UNIMORE
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

Dipartimento di Scienze Biomediche,
Metaboliche e Neuroscienze
Università di Modena e Reggio Emilia

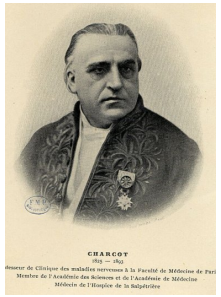
SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Modena

Dipartimento di Neuroscienze
Ospedale Civile S. Agostino-Estense
Azienda Ospedaliero Universitaria di Modena

Sla e prospettive terapeutiche: dal laboratorio ai trials clinici

Jessica Mandrioli

SLA & 2021: a che punto siamo?

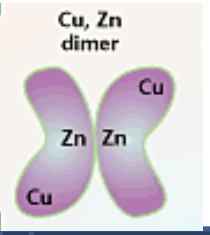


1869

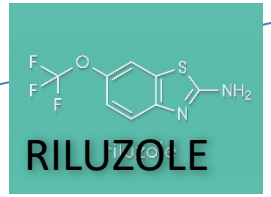
2021

ALS discovery

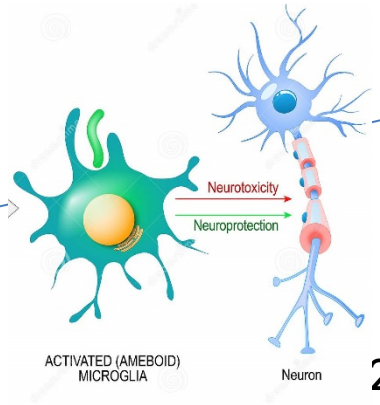
SOD1



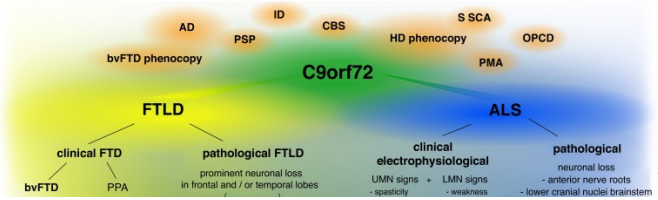
1996



2000



2007



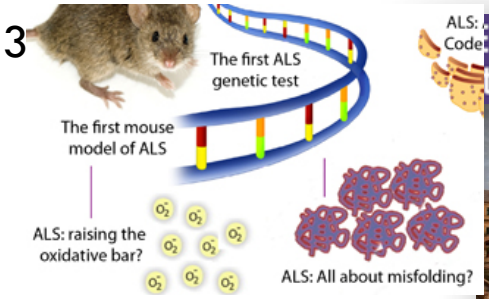
IMMUNITY,
BIOMARKERS

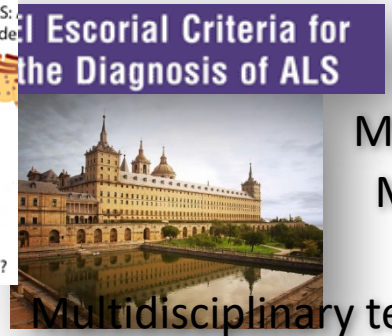
2015

Neurofilament

PRECISION MEDICINE

1993

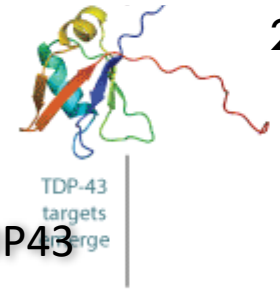




2006

MICROGLIA
MATTERS

TDP43

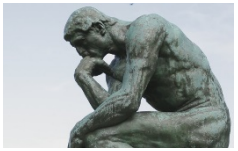


Awaji more
sensitive than
El Escorial?

2011

C9ORF72,
ALS OF A
DIFFERENT
KIND?

2013



ECAS: RETHINKING
COGNITION IN ALS

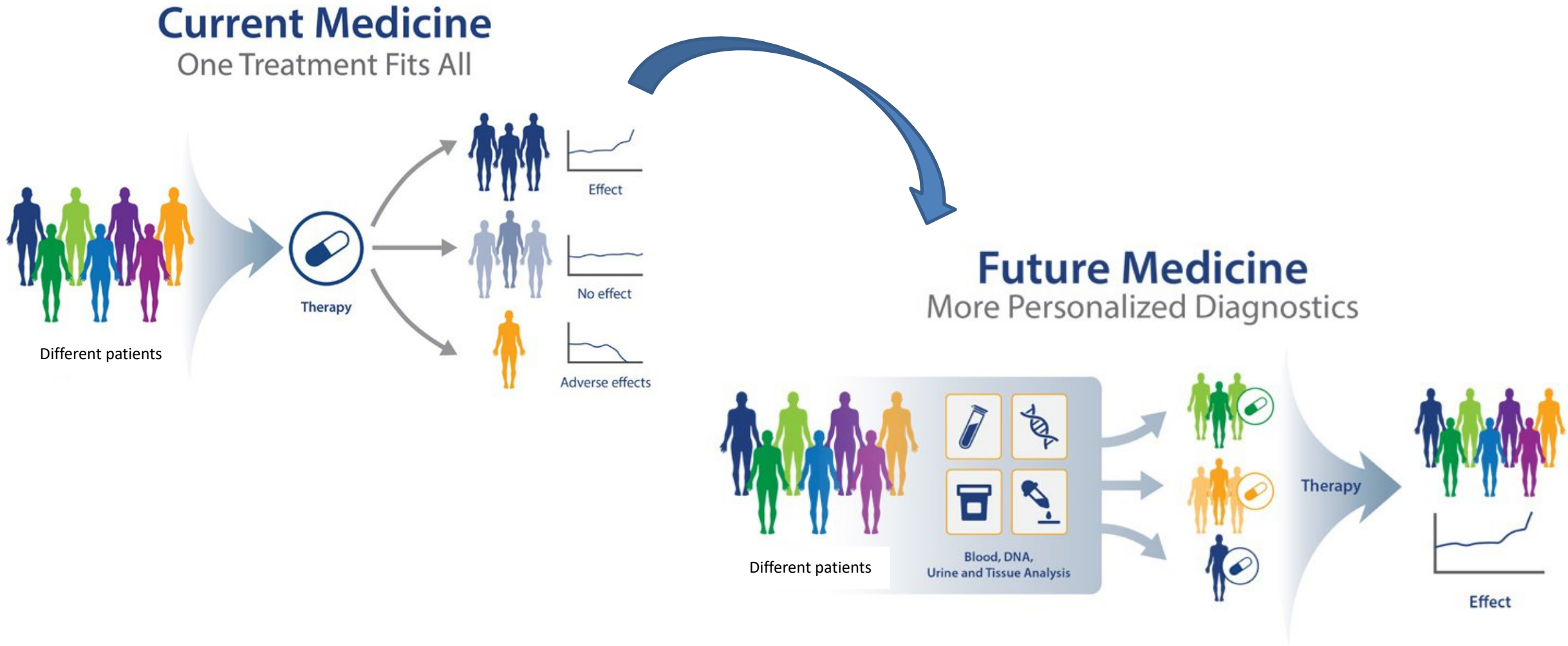
2020

CRISPR

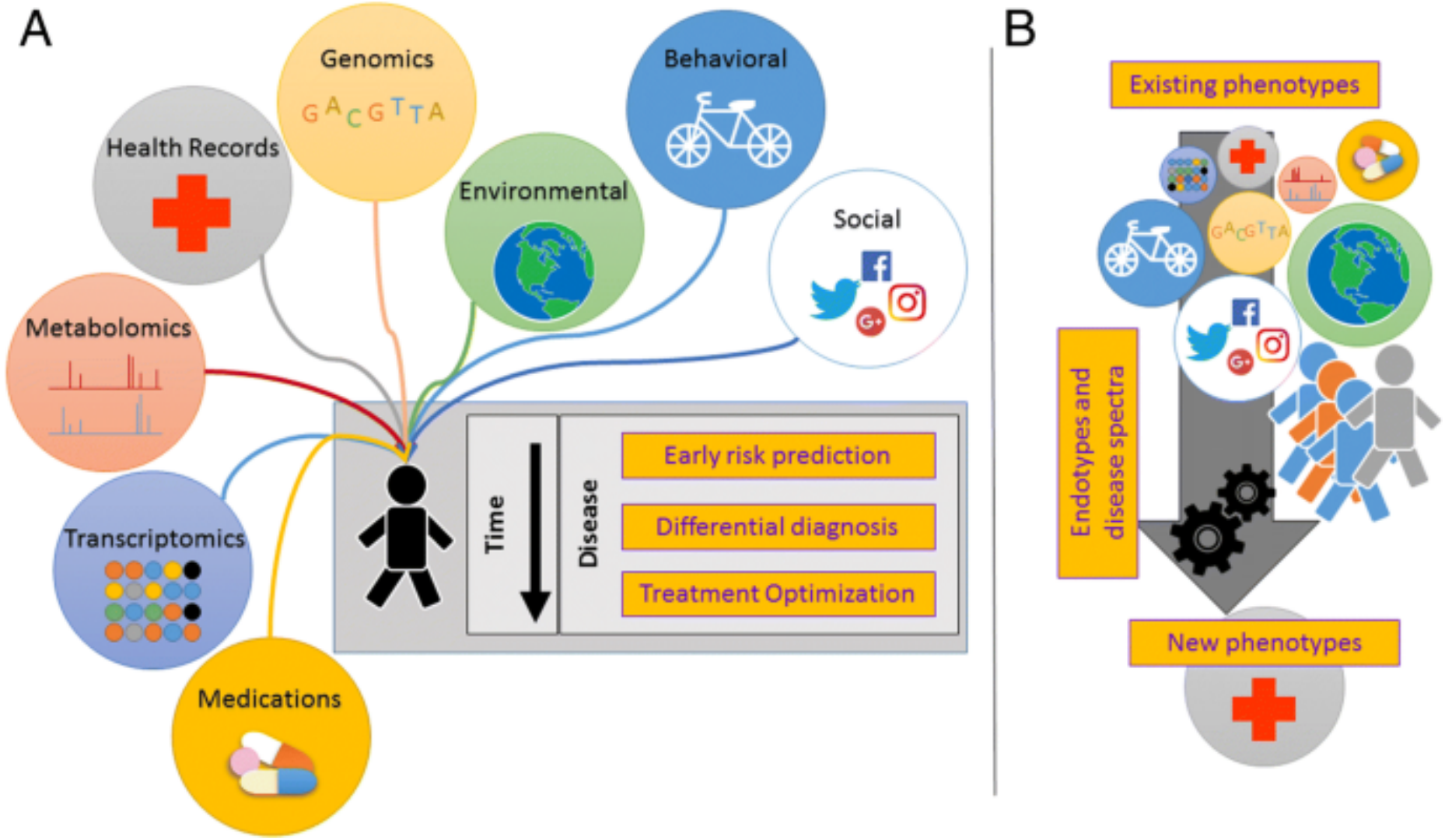
Multidisciplinary teams

NIV

SLA & medicina di precisione



SLA & medicina di precisione

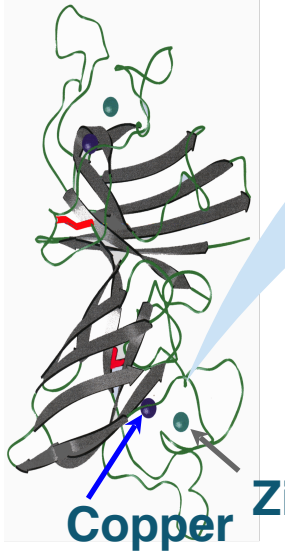


SLA & SOD1

SOD1: gene costituito da 5 esoni, >180 mutazioni crm21
SOD1: proteina ubiquitaria, prevalentemente citosolica
153 aa, 32 Kda, omodimero.
0.1–0.2% delle proteine cellulari del SNC

Rosen et al., Nature 1993

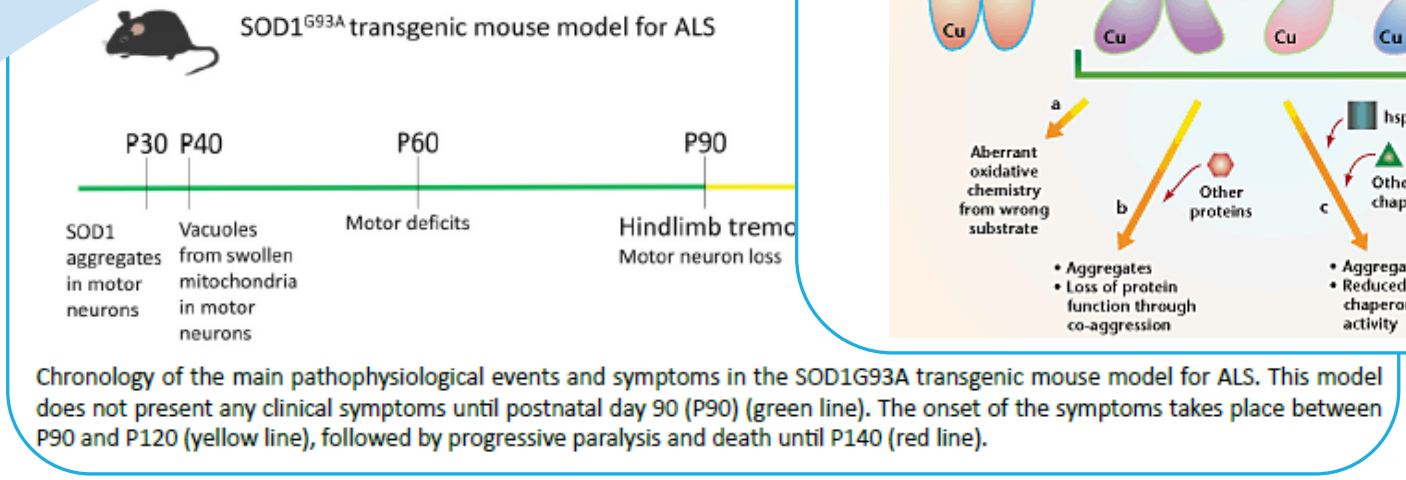
Electrostatic loop

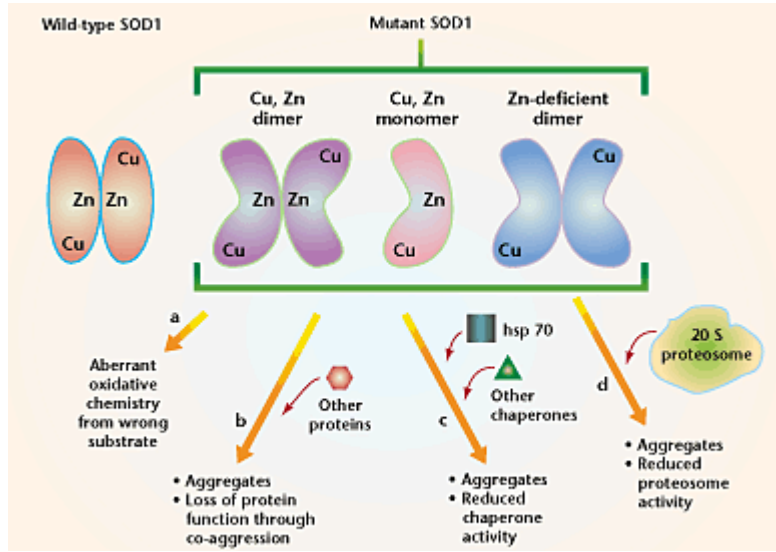


Zinc loop

ALS treatment

Preclinical trials for drugs, drug repurposing





Chronology of the main pathophysiological events and symptoms in the SOD1G93A transgenic mouse model for ALS. This model does not present any clinical symptoms until postnatal day 90 (P90) (green line). The onset of the symptoms takes place between P90 and P120 (yellow line), followed by progressive paralysis and death until P140 (red line).

ASOs nella SLA: dalla genetica al trattamento

INSIGHTS | PERSPECTIVES

MEDICINE

Antisense oligonucleotides for neurodegeneration

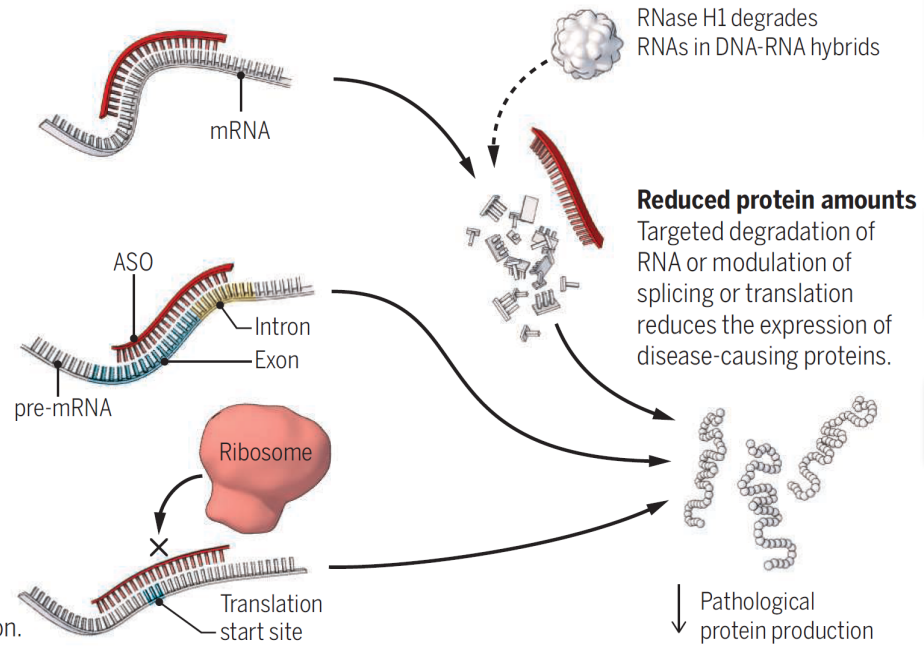
Reducing pathological protein expression

Antisense oligonucleotides (ASOs) are small, single-stranded DNAs that can bind specific RNA sequences on precursor messenger RNAs (pre-mRNAs) and mRNAs. The resulting RNA-DNA hybrid can induce ribonuclease H1 (RNase H1) degradation of the targeted RNA, modulation of splicing, or blockade of translation.

Target mutations
ASOs can target RNA transcripts that produce disease-causing proteins.

Target splice sites
Unique sequences at splice sites in pre-mRNAs can allow ASOs to modulate RNA splicing.

Target translation start sites
ASOs can selectively target translation start sites in mRNAs, which prevents protein translation.



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

Induction of Rnase H endonuclease activity

Inhibition of mRNA splicing

Reduced translation

Sex	Age (years)	Family history of amyotrophic lateral sclerosis	SOD1 mutation	Age at onset (years)	Site of c
Female	49	Yes	Glu49Lys	47	Limb
Male	59	Yes	Ala4Val	59	Limb
Female	36	Yes	Gly37Arg	23	Limb
Male	41	Yes	Ala4Thr	41	Limb
Male	47	Yes	Leu38Val	45	Limb
Male	51	Yes	Ile113Thr	47	Limb
Female	50	Yes	Ala4Val	50	Limb
Female	58	Yes	Ala4Val	58	Limb
Male	63	Yes	Gly85Arg	63	Limb
Male	52	Yes	Ala4Val	51	Limb
Male	48	Yes	Asn139Lys	45	Limb
Male	54	Yes	Ile113Thr	48	Limb
Male	44	No	Ala89Val	42	Limb
Female	56	Yes	Ile113Thr	43	Limb
Male	55	Yes	Gly93Ser	45	Limb
Male	46	Yes	Ala4Val	46	Bulbar
Male	22	Yes	Gly41Ser	22	Limb
Male	56	Yes	Asp90Ala	55	Limb
Male	51	Yes	Leu8Val	43	Limb
Female	38	Yes	Gly93Ala	37	Limb
Female	49	Yes	Gln22Leu	45	Limb

Lancet Neurol 2013; 12: 435–42

ASOs nella SLA: Tofersen

Tofersen phase 1/2 multiple ascending dose study

OBJECTIVE:
To evaluate the safety, tolerability, PK, PD, and exploratory efficacy of tofersen in people with SOD1 ALS

POPULATION

> 18 years old

Documented SOD1 mutation

Weakness attributed to ALS

FVC ≥ 50% of predicted value[b]

MAD STUDY[a]

Cohort 1: tofersen 20 mg or placebo

Cohort 2: tofersen 40 mg or placebo

Cohort 3: tofersen 60 mg or placebo

Cohort 4: tofersen 100 mg or placebo

ENDPOINTS

Primary

Safety and tolerability

PK measures of tofersen (plasma and CSF)

Secondary

Change from baseline in CSF levels of SOD1 protein

Exploratory endpoints include*

ALSFRS-R scores, SVC, HHD megascore, CSF pNFH

50 participants total, randomized 3:1 tofersen:placebo in each cohort

3 loading doses on Days 1, 15, and 29; maintenance doses on Days 57 and 85

Approximately 31 weeks including: up to 7-week screening period, 12-week dosing period, and 12-week follow-up period

Tofersen nella SLA

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

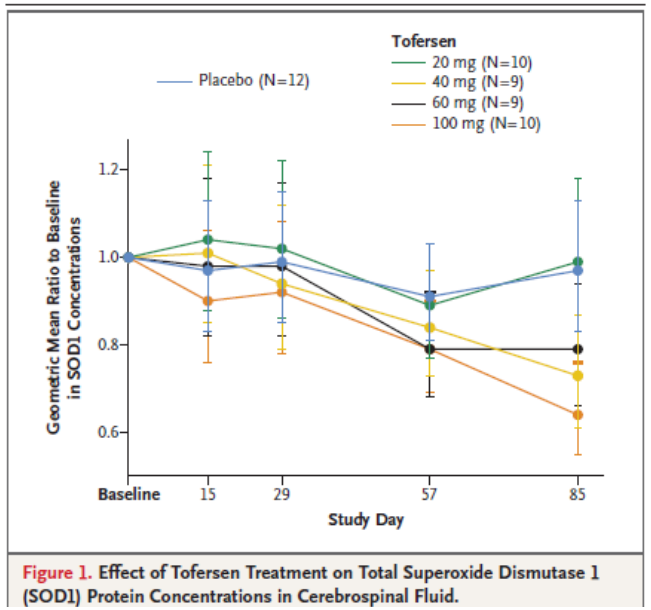
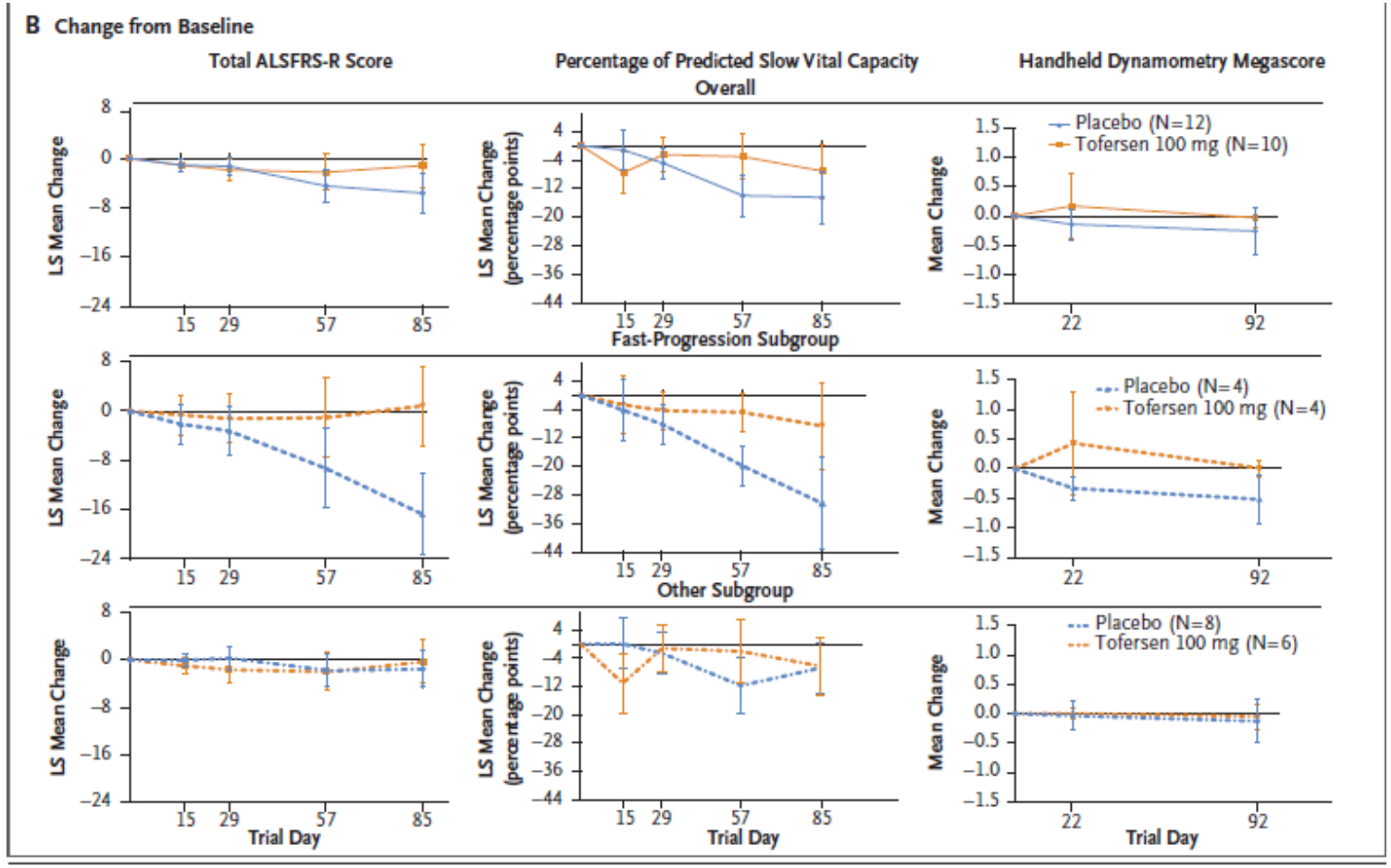


Figure 1. Effect of Tofersen Treatment on Total Superoxide Dismutase 1 (SOD1) Protein Concentrations in Cerebrospinal Fluid.



Tofersen nella SLA: lo studio VALOR

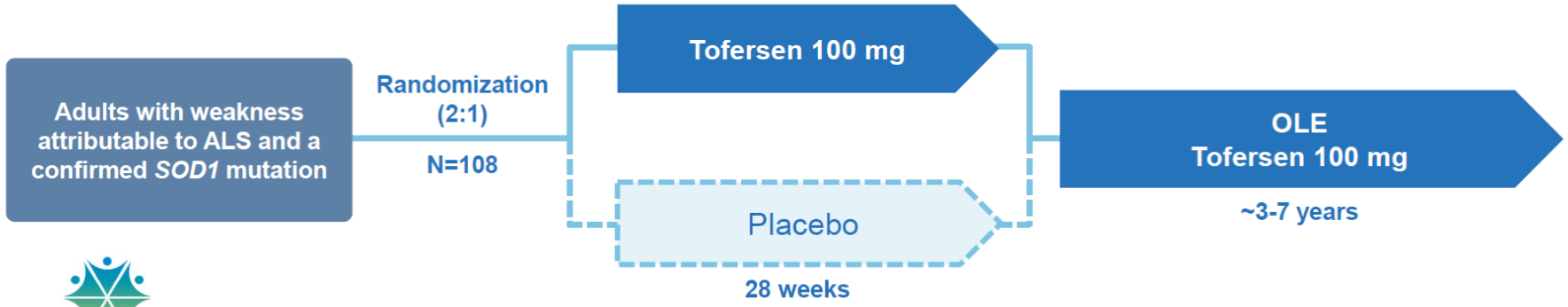
Results from the Phase 3 VALOR study and its open-label extension: evaluating the clinical efficacy and safety of tofersen in adults with ALS and confirmed *SOD1* mutation

Miller T,[1] Cudkowicz M,[2] on behalf of the VALOR Working Group

[1]Washington University, St. Louis, MO, USA

[2]Massachusetts General Hospital, Boston, MA, USA

VALOR study design[1,2]



VALOR

ENDPOINTS

	Primary	Key Secondary	Key Exploratory
Clinical	ALSFRS-R total score	% predicted SVC HHD megascore Time to death or PV Time to death	
Fluid Biomarker		Total CSF SOD1 Plasma NfL	
Quality-of-life			ALSAQ-5

Tofersen nella SLA: lo studio VALOR

Effect on clinical function

Adjusted mean (± SE) change from baseline in ALSFRS-R

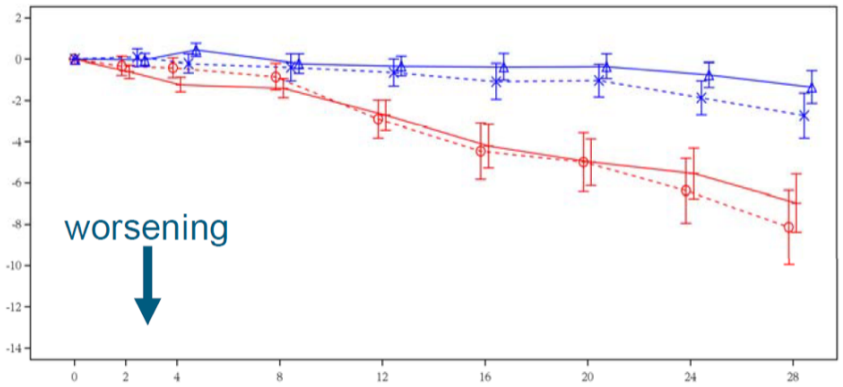
Results from the Phase 3 VALOR study and its open-label extension: evaluating the clinical efficacy and safety of tofersen in adults with ALS and confirmed *SOD1* mutation

Miller T,[1] Cudkowicz M,[2] on behalf of the VALOR Working Group

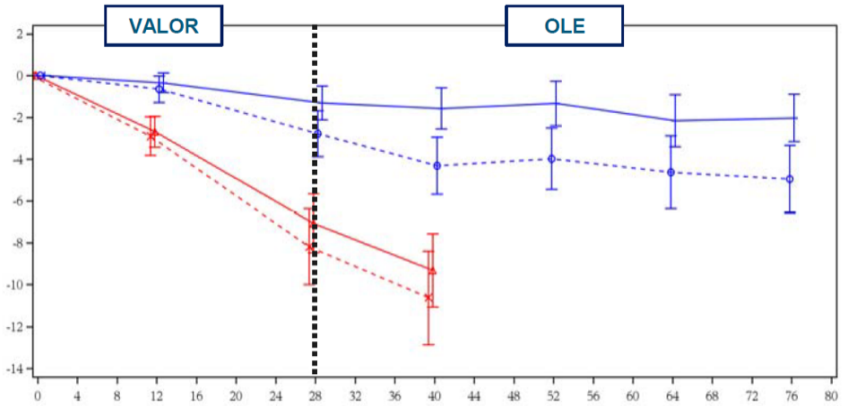
[1]Washington University, St. Louis, MO, USA

[2]Massachusetts General Hospital, Boston, MA, USA

VALOR
(Primary endpoint)



VALOR + OLE



	Placebo	Tofersen	Difference Tofersen vs Placebo (p-value)
Faster-progressing (mITT); Week 28	-8.14	-6.98	1.2 (p=0.97 joint rank)
Slower-progressing (non-mITT); Week 28	-2.73	-1.33	1.4

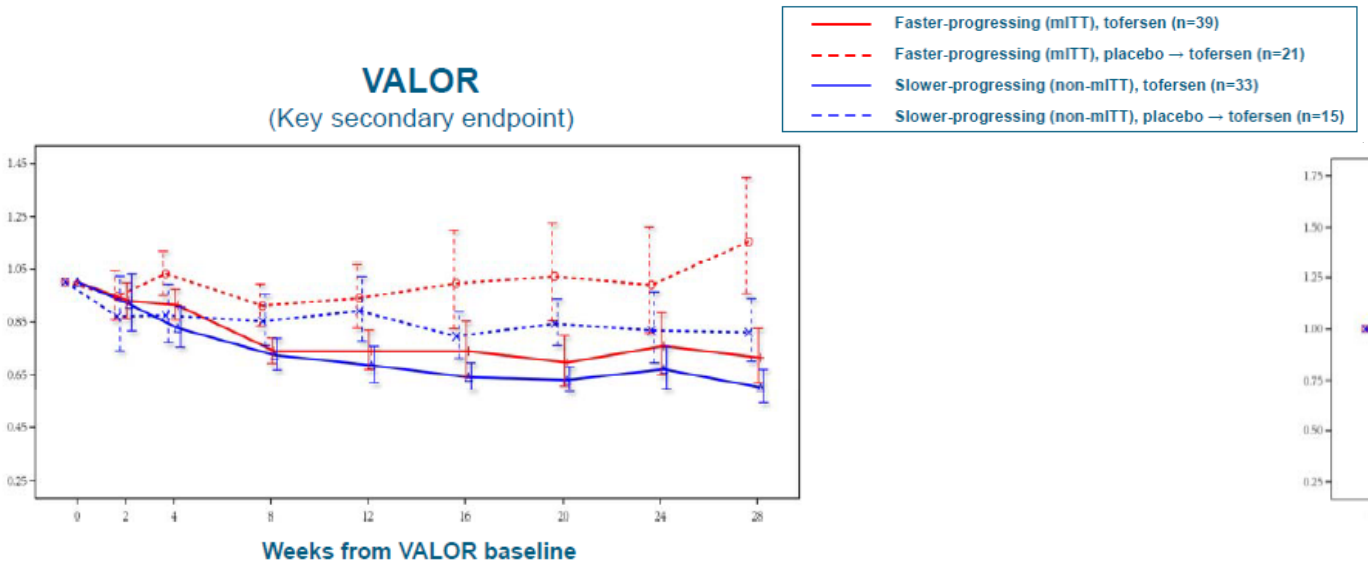
	Placebo → tofersen	Early-start tofersen	Difference Tofersen vs Placebo (95% CI)
Faster-progressing (mITT); Week 40	-10.6	-9.3	1.3 (-4.1, 6.7)
Slower-progressing (non-mITT); Week 76	-4.9	-2.0	2.9 (-0.7, 6.6)

8

Tofersen nella SLA: lo studio VALOR

Target engagement

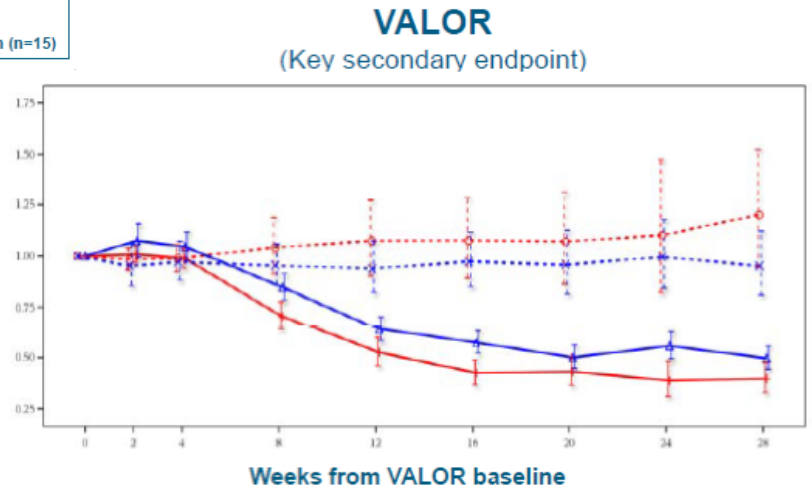
LS geometric mean ratio (95% CI) to baseline of CSF total SOD1



	Placebo	Tofersen	Geo mean ratio Tofersen:Placebo (p-value)
Faster-progressing (mITT); Week 28	1.16 (16% incr)	0.71 (29% decr)	0.62 (p<0.0001)
Slower-progressing (non-mITT); Week 28	0.81 (19% decr)	0.60 (40% decr)	0.74 (p=0.0007)

Effect on neurofilament

LS geometric mean ratio (95% CI) to baseline of plasma NfL



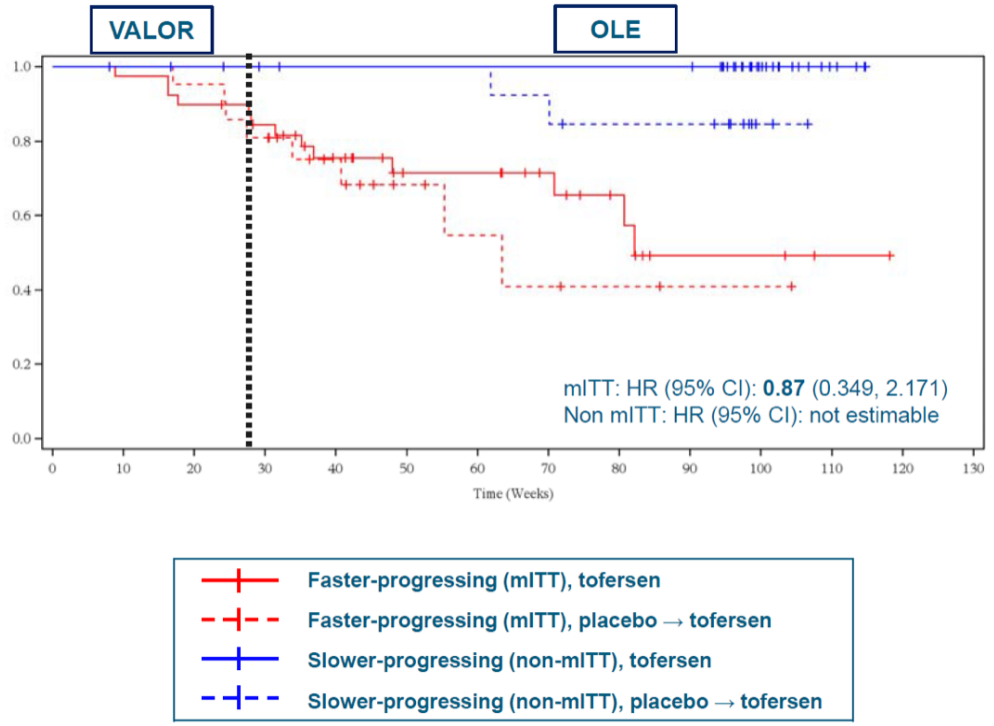
	Placebo	Tofersen	Geo mean ratio Tofersen:Placebo (p-value)
Faster-progressing (mITT); Week 28	1.20 (20% incr)	0.40 (60% decr)	0.33 (p<0.0001)
Slower-progressing (non-mITT); Week 28	0.95 (5% decr)	0.50 (50% decr)	0.52

Tofersen nella SLA: lo studio VALOR

Time-to-event analyses

Median time to death and time to death or PV were non-estimable due to the number of events; a post-hoc analysis was performed to account for withdrawal due to disease progression

Event	Early (VALOR) start tofersen (n=72)	PBO + delayed-start tofersen (n=36)
Death	4 (5.6%)	3 (8.3%)
Permanent ventilation	6 (8.3%)	2 (5.6%)
Withdrawal due to disease progression	3 (4.2%)	5 (13.9%)
Total	13 (18.1%)	10 (27.8%)



Tofersen nella SLA: lo studio VALOR

Summary

VALOR

VALOR did not achieve statistical significance on its primary endpoint of ALSFRS-R at 6 months; however, consistent effects were seen across key secondary and exploratory clinical outcome measures

These effects became more apparent with longer-term follow-up in the extension, as earlier initiation of tofersen led to:

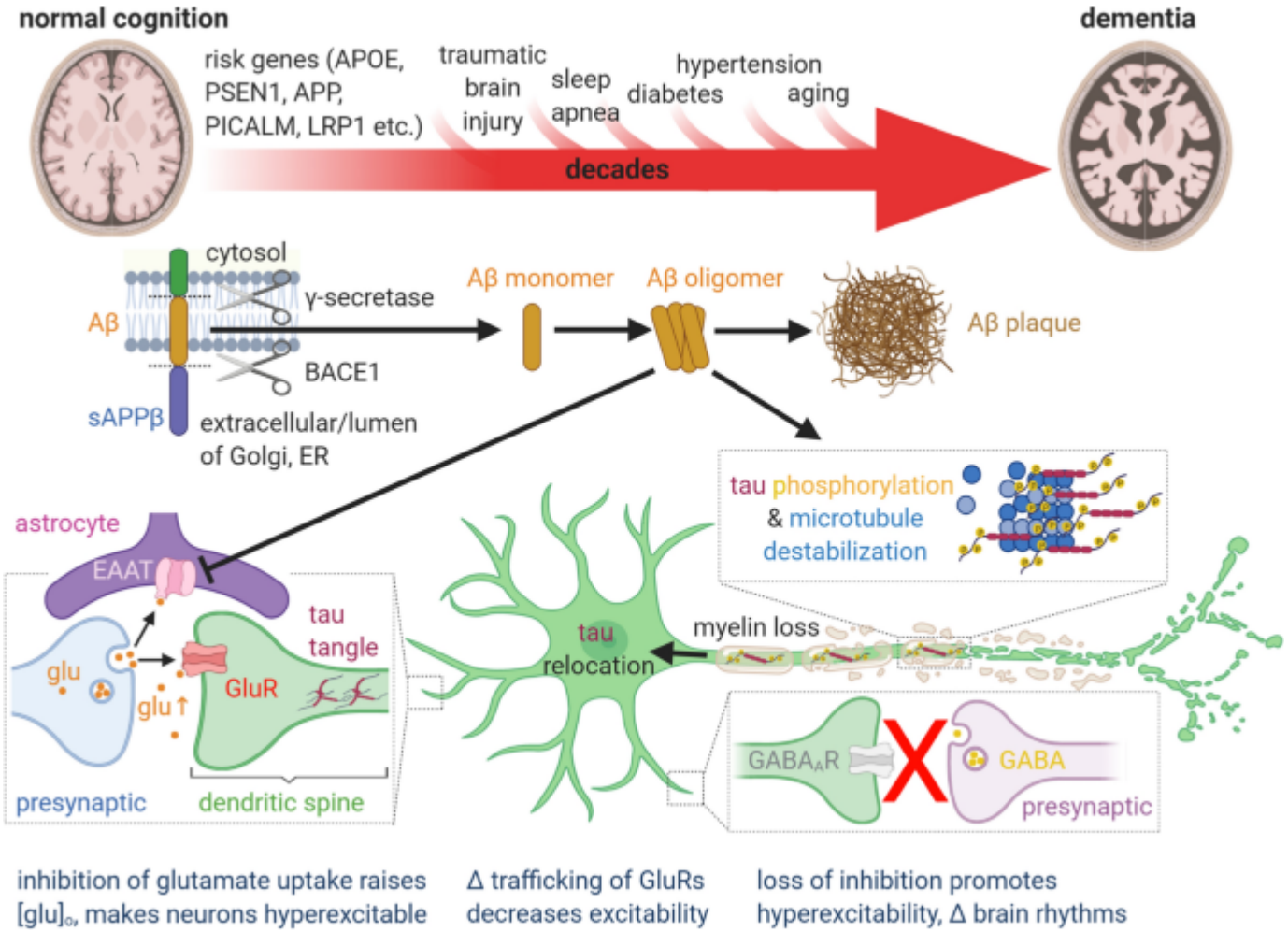
- A slowing of decline in faster progressing participants
- An apparent stabilization of clinical function in slower progressing participants

Tofersen administration led to sustained reductions in total CSF SOD1 protein demonstrating target engagement, and plasma NfL suggestive of a slowing in neuronal degeneration

Most AEs were mild to moderate in severity and many were consistent with ALS disease progression or LP-related events

Serious neurologic events, including myelitis, were seen in tofersen-treated participants

Clinica e biologia non sempre concordi: il caso dell'AD

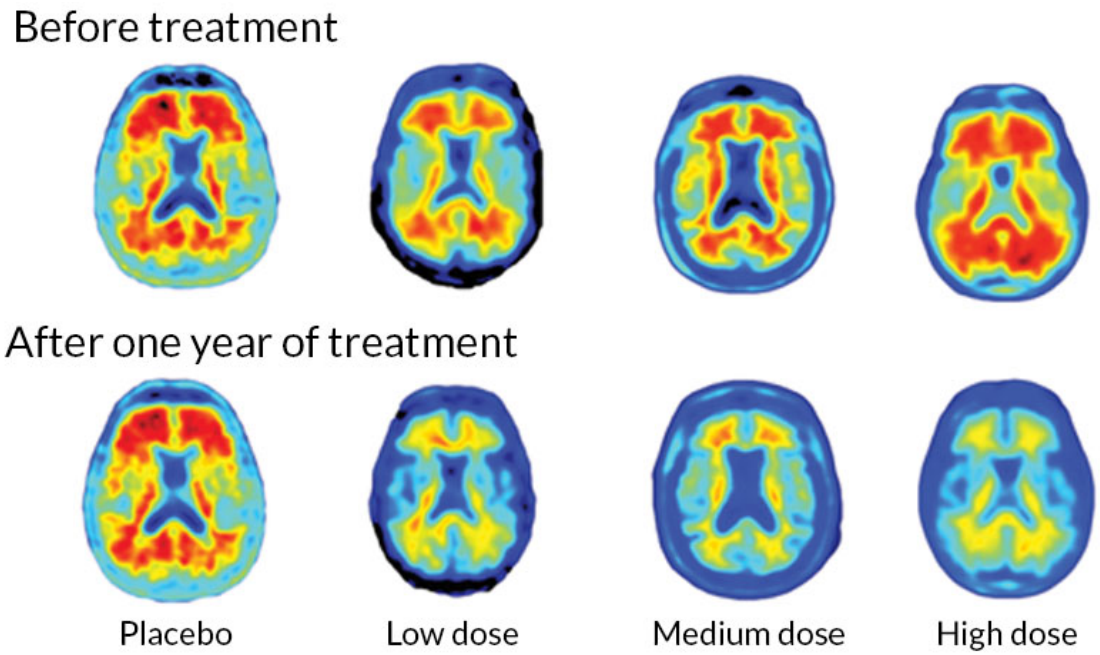


Aducanumab

Aducanumab is a human IgG1 anti-Aβ monoclonal antibody selective for Aβ aggregates. Biogen conducted two identically designed 18-month-long randomized, double-blind, placebo-controlled, parallel-group studies (301 and 302) that evaluated the efficacy, safety and pharmacokinetic and pharmacodynamic properties of aducanumab. The double-blind placebo-controlled period was followed by a dose-blinded long-term extension. The two studies enrolled a total of 3285 participants at 348 sites in 20 countries.

Aducanumab e M. di Alzheimer

Aducanumab



Biomarkers	Difference amongst individuals treated with aducanumab versus placebo, p value	
	Low dose	High dose
Amyloid PET	−0.179, p<0.0001 n=100	−0.278, p<0.0001 n=109
CSF analyte		
β-amyloid1-42 CSF	179.57, p<0.0001 n=33	318.88, p<0.0001 n=17
p-Tau CSF	−15.64, p=0.0035 n=33	−22.44, p=0.0005 n=17
t-Tau CSF	−86.74, p=0.0148 n=33	−112.05, p=0.0008 n=17
Tau PET composite region		
Frontal	−0.049, *p*=0.0876 *n*=14	−0.073, *p*=0.0212 *n*=11
Medial temporal	−0.115, *p*=0.0012 *n*=14	−0.132, *p*=0.0005 *n*=11
Temporal	−0.065, *p*=0.1174 *n*=14	−0.096, *p*=0.0304 *n*=17

Aducanumab e M. di Alzheimer

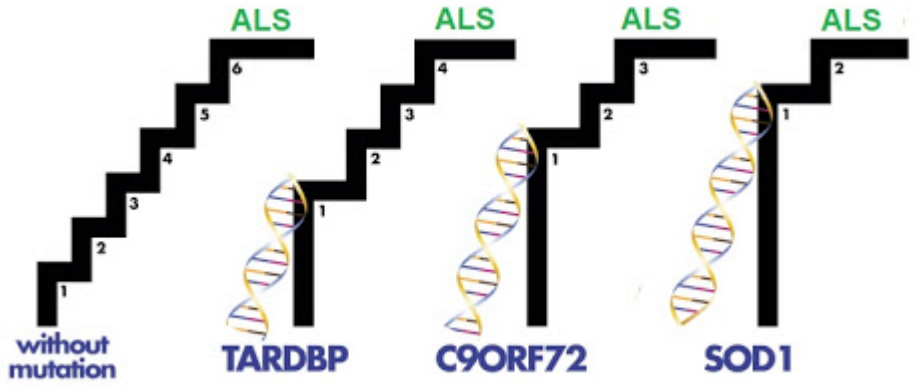
Aducanumab

Study/scales	301		302	
	Difference amongst individuals treated with aducanumab versus placebo			
	Low dose	High dose	Low dose	High dose
CDR-SB	0.18 (−12%), p=0.2250	0.03 (2%), p=0.8330	−0.26 (−15%), p=0.0901	−0.39 (−22%), p=0.0120
MMSE	0.2 (−6%), p=0.4795	−0.1 (3%), p=0.8106	−0.1 (3%), p=0.7578	0.6 (−18%), p=0.0493
ADAS-Cog13	−0.583 (−11%), p=0.2536	−0.588 (−11%), p=0.2578	−0.701 (−14%), p=0.1962	−1.400 (−27%), p=0.0097
ADCS-ADL-MCI	0.7 (−18%), p=0.1225	0.7 (−18%), p=0.1506	0.7 (−16%), p=0.1515	1.7 (−40%), p=0.0006
NPI-10	Not available	Not available	−0.5 (−33%), p=0.3921	−1.3 (−87%), p=0.0215

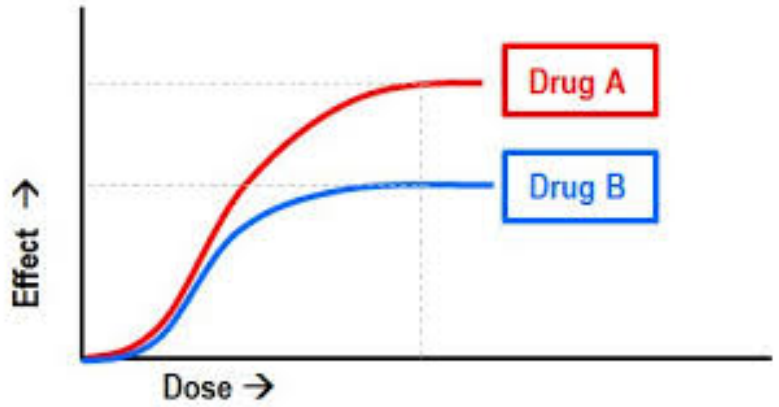
Clinica e biologia non sempre concordi: perchè?

?

Other factors matter



Dose matters



Pathways matter

Time matters

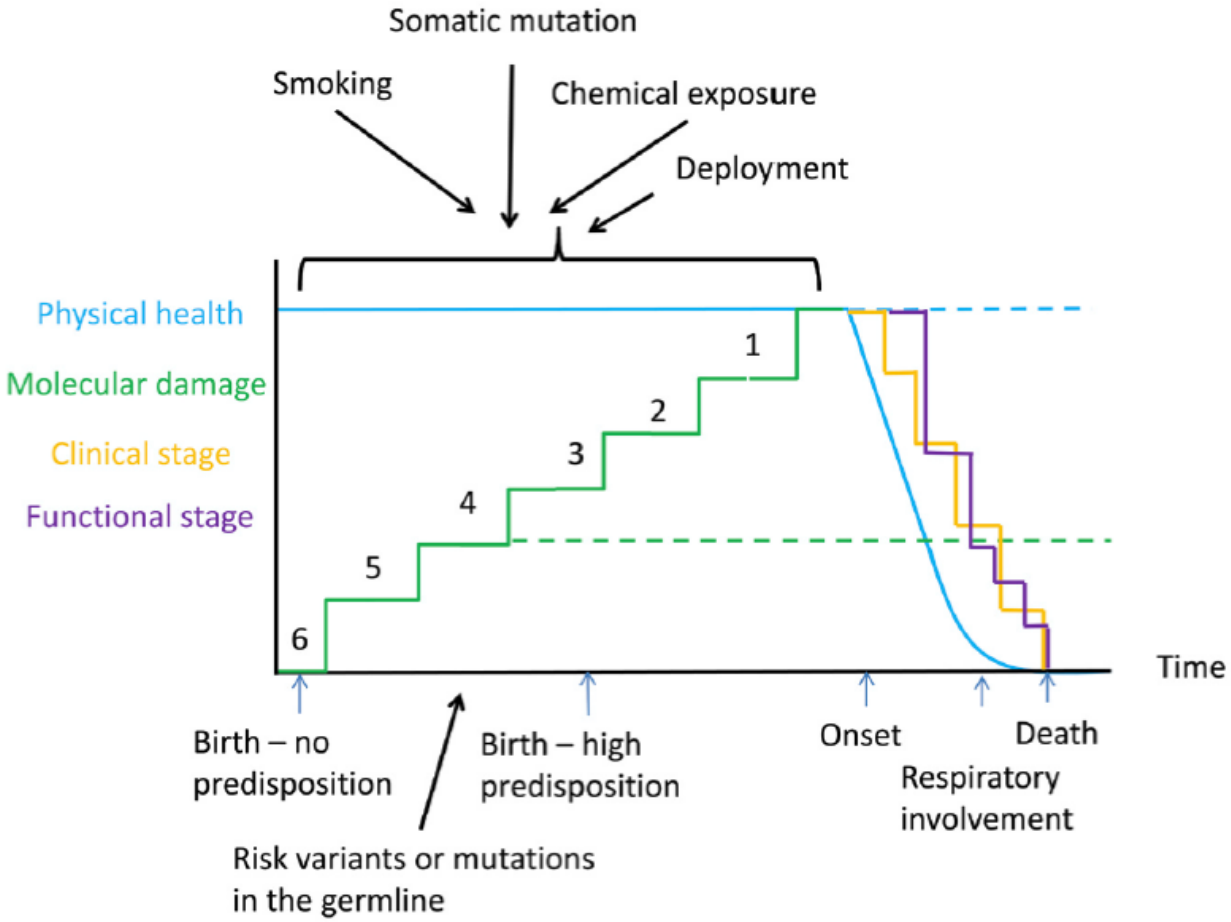
Cosa determina la malattia: l'ipotesi multistep

REVIEW

What causes amyotrophic lateral sclerosis?

Martin S, Al Khleifat A and Al-Chalabi A.

F1000Research 2017, **6**(F1000 Faculty Rev):371

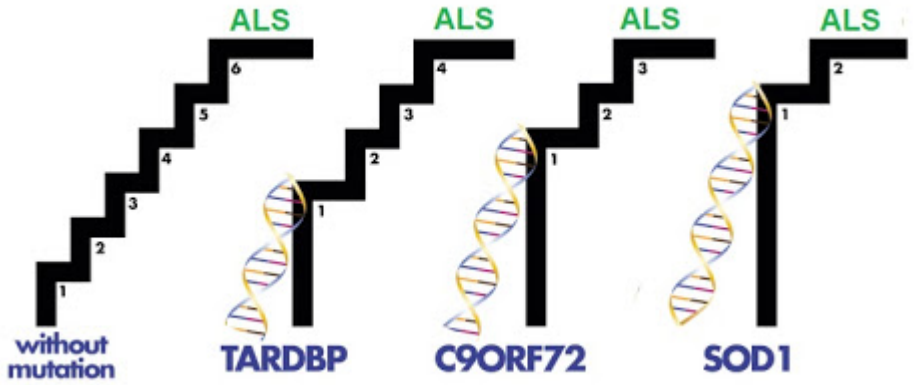


The multistep hypothesis of ALS revisited

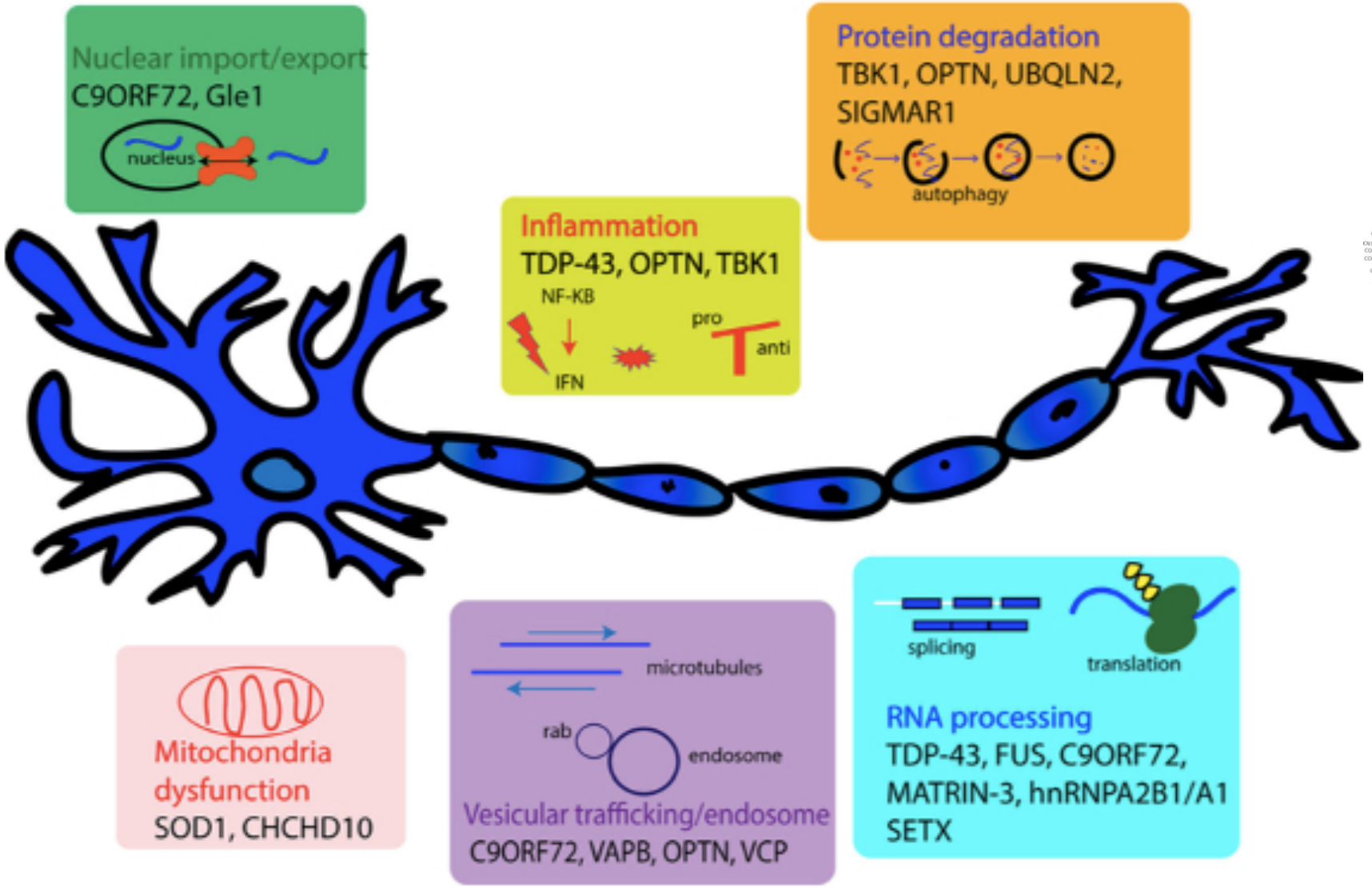
The role of genetic mutations

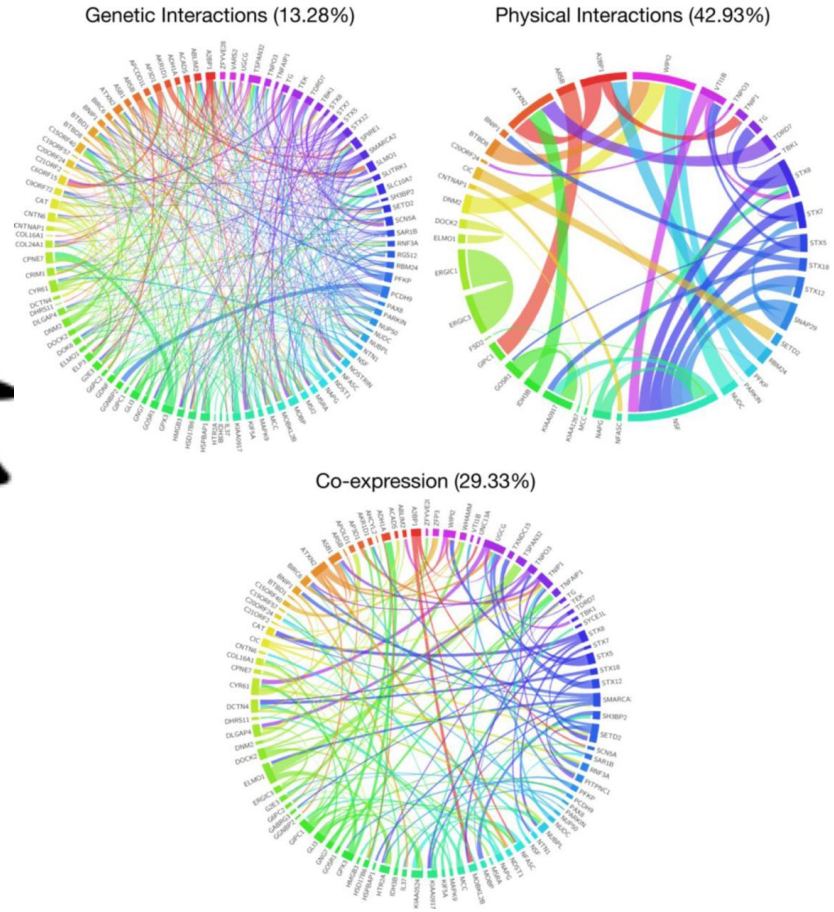
Adriano Chiò, MD, FAAN, Letizia Mazzini, MD, Sandra D'Alfonso, PhD, Lucia Corrado, PhD, Antonio Canosa, MD, PhD, Cristina Moglia, MD, PhD, Umberto Manera, MD, Enrica Bersano, MD, Maura Brunetti, BSc, Marco Barberis, BSc, PhD, Jan H. Veldink, MD, PhD, Leonard H. van den Berg, MD, PhD, Neil Pearce, DSc, William Sproviero, PhD, Russell McLaughlin, PhD, Alice Vajda, PhD, Orla Hardiman, MD, PhD, James Rooney, MSc, Gabriele Mora, MD, Andrea Calvo, MD, PhD, and Ammar Al-Chalabi, PhD, FRCP

Neurology® 2018;0:e1-e8. doi:10.1212/WNL.0000000000005996



Cosa determina la SLA: patogenesi e interazioni





Patogenesi e interazioni: ATXN2

LETTER

doi:10.1038/nature22038

Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice

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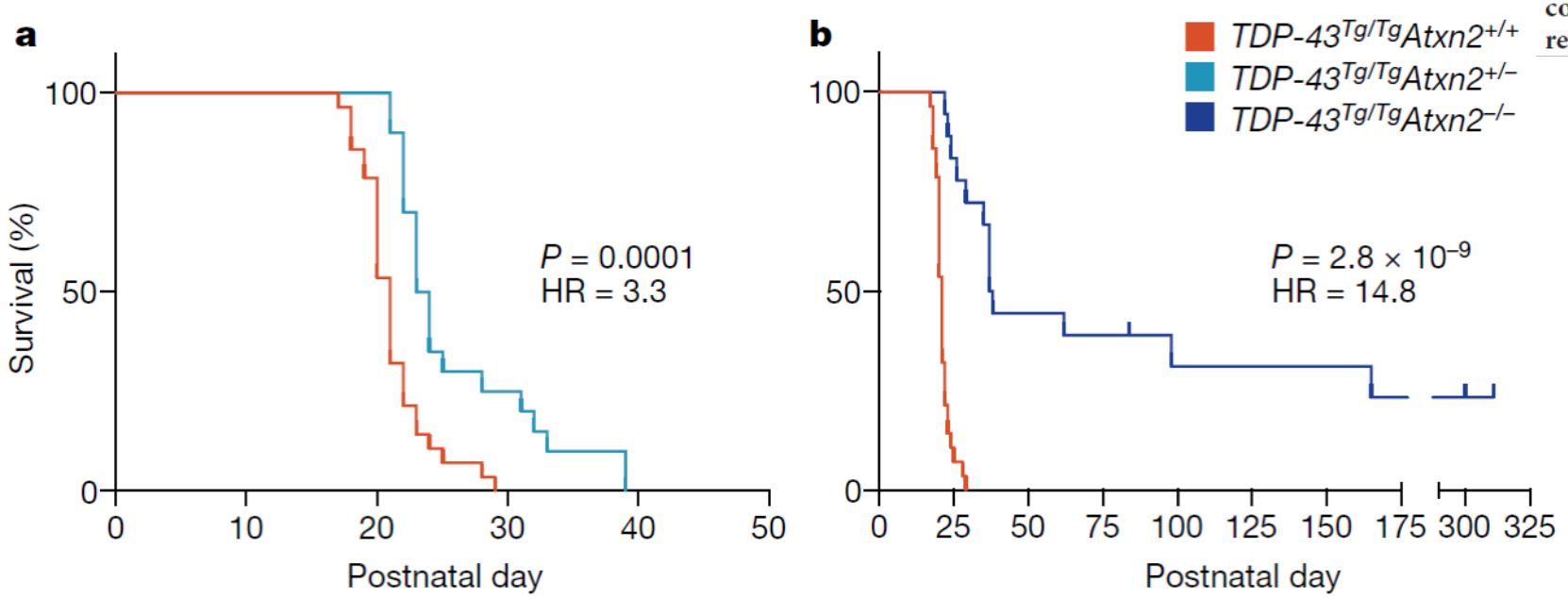


expansions in the ataxin-2 gene increase risk of ALS[7,8]. We used two independent approaches to test whether decreasing ataxin-2 levels could mitigate disease in a mouse model of TDP-43 proteinopathy[9]. First, we crossed ataxin-2 knockout mice with *TDP-43* (also known as *TARDBP*) transgenic mice. The decrease in ataxin-2 reduced aggregation of TDP-43, markedly increased survival and improved motor function. Second, in a more therapeutically applicable approach, we administered ASOs targeting ataxin-2 to the central nervous system of *TDP-43* transgenic mice. This single treatment markedly extended survival. Because TDP-43 aggregation is a component of nearly all cases of ALS[6], targeting ataxin-2 could represent a broadly effective therapeutic strategy.

Patogenesi e interazioni:ATXN2

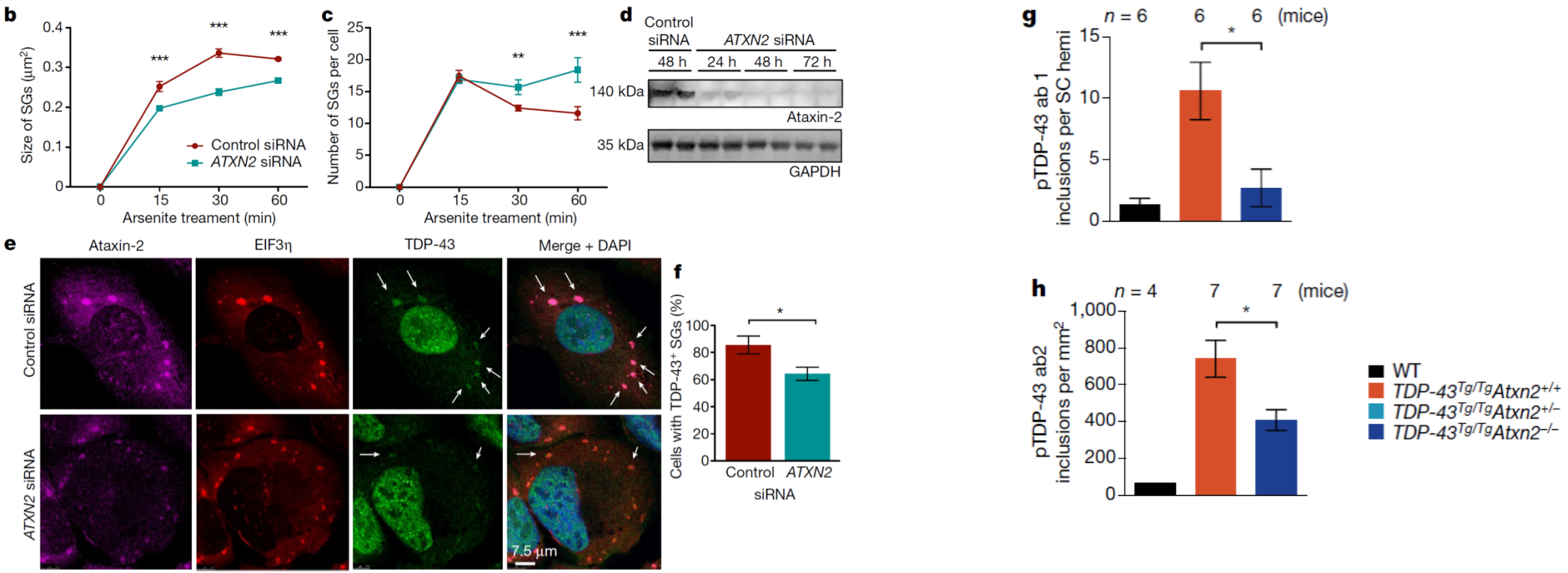


Figure 2 | Knockdown of ataxin-2 delays the maturation of stress granules and decreases recruitment of TDP-43 to stress granules. a, An EIF3η antibody was used to visualize stress granules (SGs) in U2OS cells. During continued stress, stress granules fuse to form larger structures. *ATXN2* siRNA inhibited maturation of stress granules, resulting in smaller, more numerous stress granules at each time point. **b**, **c**, Quantification of size (**b**) and number (**c**) of stress granules. **d**, Western blot of ataxin-2 knockdown. Gel source data can be found in Supplementary Fig. 1. **e**, **f**, Ataxin-2 siRNA treatment caused fewer cells to have TDP-43-positive stress granules at 60 min of arsenite exposure. **b**, **c**, **f**, The mean ± s.e.m. of three separate wells (116–146 cells per well) is plotted for each data point. Two-tailed *t*-tests were used to compare treatment groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Patogenesi e interazioni: ATXN2

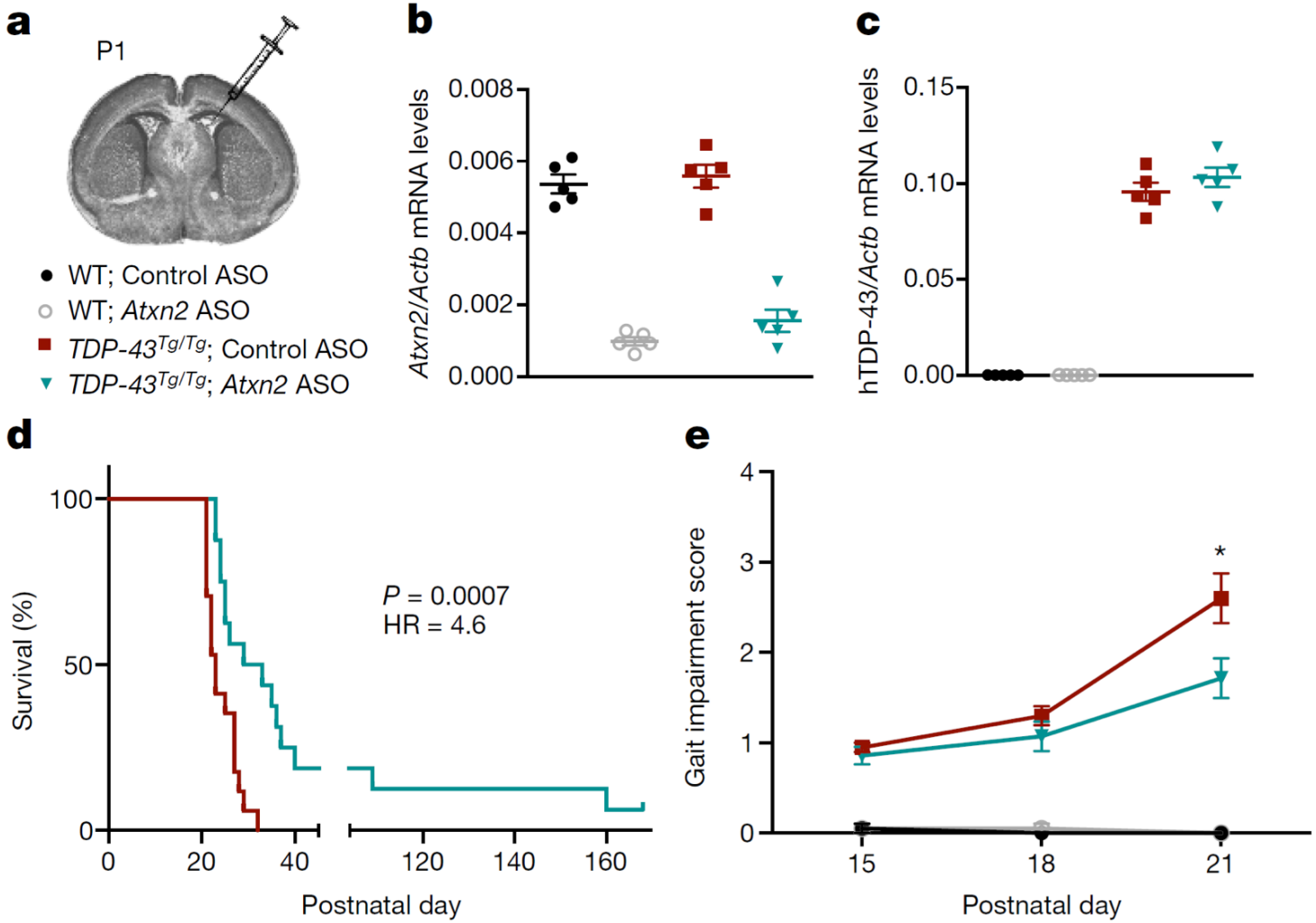
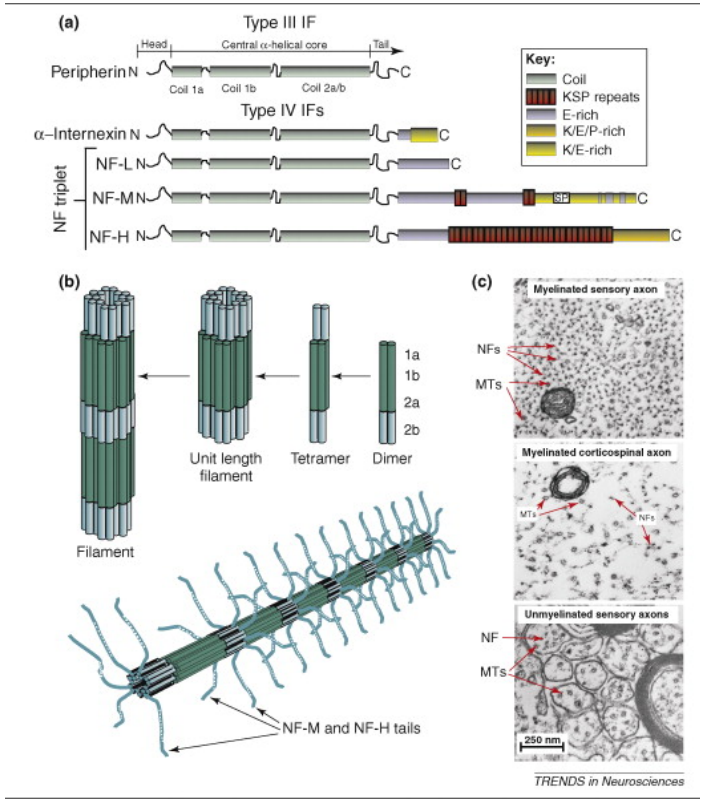


Figure 4 | ASOs that target ataxin-2 extend lifespan and improve motor performance in TDP-43 transgenic mice. a, P1 mouse pups were treated with control or *Atxn2* ASOs by ICV injection. **b**, **c**, At P21, mRNA levels of *Atxn2* were decreased by 77% in the brains of mice injected with the *Atxn2* ASO (**b**) without affecting mRNA levels of the human *TDP-43* transgene (**c**). **d**, Lifespan was significantly extended by treatment with the *Atxn2* ASO ($n = 16$) compared to the control ASO ($n = 17$). Curves were compared by log-rank test, and effect size was estimated using a Cox proportional hazards model. The tick indicates a mouse still alive at the time of submission. **e**, Gait impairment score was also improved in mice treated with the *Atxn2* ASO ($n = 14$) by P21 compared to treatment with the control ASO ($n = 20$). Two-tailed *t*-tests were used to compare the two treatment groups at each age. Data are mean ± s.e.m. $^*P < 0.05$.

Il tempo e il ruolo dei biomarcatori



Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS

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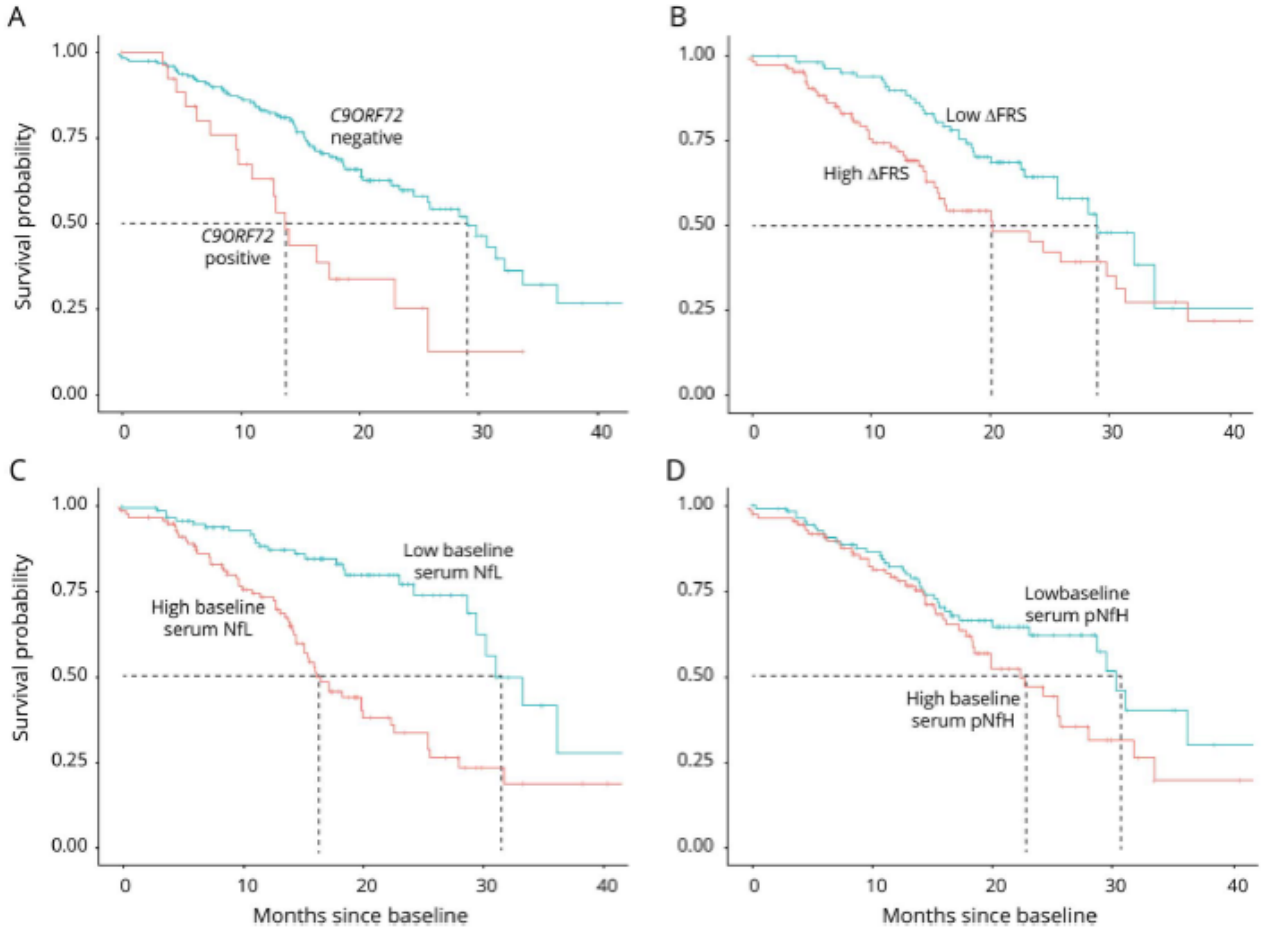
Richard Barohn, MD, Andrea Swe
Jaya Trivedi, MD, Erik P. Pioro, MD
Rosa Rademakers, PhD, Andrea M
behalf of the CReATe Consortium

Neurology® 2020;95:1-11. doi:10.1



Conclusions

Serum NfL may be considered a clinically validated prognostic biomarker for ALS. Serum NfL (and perhaps pNfH), quantified using the Simoa assay, has potential utility as a pharmacodynamic biomarker of treatment effect.

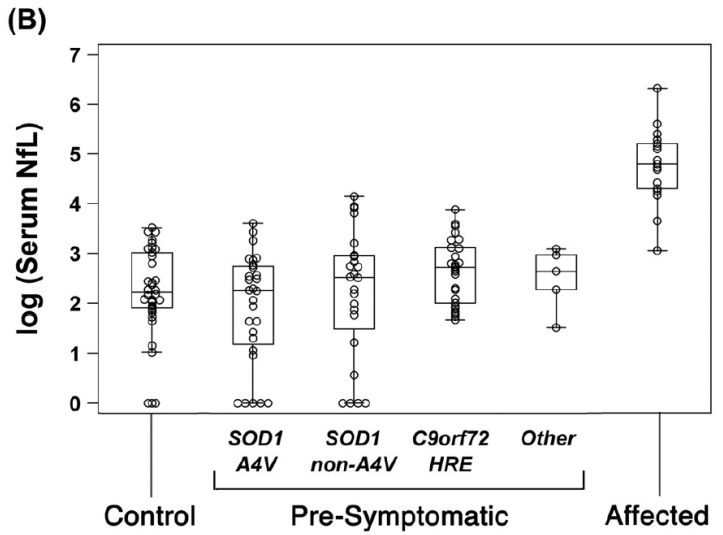
Biomarkers di fenoconversione

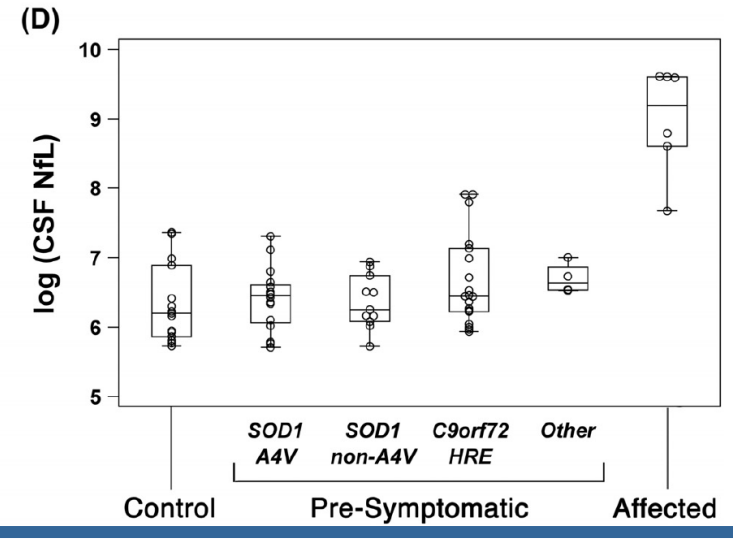
Neurofilament Light: A Candidate Biomarker of Presymptomatic Amyotrophic Lateral Sclerosis and Phenoconversion

Michael Benatar, MD, PhD,[1]* Joanne Wuu, ScM,[1]* Peter M. Andersen, MD, PhD,[2] Vittoria Lombardi, PhD,[3] and Andrea Malaspina, MD, PhD[3]

ANN NEUROL 2018;84:130–139

		Control (N = 34)	At-Risk (N = 84)	Converter (N = 10)	Affected (N = 17)
Baseline age (years)	Mean ± SD	46.4 ± 11.4	45.1 ± 12.3	50.3 ± 11.6	59.8 ± 8.2
	(range)	(24.2–69.2)	(18.9–77.0)	(31.9–74.8)	(45.6–75.8)
Male	N (%)	15 (44)	31 (37)	4 (40)	9 (53)
Genotype	*SOD1* A4V	(n/a)	28	8	1
	SOD1 nonA4V	(n/a)	24	1	4
	C9orf72 HRE	(n/a)	27	0	0
	Other	(n/a)	5	1	11
	Unknown	(n/a)	0	0	2





Biomarkers di fenoconversione

Serum (and CSF) NfL are informative biomarkers of presymptomatic ALS, providing a tool to quantify presymptomatic disease progression and to predict the timing of clinical phenoconversion.

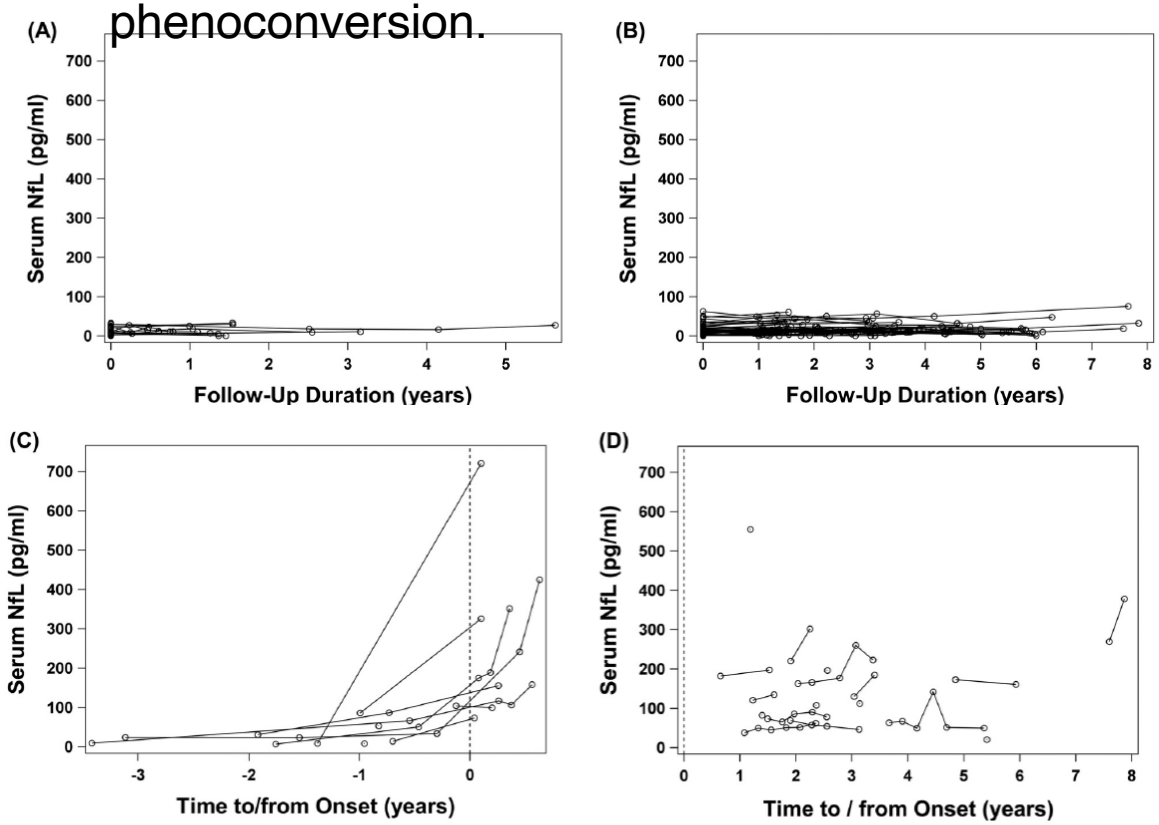
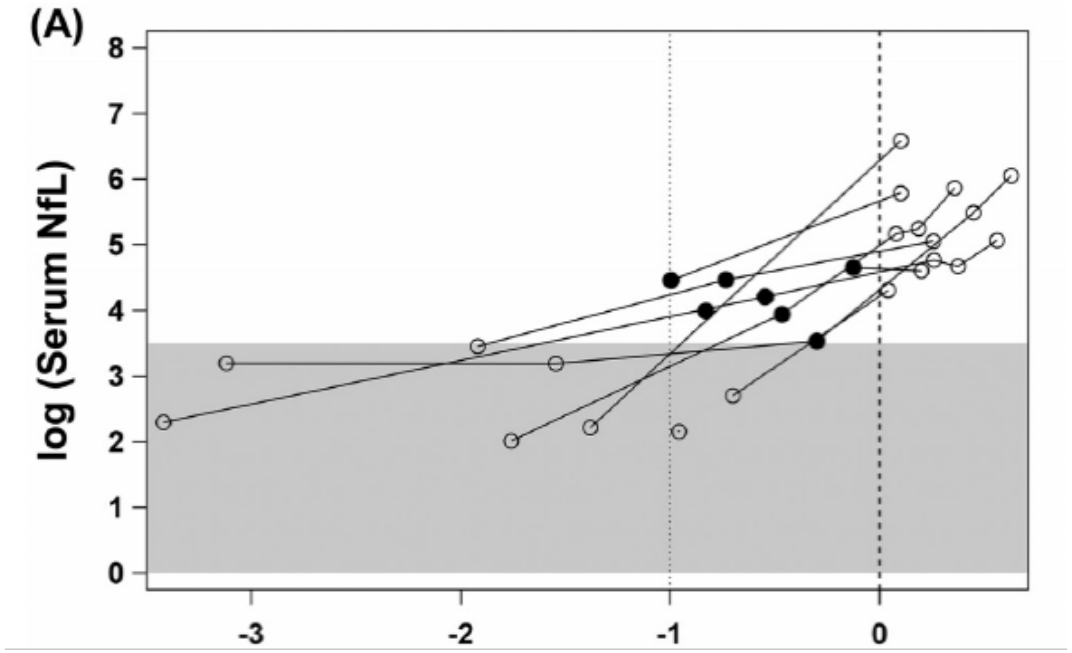


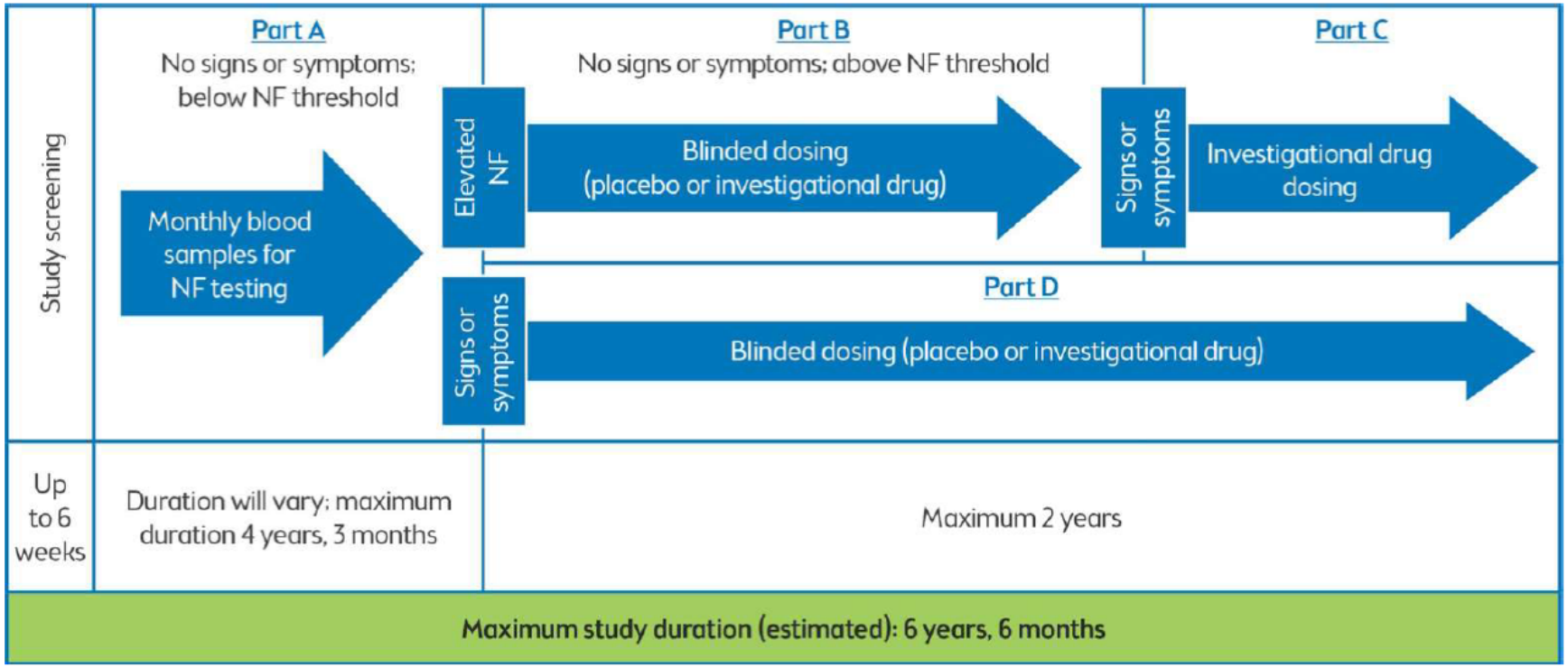
FIGURE 2: Longitudinal changes in serum NfL concentration (pg/ml): (A) controls; (B) at-risk individuals who remain pre-symptomatic throughout follow-up; (C) phenoconverters; and (D) ALS patients. The x-axis in (A) and (B) shows years since baseline. The x-axis in (C) and (D) shows years to or since the onset of symptoms or signs, which is marked by the vertical dashed line at year = 0. ALS = amyotrophic lateral sclerosis; NfL = neurofilament light.



ANN NEUROL 2018;84:130–139

Times matters: lo studio ATLAS

A Study of BIIB067 When Initiated in Clinically Presymptomatic Adults With a Confirmed Superoxide Dismutase 1 Mutation (ATLAS)



ClinicalTrials.gov Identifier: NCT04856982

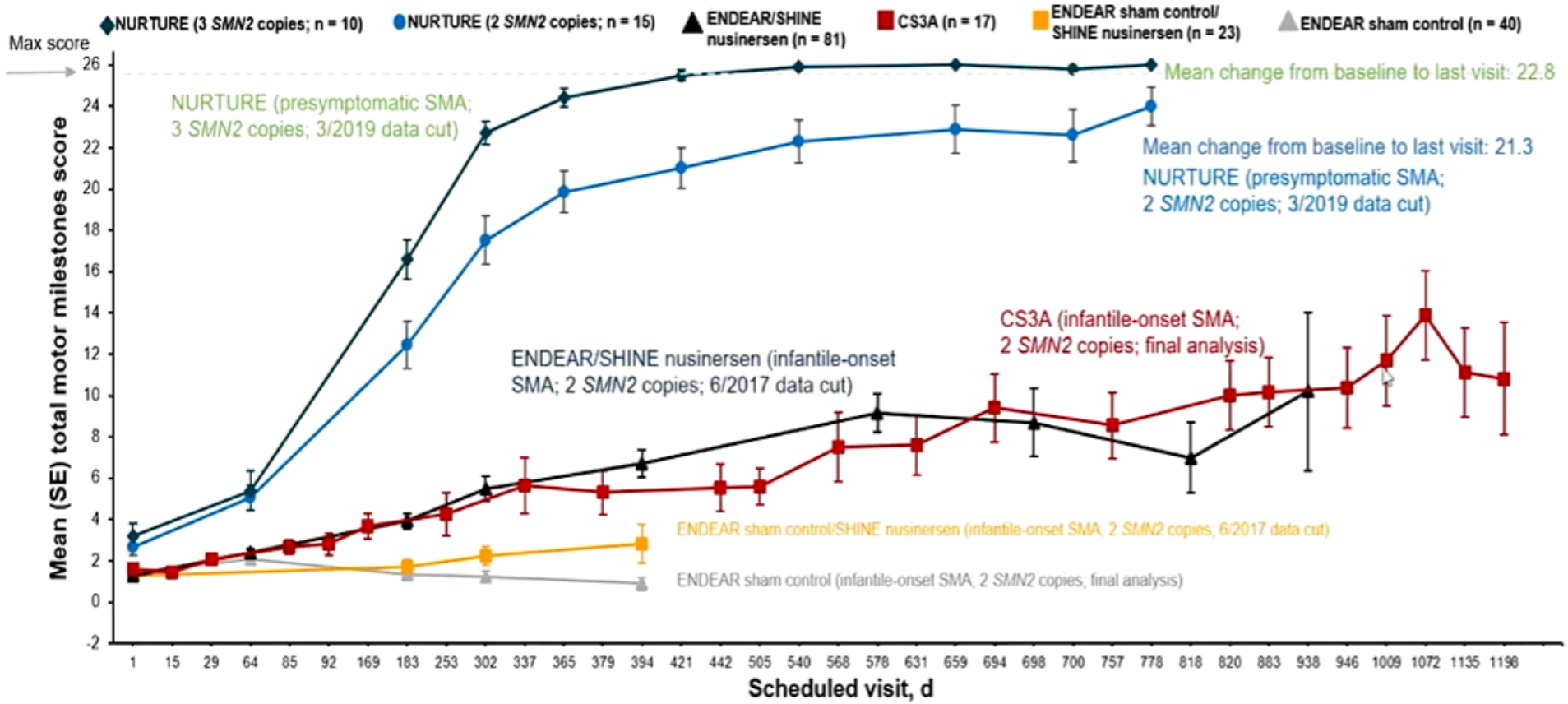
Recruitment Status : Recruiting
First Posted : April 23, 2021
Last Update Posted : November 8, 2021

See **Contacts and Locations**

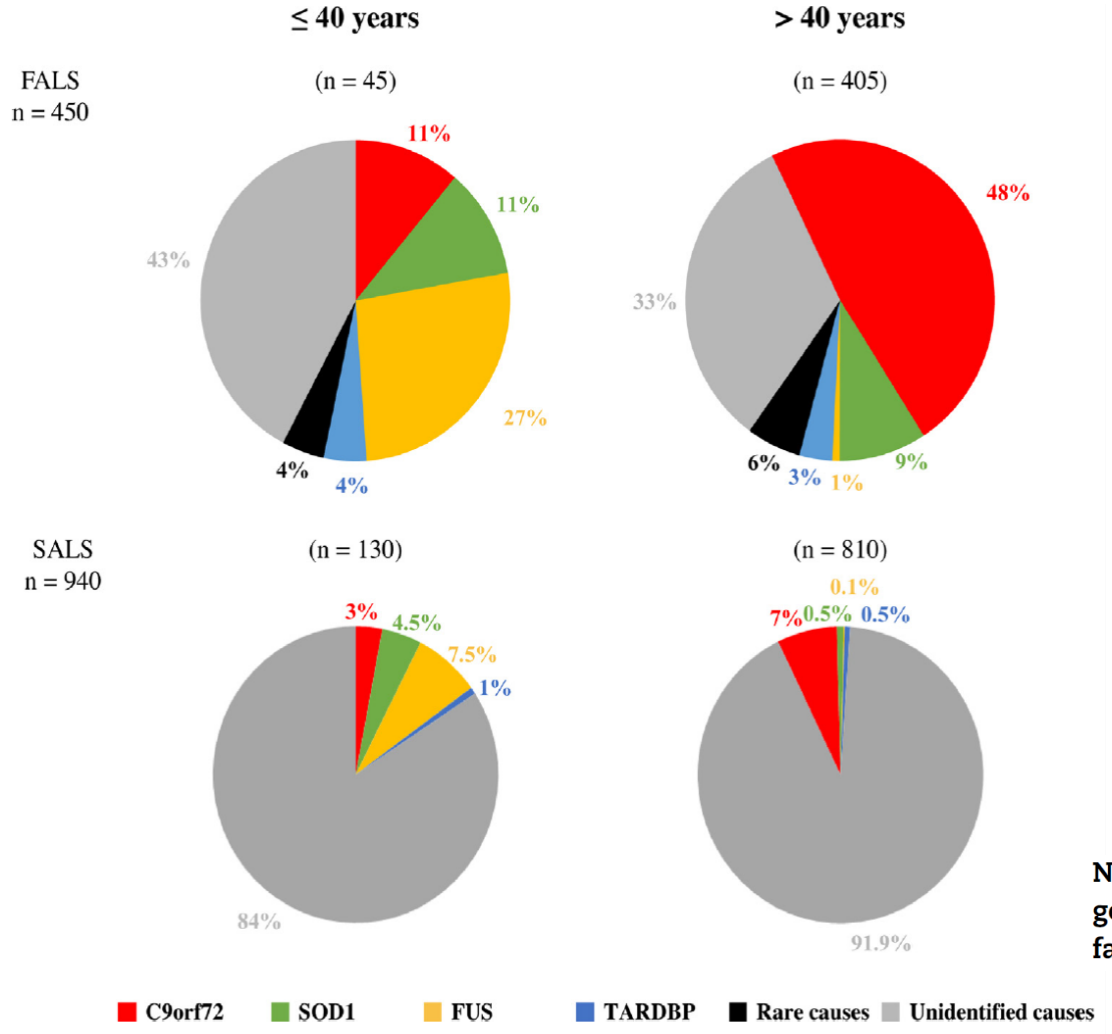
ATLAS STUDY

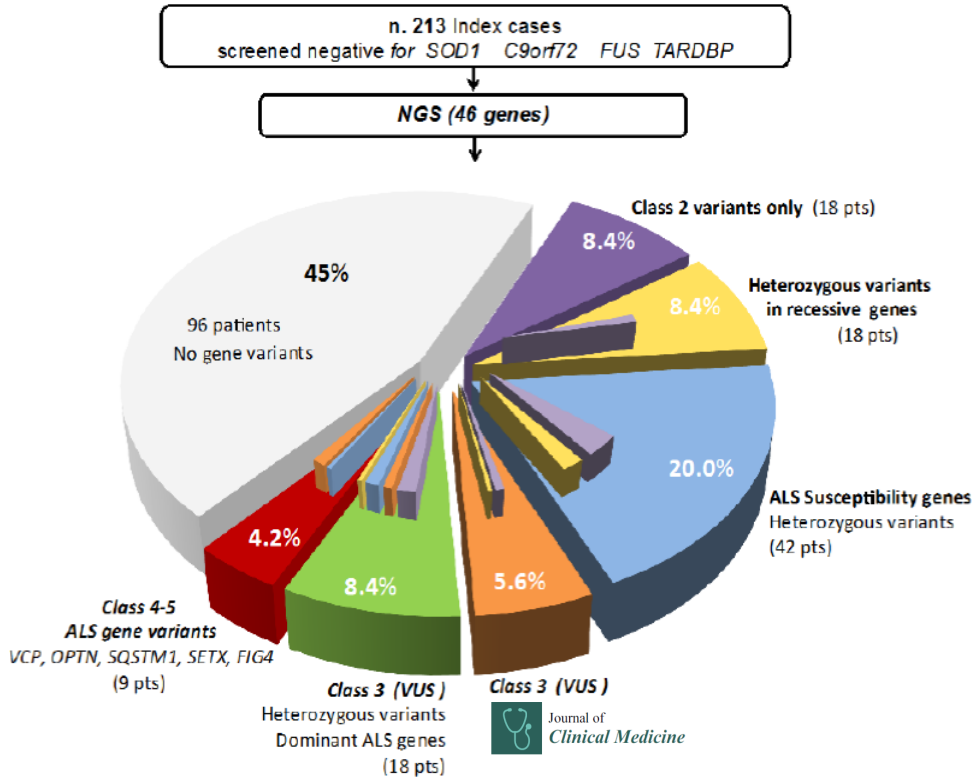
Learning from SMA

The greatest improvements in HINE-2 motor milestone scores in infants with presymptomatic SMA treated with nusinersen (NURTURE)



Il ruolo della genetica OGGI, al tempo dell'NGS





New advances in Amyotrophic Lateral Sclerosis genetics: Towards gene therapy opportunities for familial and young cases

REVUE NEUROLOGIQUE 177 (2021) 524–535

Journal of *Clinical Medicine*

MDPI

Article

Sorting Rare ALS Genetic Variants by Targeted Re-Sequencing Panel in Italian Patients: *OPTN*, *VCP*, and *SQSTM1* Variants Account for 3% of Rare Genetic Forms

J. Clin. Med. **2020**, *9*, 412; doi:10.3390/jcm9020412

Trattamento con ASOs: cosa è attivo