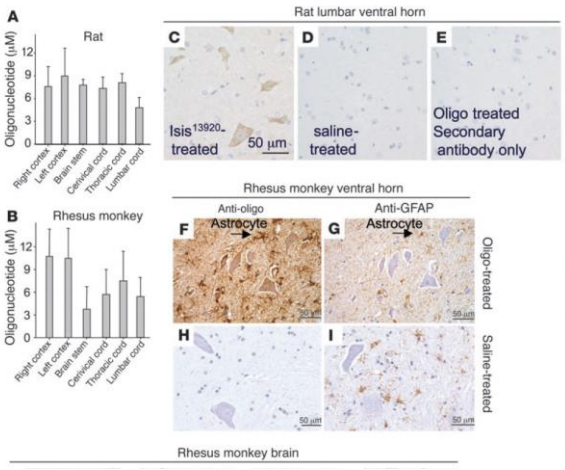
Test in Primary Hepatocytes from Transgenic (A4V/G93A) Mice & Rats

Test Lead ASOs for SOD-1 Inhibition in Transgenic Mice/Rats via Systemic & ICV TX

Select Human Candidate

Primate PK & Toxicology

A critical achievement

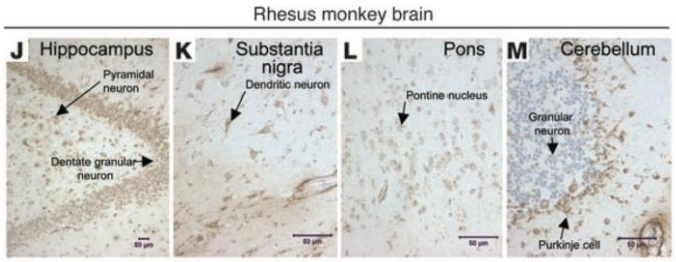


Research article

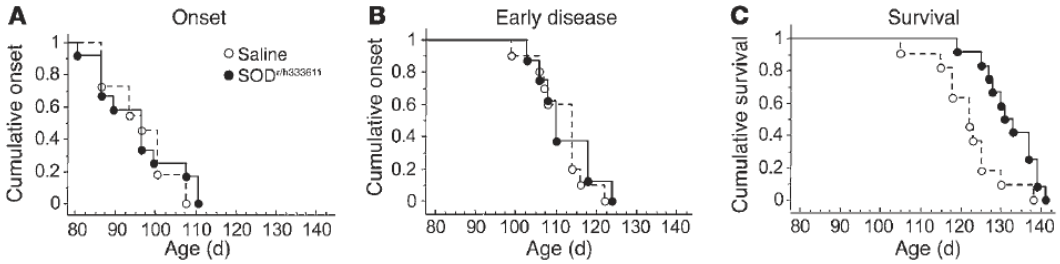
Antisense oligonucleotide therapy for neurodegenerative disease

Richard A. Smith,[1,2,3] Timothy M. Miller,[1,4] Koji Yamanaka,[1] Brett P. Monia,[5] Thomas P. Condon,[5] Gene Hung,[5] Christian S. Lobsiger,[1] Chris M. Ward,[1] Melissa McAlonis-Downes,[1] Hongbing Wei,[5] Ed V. Wancewicz,[5] C. Frank Bennett,[5] and Don W. Cleveland[1,4]

J. Clin. Invest. **116**:2290–2296 (2006). doi:10.1172/JCI25424.



presymptomatic treatment



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

Ionis Pharmaceuticals

Lancet Neurol 2013; 12: 435–42

Methods In this randomised, placebo-controlled, phase 1 trial, we delivered ISIS 333611 by intrathecal infusion using an external pump over 11·5 h at increasing doses (0·15 mg, 0·50 mg, 1·50 mg, 3·00 mg) to four cohorts of eight patients with *SOD1*-positive amyotrophic lateral sclerosis (six patients assigned to ISIS 333611, two to placebo in each cohort). We did the randomisation with a web-based system, assigning patients in blocks of four. Patients and investigators were masked to treatment assignment. Participants were allowed to re-enrol in subsequent cohorts. Our primary objective was to assess the safety and tolerability of ISIS 333611. Assessments were done during infusion and over 28 days after infusion. This study was registered with Clinicaltrials.gov, number NCT01041222.

Findings Seven of eight (88%) patients in the placebo group versus 20 of 24 (83%) in the ISIS 333611 group had adverse events. The most common events were post-lumbar puncture syndrome (3/8 [38%] *vs* 8/24 [33%]), back pain (4/8 [50%] *vs* 4/24 [17%]), and nausea (0/8 [0%] *vs* 3/24 [13%]). We recorded no dose-limiting toxic effects or any safety or tolerability concerns related to ISIS 333611. No serious adverse events occurred in patients given ISIS 333611. Re-enrolment and re-treatment were also well tolerated.

	Sex	Age (years)	Family history of amyotrophic lateral sclerosis	SOD1 mutation	Age at onset (years)	Site of onset
1	Female	49	Yes	Glu49Lys	47	Limb
2	Male	59	Yes	Ala4Val	59	Limb
3	Female	36	Yes	Gly37Arg	23	Limb
4	Male	41	Yes	Ala4Thr	41	Limb
5	Male	47	Yes	Leu38Val	45	Limb
6	Male	51	Yes	Ile113Thr	47	Limb
7	Female	50	Yes	Ala4Val	50	Limb
8	Female	58	Yes	Ala4Val	58	Limb
9	Male	63	Yes	Gly85Arg	63	Limb
10	Male	52	Yes	Ala4Val	51	Limb
11	Male	48	Yes	Asn139Lys	45	Limb
12	Male	54	Yes	Ile113Thr	48	Limb
13	Male	44	No	Ala89Val	42	Limb
14	Female	56	Yes	Ile113Thr	43	Limb
15	Male	55	Yes	Gly93Ser	45	Limb
16	Male	46	Yes	Ala4Val	46	Bulbar
17	Male	22	Yes	Gly41Ser	22	Limb
18	Male	56	Yes	Asp90Ala	55	Limb
19	Male	51	Yes	Leu8Val	43	Limb
20	Female	38	Yes	Gly93Ala	37	Limb
21	Female	49	Yes	Gln21Leu	45	Limb

Table 1: Demographic and clinical characteristics of each participant

	Placebo group (n=8)	ISIS 333611 group (n=24)	Events in the ISIS 333611 group (n)			
			Cohort 1	Cohort 2	Cohort 3	Cohort 4
Any serious adverse event	1 (13%; 2)	0 (0%; 0)	0	0	0	0
Any adverse event	7 (88%; 23)	20 (83%; 50)	23	9	7	11
Post-lumbar puncture syndrome	3 (38%; 5)	8 (33%; 8)	4	2	1	1
Back pain	4 (50%; 4)	4 (17%; 4)	2	1	1	0
Nausea	0 (0%; 0)	3 (13%; 3)	2	0	1	0
Vomiting	0 (0%; 0)	2 (8%; 2)	2	0	0	0
Headache	1 (13%; 1)	2 (8%; 2)	0	2	0	0
Fall	0 (0%; 0)	2 (8%; 2)	1	1	0	0
Dizziness	0 (0%; 0)	2 (8%; 2)	1	0	0	1

Data are number of patients (%; number of events) unless otherwise stated. Events listed are those that occurred in >5% of ISIS 333611 treated patients (ie, occurred in >1 patient).

Table 3: Adverse events by group and cohort

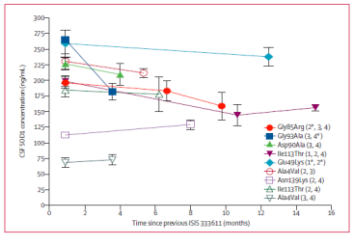
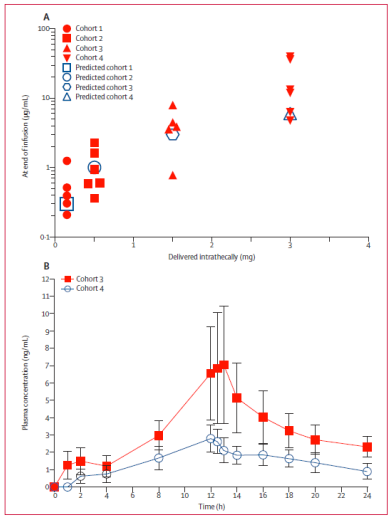
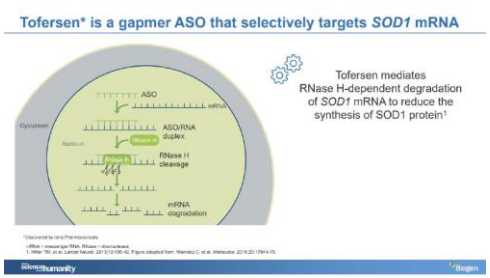


Figure 3: SOD1 protein concentrations in CSF of patients enrolled in more than one cohort



Autoptic Case: elimination half-life 30 days

We analysed spinal cord tissue samples obtained at autopsy from a patient with a SOD1 Ala4Val mutation. ISIS 333611 concentrations were 218 ng/g in a lumbar spinal cord sample, 122 ng/g in a thoracic spinal cord sample, and 39 ng/g in a cervical spinal cord sample. These results and the gradient between lumbar and cervical samples are consistent with expected tissue concentrations based on preclinical studies of Rhesus monkeys (predicted concentration *vs* measured concentration: 344 ng/g *vs* 218 ng/g in the lumbar sample, 282 ng/g *vs* 122 ng/g in the thoracic sample, 36 ng/g *vs* 39 in the cervival sample; appendix). The CSF concentration of ISIS 333611 at the end of infusion for this patient was 3·5 µg/mL during cohort 3 and 6·3 µg/mL during cohort 4.



The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 JULY 9, 2020 VOL. 383 NO. 2

Phase 1–2 Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS

T. Miller, M. Cudkowicz, P.J. Shaw, P.M. Andersen, N. Atassi, R.C. Bucelli, A. Genge, J. Glass, S. Ladha, A.L. Ludolph, N.J. Maragakis, C.J. McDermott, A. Pestronk, J. Ravits, F. Salachas, R. Trudell, P. Van Damme, L. Zinman, C.F. Bennett, R. Lane, A. Sandrock, H. Runz, D. Graham, H. Houshyar, A. McCampbell, I. Nestorov, I. Chang, M. McNeill, L. Fanning, S. Fradette, and T.A. Ferguson

Table 1. Adverse Events Occurring in at Least Three Participants in Any Group.*

Event	Placebo (N=12)	Tofersen, 20 mg (N=10)	Tofersen, 40 mg (N=9)	Tofersen, 60 mg (N=9)	Tofersen, 100 mg (N=10)
	number of participants (percent)				
Any adverse event	12 (100)	10 (100)	9 (100)	9 (100)	10 (100)
Headache	7 (58)	4 (40)	2 (22)	4 (44)	6 (60)
Procedural pain	5 (42)	4 (40)	1 (11)	4 (44)	7 (70)
Post-lumbar puncture syndrome	3 (25)	4 (40)	3 (33)	3 (33)	3 (30)
Fall	3 (25)	3 (30)	3 (33)	2 (22)	5 (50)
Back pain	0	1 (10)	1 (11)	1 (11)	5 (50)
Nasopharyngitis	1 (8)	2 (20)	1 (11)	3 (33)	1 (10)
Upper respiratory tract infection	0	4 (40)	0	2 (22)	0
CSF protein concentration increased	1 (8)	0	0	4 (44)	1 (10)
CSF white-cell count increased	0	0	1 (11)	3 (33)	0
Pain in arm or leg	2 (17)	0	1 (11)	0	3 (30)
Dizziness	3 (25)	0	0	0	1 (10)
Neck pain	3 (25)	0	0	1 (11)	0

* The placebo group includes all the participants who had been assigned to receive placebo in any dose-matched cohort. CSF denotes cerebrospinal fluid.

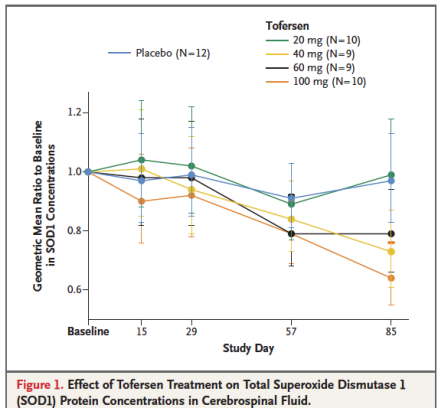
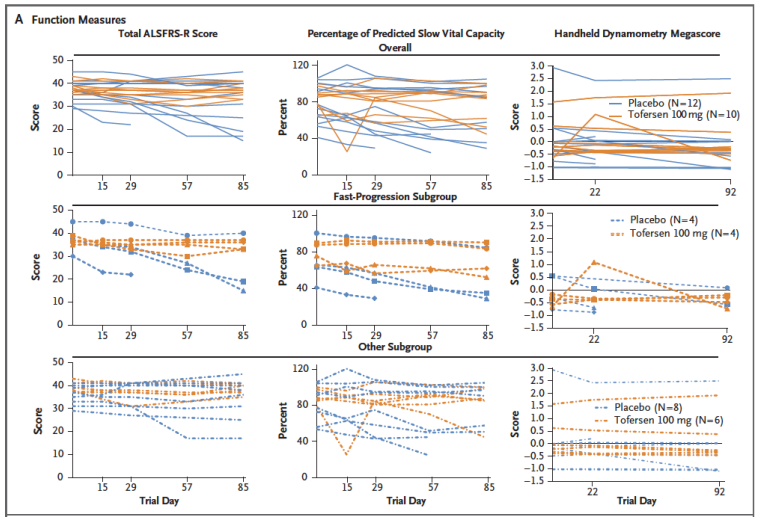
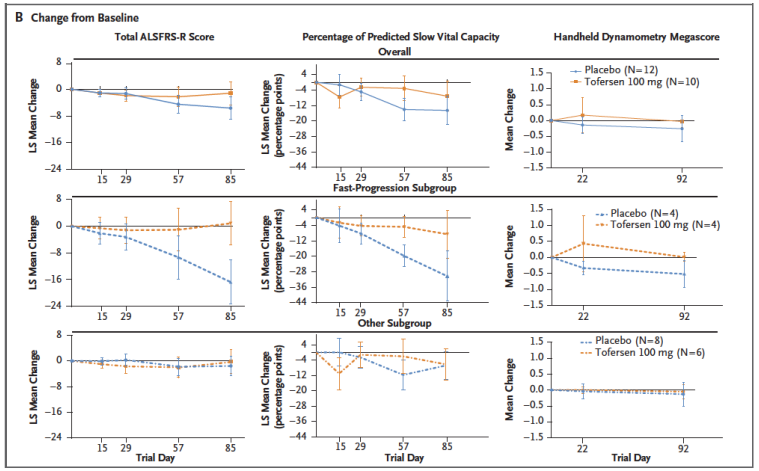
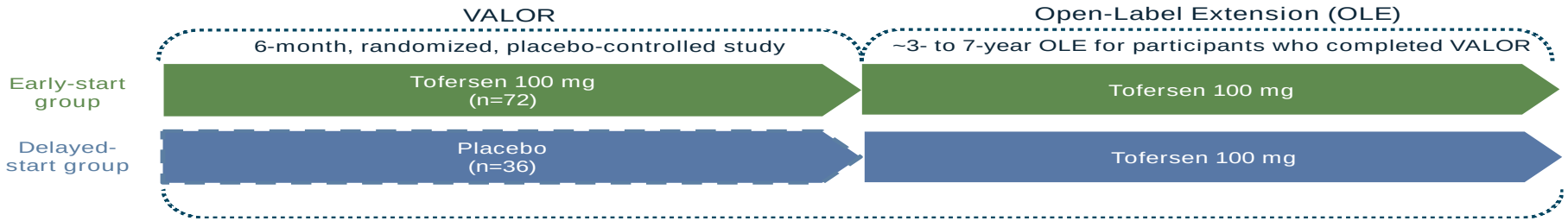


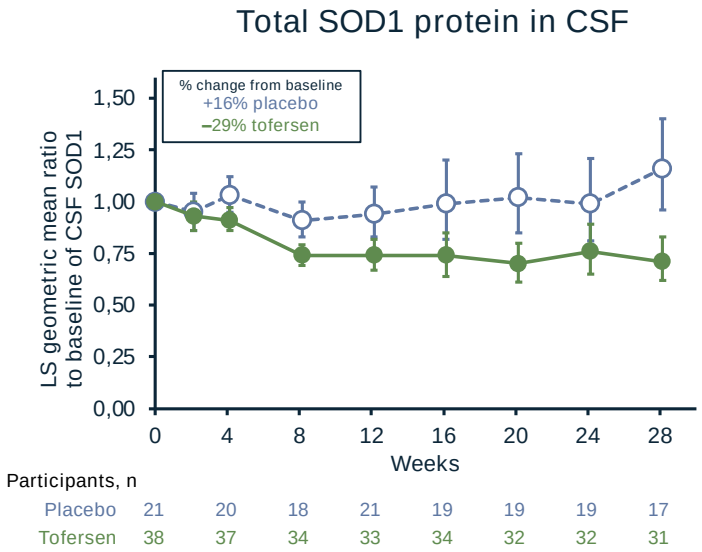
Figure 1. Effect of Tofersen Treatment on Total Superoxide Dismutase 1 (SOD1) Protein Concentrations in Cerebrospinal Fluid.

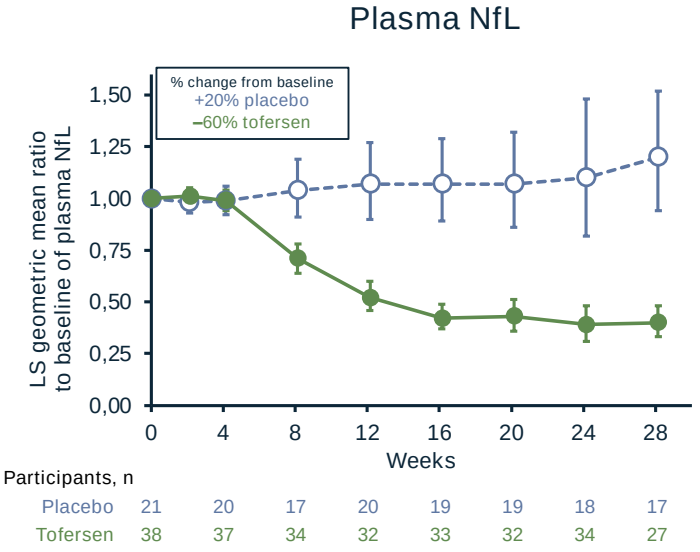




Tofersen in SOD1-ALS (April 25, 2023 FDA approuval)







courtesy of Biogen

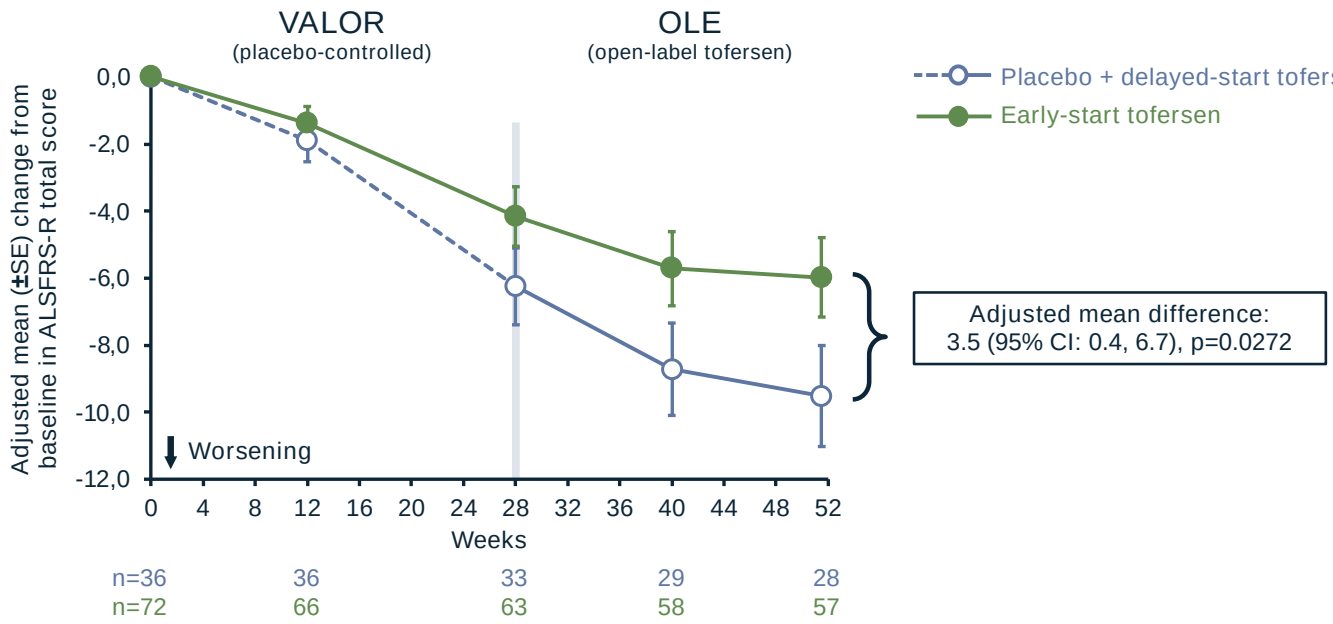
Tofersen in SOD1-ALS

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS

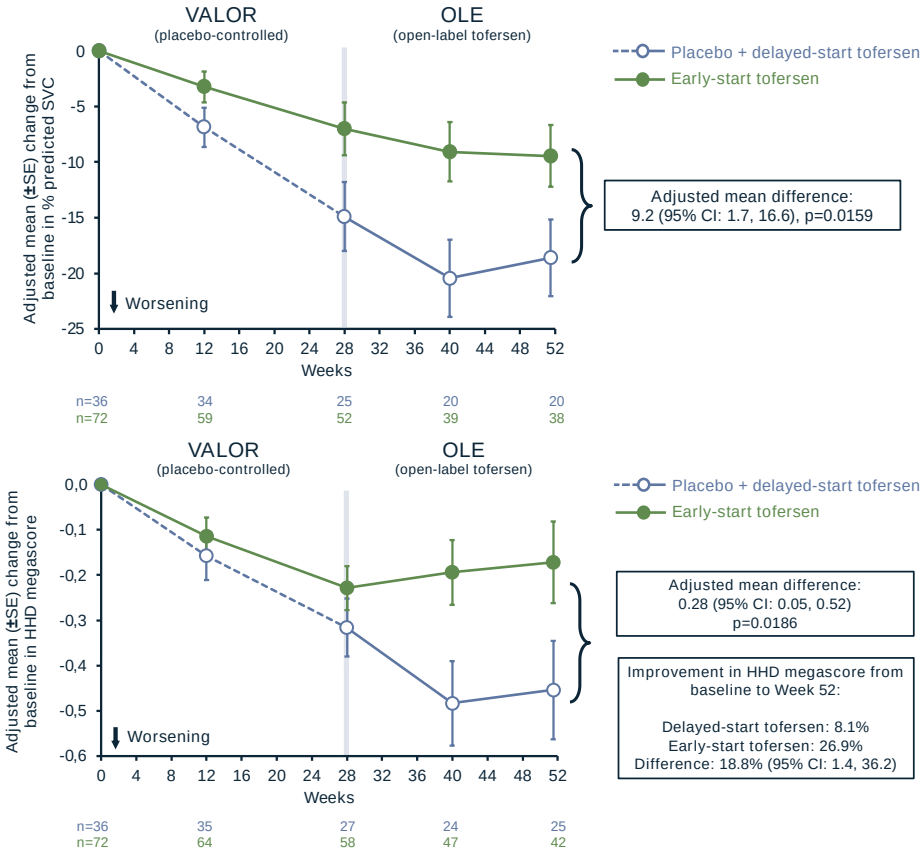
T.M. Miller, M.E. Cudkowicz, A. Genge, P.J. Shaw, G. Sobue, R.C. Bucelli, A. Chiò, P. Van Damme, A.C. Ludolph, J.D. Glass, J.A. Andrews, S. Babu, M. Benatar, C.J. McDermott, T. Cochrane, S. Chary, S. Chew, H. Zhu, F. Wu, I. Nestorov, D. Graham, P. Sun, M. McNeill, L. Fanning, T.A. Ferguson, and S. Fradette, for the VALOR and OLE Working Group*



Effect on clinical function (ALSFRS-r)

courtesy of Biogen

Tofersen in SOD1-ALS



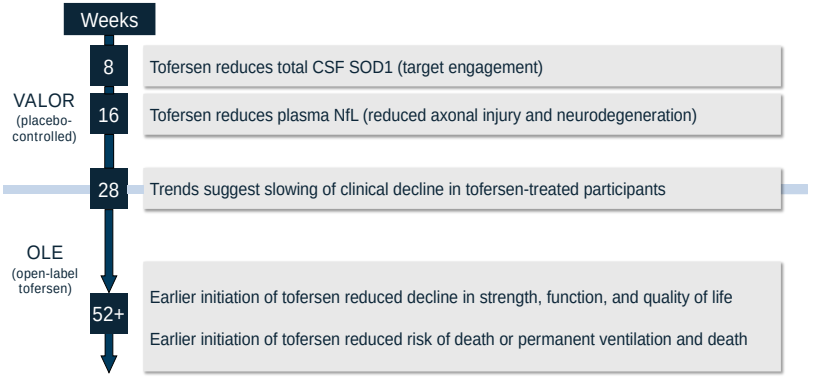
Effect on respiratory function (SVC)

Effect on muscle strenght (HDD megascore)

courtesy of Biogen

Open questions

- effect on survival (death, permanent ventilation)
- magnitude of effect (stabilization? reversal?)
- effect on different *SOD1* mutations
- timing of intervention (pre-symptomatic individuals?)
- side effects?
- use of NfL as surrogate biomarker capable to predict clinical benefit



Predicted benefit on clinical outcomes (at Week 28) for each 10 pg/mL reduction of plasma NfL (at Week 16)*	
ALSFRS-R total score	0.77 (p=0.0038)
Percent-predicted SVC	1.45 (p=0.0706)
HHD overall megascore	0.029 (p=0.1303)
ALSAQ-5 total score	2.194 (p=0.0056)
EQ-5D-5L utility score	0.017 (p=0.0894)

*Example for a participant with a baseline plasma NfL level equivalent to the sample mean for ITT completers (96.78 pg/mL)

courtesy of Biogen

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

SOD1 Suppression with Adeno-Associated Virus and MicroRNA in Familial ALS

Christian Mueller, Ph.D., James D. Berry, M.D., Diane M. McKenna-Yasek, R.N., Gwladys Gernoux, Ph.D., Margaret A. Owegi, M.D., Lindsay M. Pothier, B.S., Catherine L. Douthwright, Ph.D., Dario Gelevski, B.S., Sarah D. Luppino, B.S.N., R.N., Meghan Blackwood, B.S., Nicholas S. Wightman, B.S., Derek H. Oakley, M.D., Ph.D., Matthew P. Frosch, M.D., Ph.D., Terrence R. Flotte, M.D., Merit E. Cudkowicz, M.D., and Robert H. Brown, Jr., D.Phil., M.D.

N ENGL J MED 383;2 NEJM.ORG JULY 9, 2020

immunosuppression needed

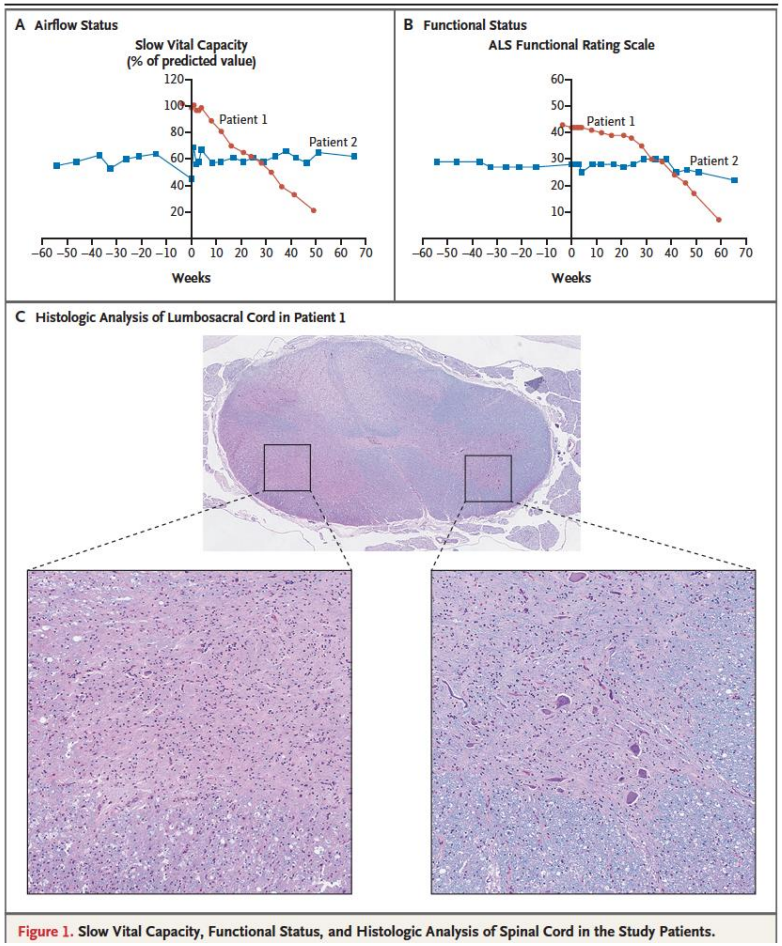


Figure 1. Slow Vital Capacity, Functional Status, and Histologic Analysis of Spinal Cord in the Study Patients.

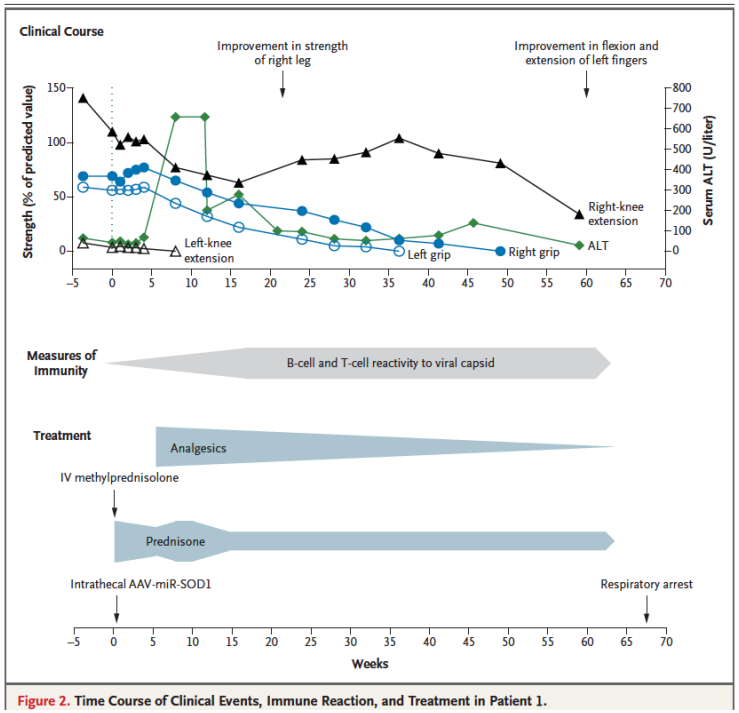


Figure 2. Time Course of Clinical Events, Immune Reaction, and Treatment in Patient 1.

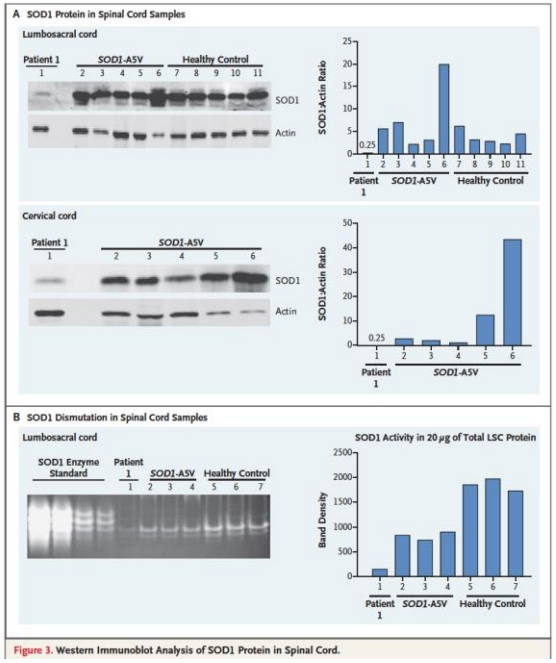


Figure 3. Western Immunoblot Analysis of SOD1 Protein in Spinal Cord.

ASO-based therapies for *c9*-ALS

BIIB078

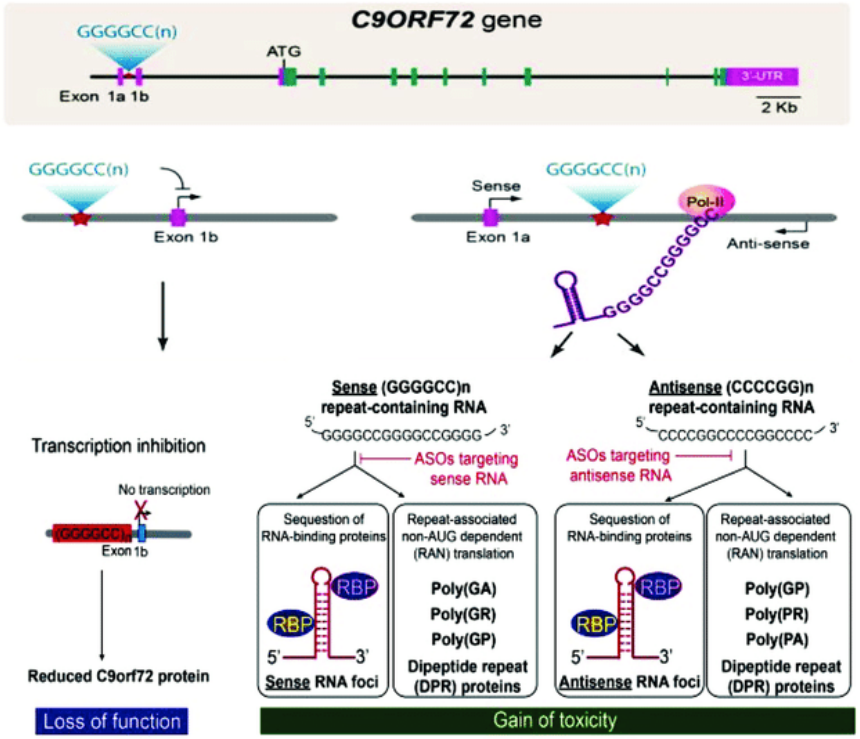
- no clinical benefit
- 60 mg dose = placebo
- 90 mg worse than placebo
- decreased DPR CSF levels
- increased NfL plasma levels

WVE-004

- safe
- no clinical benefit
- decreased DPR CSF levels

ASO5-2

- individual patient
- finished



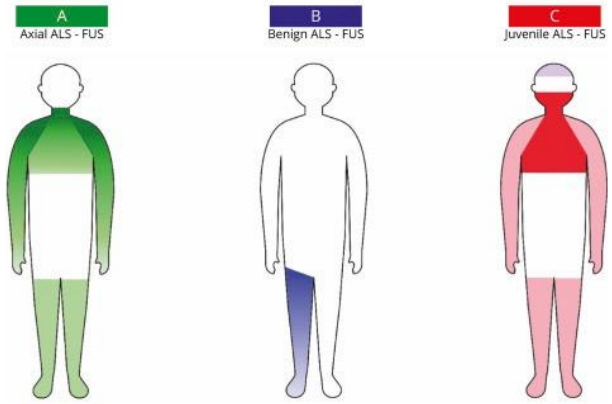
Kumar V et al, *Front Neurosci* 2017

Jacifusen in *FUS*-ALS

Analysis of *FUS* gene mutation in familial amyotrophic lateral sclerosis within an Italian cohort — Ticozzi et al., *Neurology* 2009

(Gourie-Devi et al., JNPP 2003)

Phenotype Analysis of Fused in Sarcoma Mutations in Amyotrophic Lateral Sclerosis — Grassano et al., *Neurol Genet* 2022



ARTICLES
https://doi.org/10.1038/s41591-021-01615-z
nature medicine

OPEN

Antisense oligonucleotide silencing of FUS expression as a therapeutic approach in amyotrophic lateral sclerosis

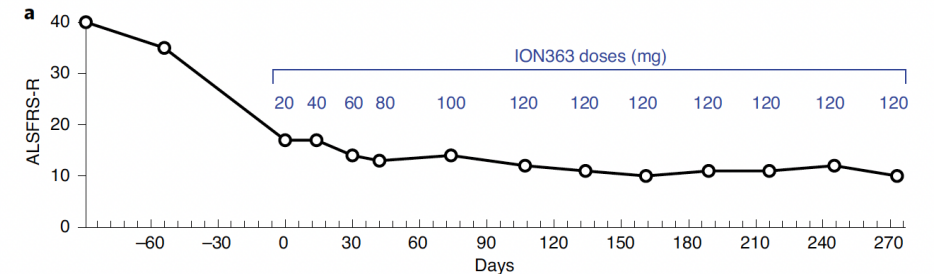
Vladislav A. Korobeynikov[1,2,6], Alexander K. Lyashchenko[1,2,6], Beatriz Blanco-Redondo[1,5,6], Paymaan Jafar-Nejad[3] and Neil A. Shneider[1,4]

FUSION

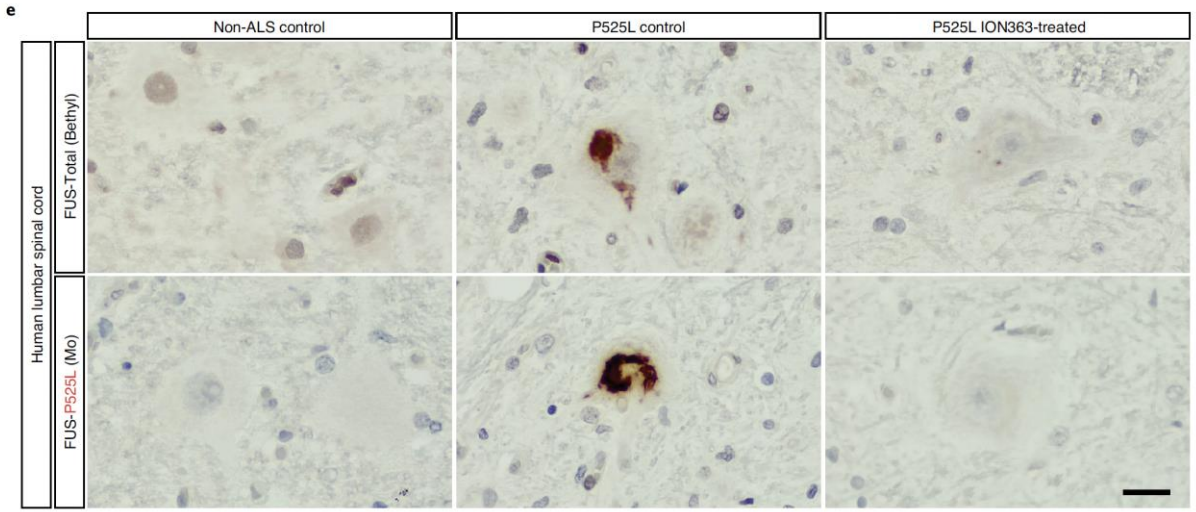
Phase III clinical trial
(ION-363)

FUS-ALS

- <1% of ALS cases
- early onset
- axial and proximal (shoulder and pelvic girdle) symmetrical weakness and wasting
- phenotype: LMN>>>UMN
- rapid progression



25-year-old woman



Korobeynikov et al., 2022

Challenges to gene therapy in ALS

Gain vs Loss of Function

GOF+LOF genes

TARDBP

non degrading ASOs
(splicing of UNC13A, STMN2)

LOF genes

TBK1, NEK1, GRN, OPTN…

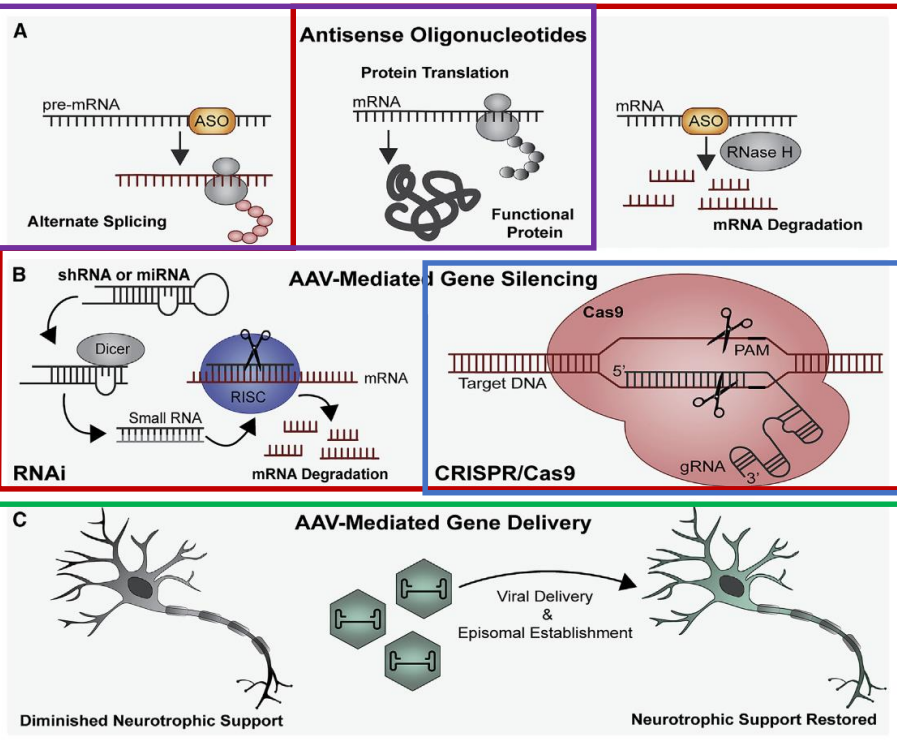
gene delivery

suppressor tRNAs
(nonsense mutations)

small activating RNAs
(promoters, enhancers)

Non-Viral Strategy

Viral-Based Strategies



GOF genes

SOD1, (FUS?)

degrading ASOs
(RNAi, genome editing)

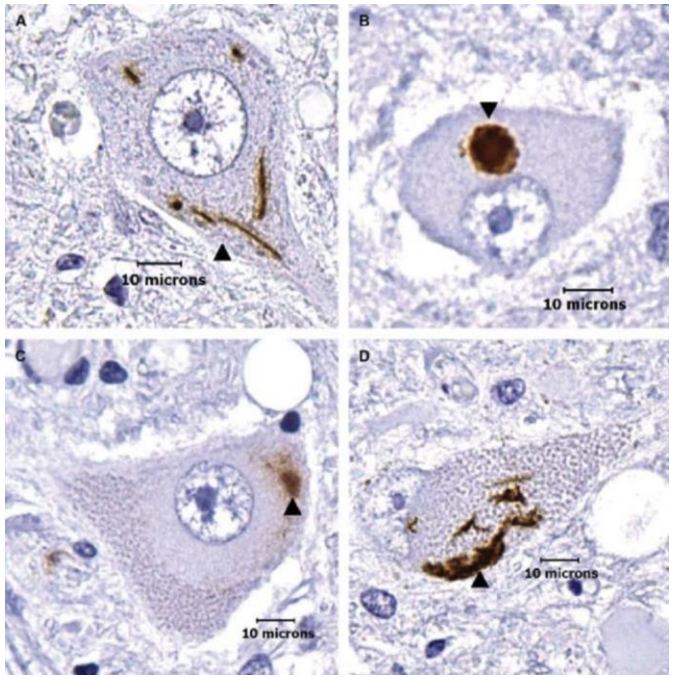
All ALS genes?

genome editing
(CRISPR/Cas9, ZFP-TF, TALEN)

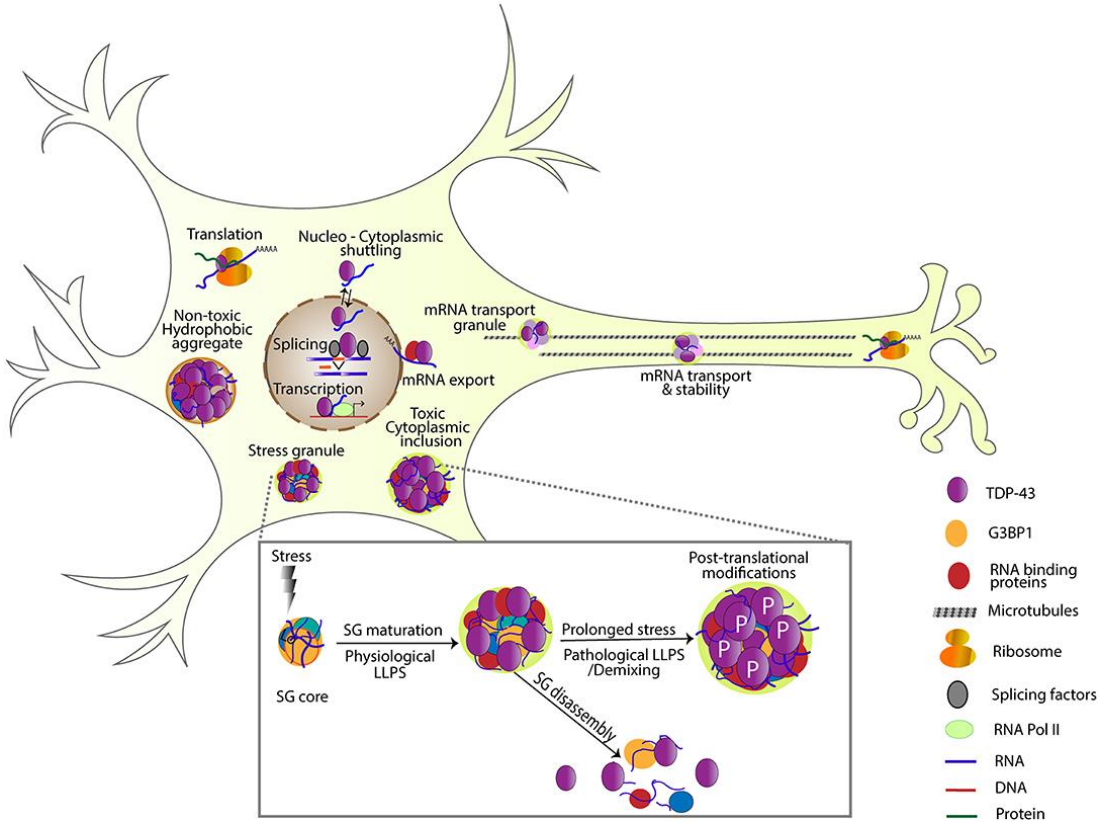
RNA base editing
(AIMers, CRISPR/Cas13, …)

Amado DA & Davidson BL, *Mol Therapy* 2021

ALS as TDP-43 protheinopathy

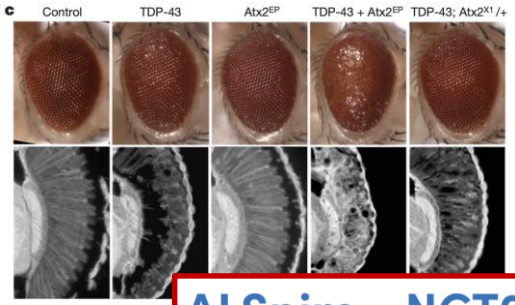


TDP43+ cytoplasmic inclusions in >95% of all ALS cases
(except *SOD1*, *FUS*)



Upstream modulation of TDP-43

- *ATXN2* intermediate-lenght polyQ expansions are a risk factor of ALS
- Risk alleles increase ATXN2 stability and reduce its degradation
- ATXN2 recruits TDP-43 into stress granules and promote aggregation
- ATXN2 levels directly correlate with TDP-43 toxicity in yeast, fly, mouse models
- Phenotypic rescue of TDP-43 mouse model using ATXN2-ASOs or RNA-targeting CRISPR platform against ATXN2



Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS

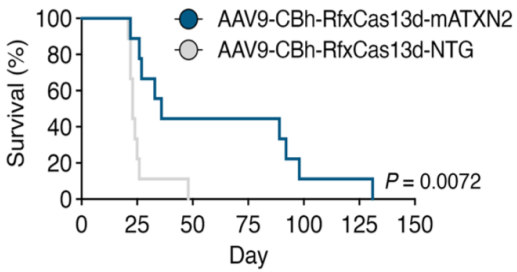
Elden AC et al, *Nature* 2012

ALSpire – NCT04494256

A Study to Assess the Safety, Tolerability, and Effect on Disease Progression of BIIB105 in Participants With Amyotrophic Lateral Sclerosis (ALS) and Participants With the ALS Ataxin-2 (ATXN2) Genetic Mutation

Therapeutic redu
and reduces path

Becker LA et al, *Na*



Mitigating a TDP-43 proteinopathy by targeting ataxin-2 using RNA-targeting CRISPR effector proteins

Zeballos C. MA et al, *Nat Commun* 2023

BIIB105* is an intrathecally administered ASO being investigated for the treatment of broad ALS

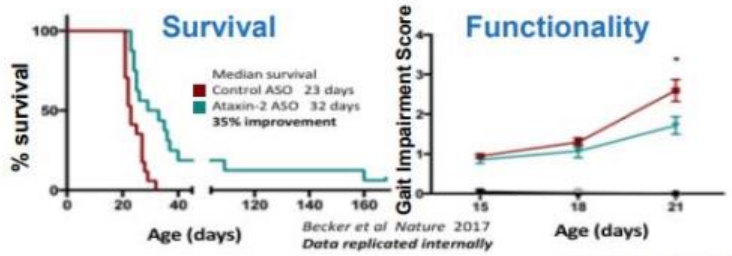
Reduction of ATXN2 may improve TDP-43 toxicity and clinical outcomes in ALS[1,2,3]

Preclinical observations[1]

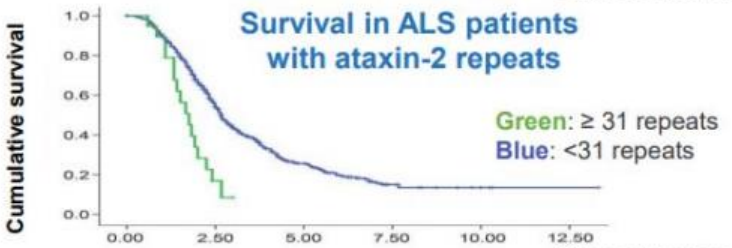
Wildtype Atxn2 reduction increases survival and functionality and reduces TDP-43 pathology in yeast, fly, and mouse models

Human genetic evidence[2,3]

Intermediate repeat PolyQ expansion (30–33) in *ATXN2* results in 7× increased risk of ALS and is associated with a more aggressive phenotype



Becker et al. Nature 2017



Chiò et al. Neurology 2015

BIIB105 Studies

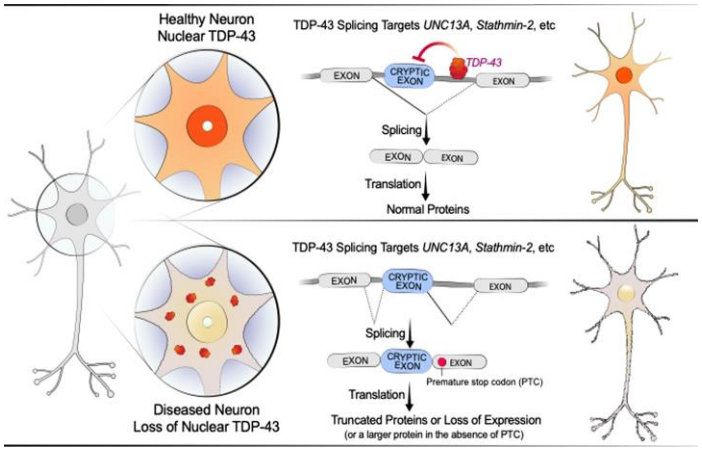
Phase 1[4] | Phase 2 | Phase 3 | Phase 4

* Discovered by Ionis Pharmaceuticals

ALS, amyotrophic lateral sclerosis.

1. Becker LA, et al. *Nature*. 2017;544:367-71. 2. Elden AC, *Nature*. 2010;466:1069-75. 3. Chio A, et al. *Neurology*. 2015;84:251-8.
4. NCT04494256. Available from: https://clinicaltrials.gov/ct2/show/NCT04494256. Accessed October 2020.

Downstream modulation of TDP-43

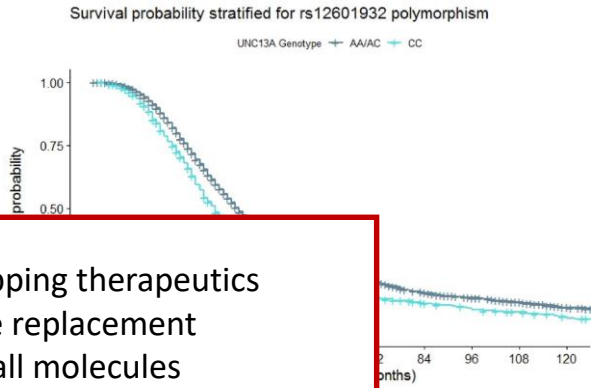


TDP-43 physiologically suppresses the splicing of crypting exons (CE)

LoF leads to abnormal inclusions of CE with formation of truncated proteins (STMN2) or reduced expression via RNA-mediated decay (UNC13A)

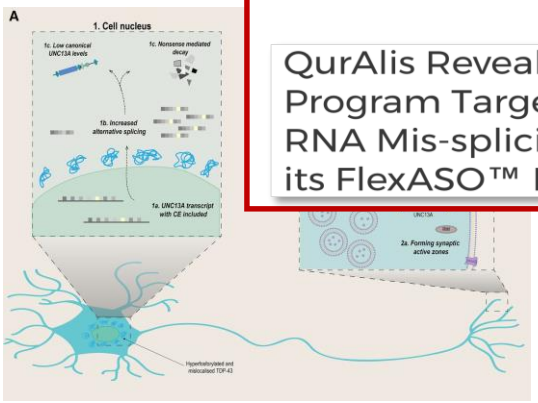
UNC13A is a risk factor for ALS susceptibility and a strong modifier of survival

Minor C allele exacerbates CE inclusion in UNC1 function is lost



exon skipping therapeutics
gene replacement
small molecules

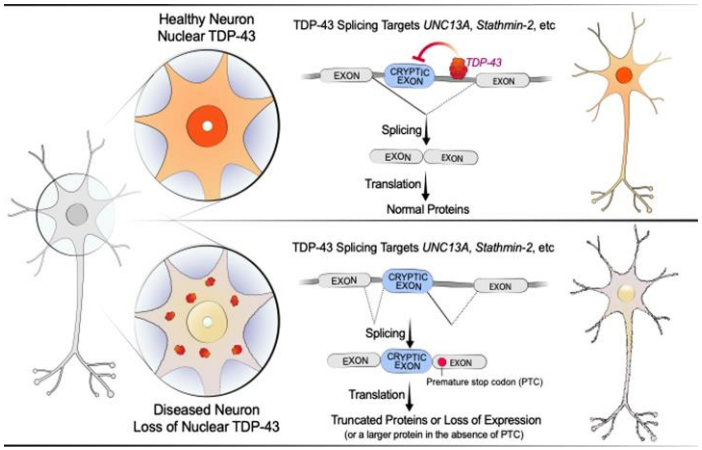
QurAlis Reveals Newest Program Targeting UNC13A RNA Mis-splicing Incorporating its FlexASO™ Platform



ral processes
structure and

Loss of UNC13A causes reduction in synaptic vesicle release and motor phenotype in animal models

Downstream modulation of TDP-43

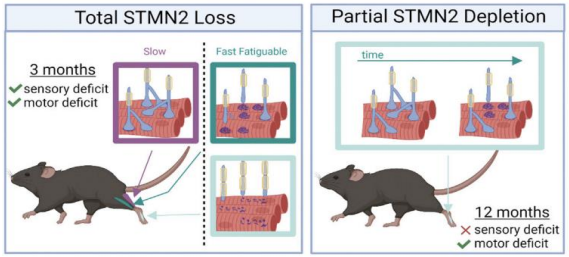


TDP-43 physiologically suppresses the splicing of crypting exons

LoF leads to abnormal inclusions of CE with formation of truncated proteins (STMN2) or reduced expression via RNA-mediated decay (UNC13A)

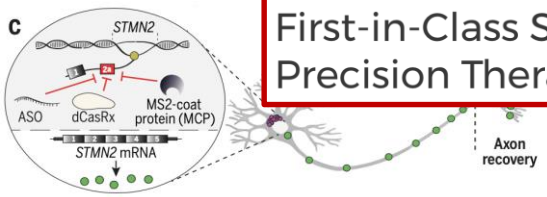
STMN2 is a CNS-specific protein that binds tubulin dimers and regulates microtubule stability.

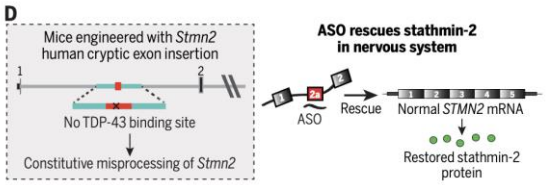
Loss of STMN2 promotes degeneration of injured axons, while overexpression of STMN2 delays degeneration after injury.



Loss of STMN2 neuropathy in mice

QurAlis Announces First Patient Dosed With QRL-201, a First-in-Class STATHMIN-2 Precision Therapy for ALS





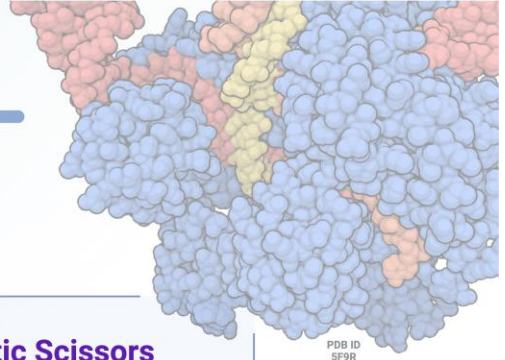
ASOs correct STMN2 pre-mRNA misprocessing and restore STMN2 expression levels

New frontiers

bio RENDER in collaboration with the Doudna lab

2020 Nobel Prize in Chemistry

A tool for genome editing: CRISPR-Cas9



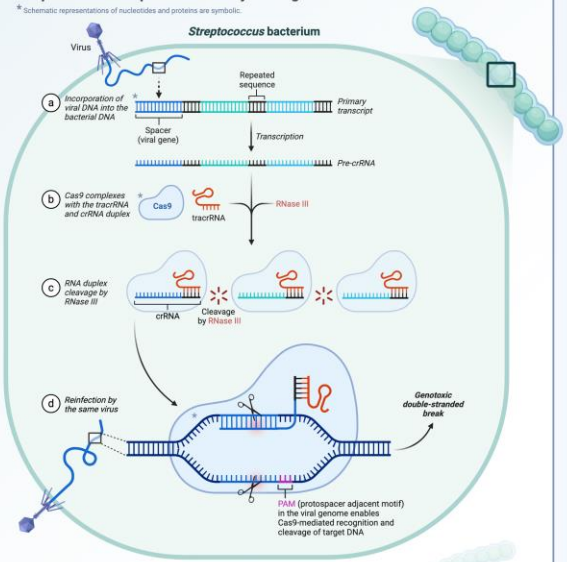
Scientist Spotlight

CRISPR-Cas9 has been touted as one of the most revolutionary discoveries of the decade. Meet Dr. Jennifer Doudna and Dr. Emmanuelle Charpentier, the first two women to ever hold the Nobel Prize in Chemistry together for their discovery of CRISPR-Cas9. Growing up in small towns, Dr. Doudna and Dr. Charpentier (in the USA and France, respectively) never expected that this would happen to them.

Dr. Jennifer Doudna — Dr. Emmanuelle Charpentier

Streptococcus' Adaptive Immune System against Viruses: CRISPR-Cas9

*Schematic representations of nucleotides and proteins are symbolic



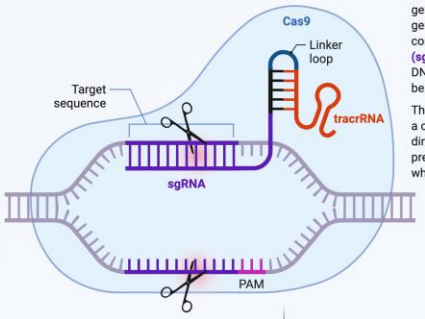
(a) Viral DNA is inserted into the bacterial genome in between the repeated CRISPR sequences.

(b) tracrRNAs recognize CRISPR RNA sequences and target Cas9 enzymes to the CRISPR RNA.

(c) The CRISPR RNA:tracrRNA duplex is cleaved off of the longer pre-crRNA.

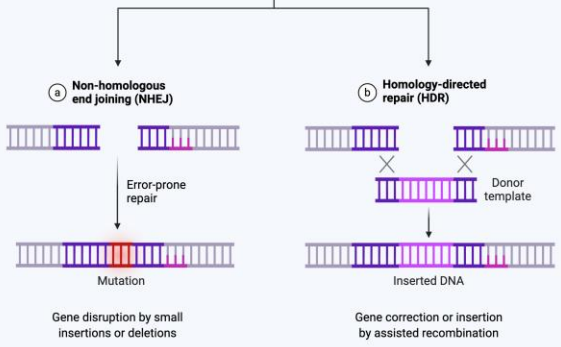
(d) If the bacterium is infected by the same virus, the Cas9:crRNA generated during the first infection recognizes PAM motifs in the viral genome, enabling target-DNA recognition and Cas9-mediated cleavage to prevent re-infection.

The CRISPR-Cas9 Genetic Scissors



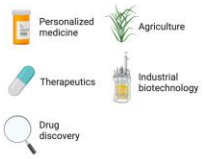
When researchers edit a genome using the CRISPR-Cas9 genetic scissors, they artificially construct a **single guide RNA (sgRNA)**, which matches the DNA code where the cut is to be made.

The scissor protein, **Cas9**, forms a complex with the **sgRNA**, and directs the scissors to the precise location in the genome where the cut will be made.

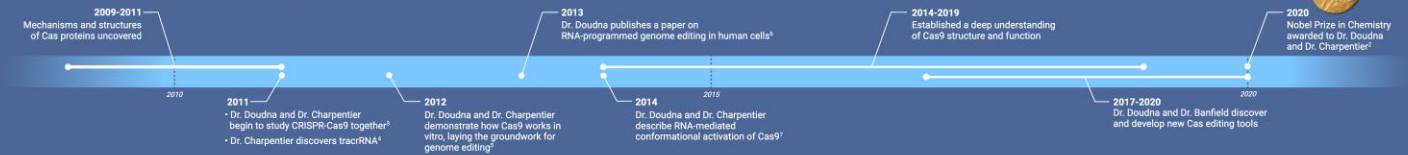


Applications

CRISPR-Cas9 revolutionized genome engineering: its precision, speed, and affordability permits its use in a nearly limitless range of applications. Since its discovery, scientists have been using the technology to cure diseases, discover new treatments, and for precision medicine. It doesn't stop there: beyond human disease, CRISPR-Cas9 is being used to manufacture biofuels, engineer better crops, and more. These advances, made possible by the discovery of CRISPR-Cas9, will change lives worldwide.



Doudna & Charpentier Key Discoveries



1. ® The Nobel Foundation
2. The Nobel Prize in Chemistry 2020. NobelPrize.org. Nobel Media AB 2020. Fri. 30 Oct 2020. <https://www.nobelprize.org/prizes/chemistry/2020/summary/>
3. CRISPR-Cas9: Timeline of key events from "What is Biotechnology?"
4. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III (2011), PMID: 21455174
5. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity (2012), PMID: 22745249
6. RNA-programmed genome editing in human cells (2013), PMID: 23395978
7. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation (2014), PMID: 24505130

Take home messages

- High genetic heterogeneity
- High pathomechanistic heterogeneity
- RNA-based therapies are powerful tools, but each ALS-associated gene likely requires a different approach
- Upstream/downstream modulation of TDP-43 could be a promising strategy also for sporadic ALS cases
- Side effects?

Thank You !