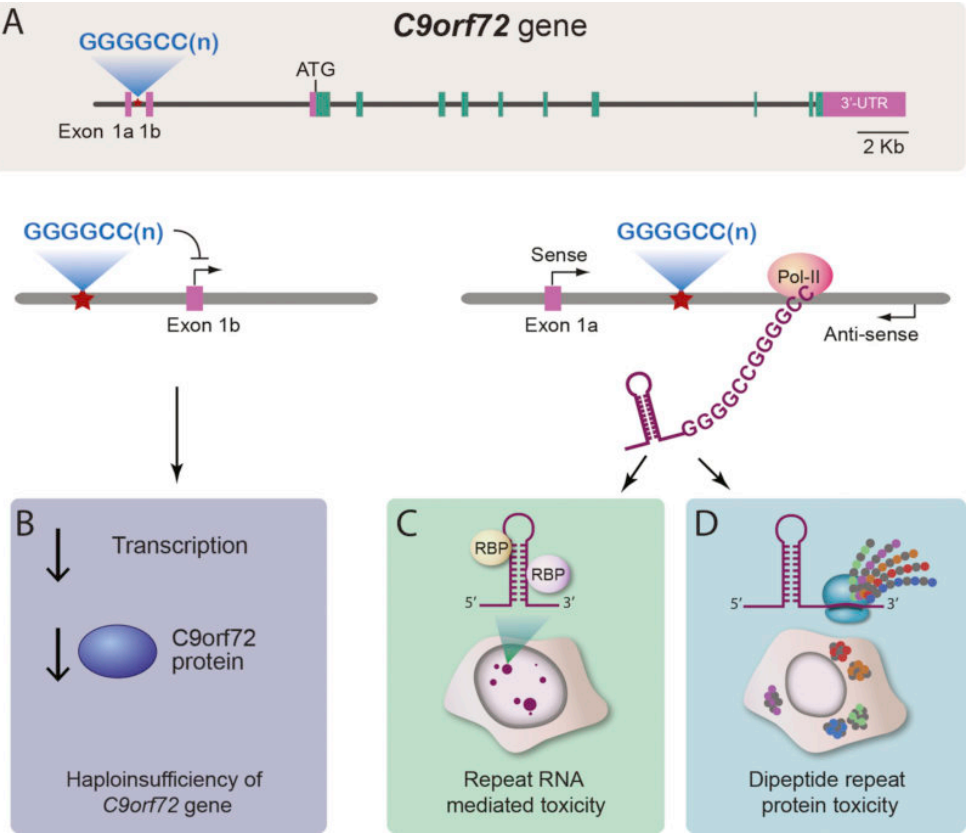
ASOs, C9ORF72 & biomarkers



Poly(GP) proteins are a useful pharmacodynamic marker for C9ORF72-associated amyotrophic lateral sclerosis

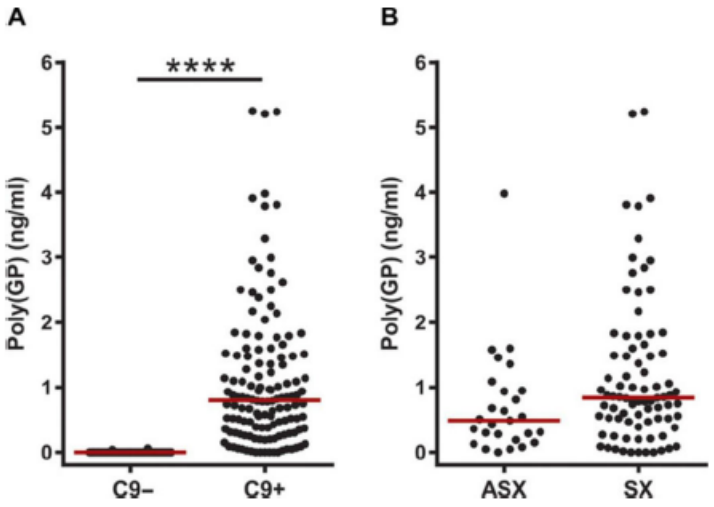


Fig. 1. Poly(GP) is detected in CSF from asymptomatic and symptomatic *C9ORF72* repeat expansion carriers
(**A**) Poly(GP) in CSF from *C9ORF72* repeat expansion carriers (C9+; $n = 134$) and noncarriers (C9−; $n = 120$). ****$P < 0.0001$, as assessed by van Elteren stratified Wilcoxon rank sum test. (**B**) CSF poly(GP) concentrations in asymptomatic *C9ORF72* mutation carriers (ASX; $n = 27$) and symptomatic c9ALS patients with or without comorbid FTD (SX; $n = 83$). No significant difference in poly(GP) between ASX and SX subjects was observed using a linear regression model adjusted for gender and age at CSF collection. Red lines denote the median.

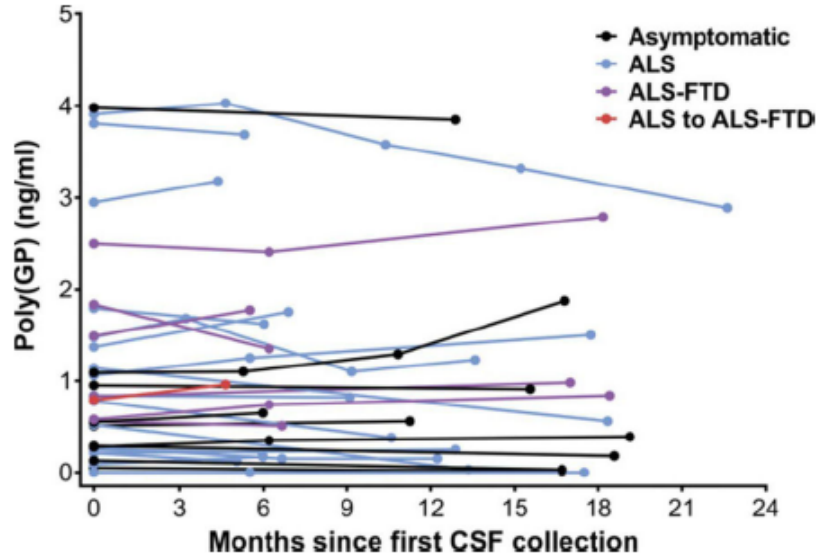


Fig. 2. Longitudinal trajectory of poly(GP) in CSF
Poly(GP) in CSF collected longitudinally from 33 *C9ORF72* repeat expansion carriers who either were asymptomatic or had c9ALS or c9ALS-FTD. Twenty-four subjects had two measurements, 6 had three measurements, 2 had four measurements, and 1 had five measurements. One patient (denoted by red circles) converted from a clinical diagnosis of ALS to ALS-FTD between the first and second CSF collection.

Sci Transl Med. 2017 March 29; 9(383)

ASOS, C9ORF72 & biomarkers

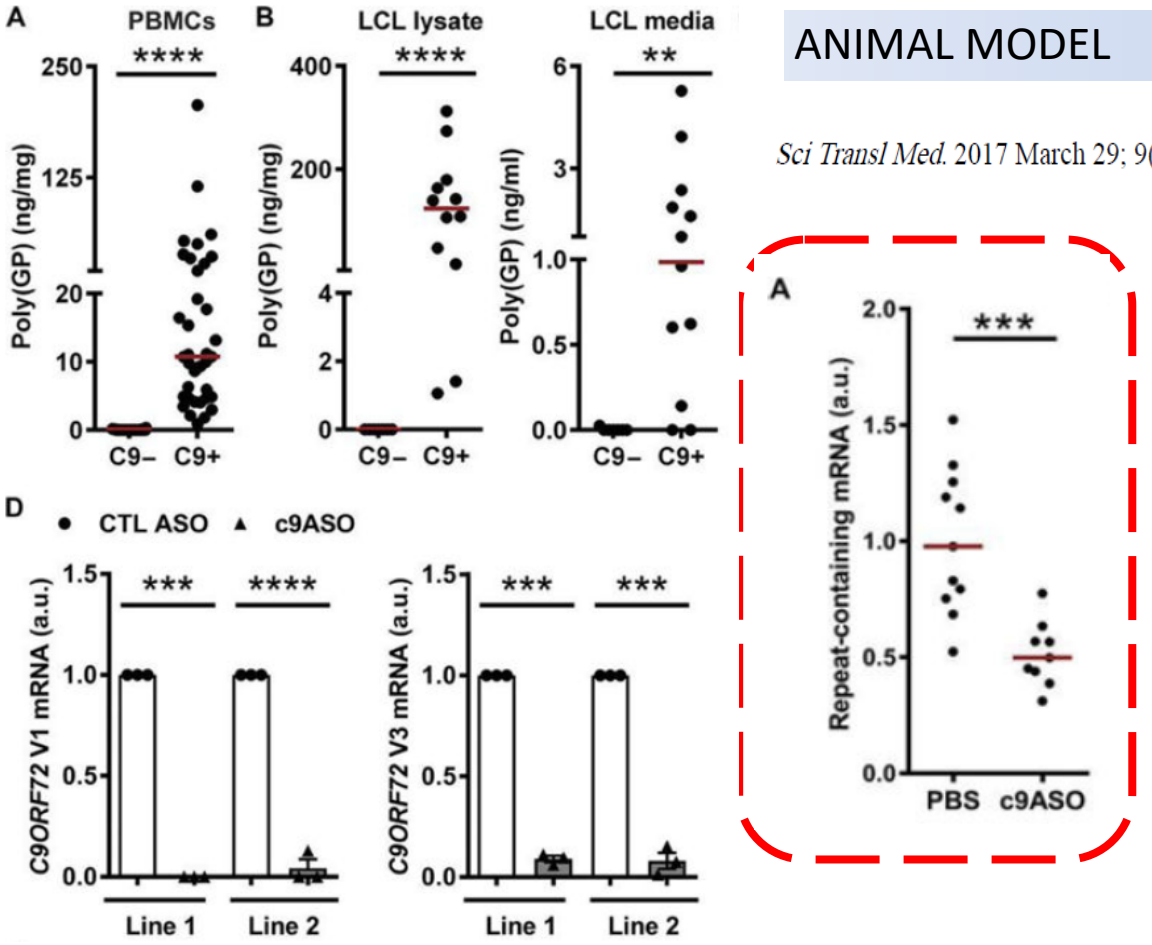


Fig. 3. Poly(GP) is detected in PBMCs from *C9ORF72* mutation carriers, and c9ASO-1 treatment decreases poly(GP) in lymphoblastoid cell lines

ANIMAL MODEL

Sci Transl Med. 2017 March 29; 9(383)

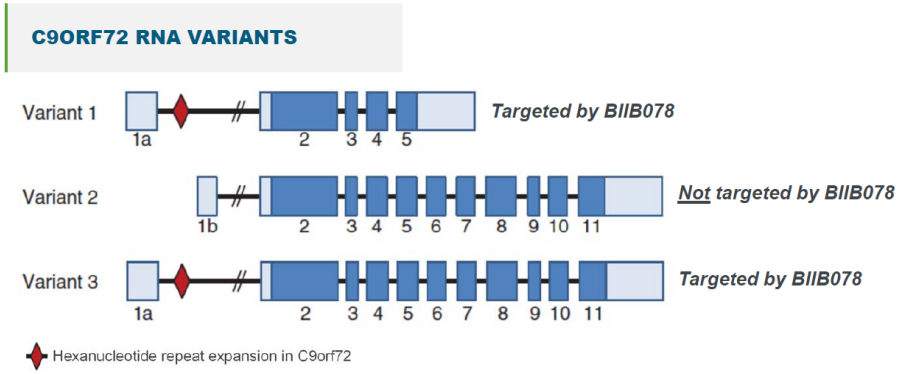
-CSF poly(GP) proteins remained relatively constant over time

-Treating c9ALS patient cells or a mouse model of c9ALS with ASOs that target G4C2 RNA resulted in decreased poly(GP) proteins.

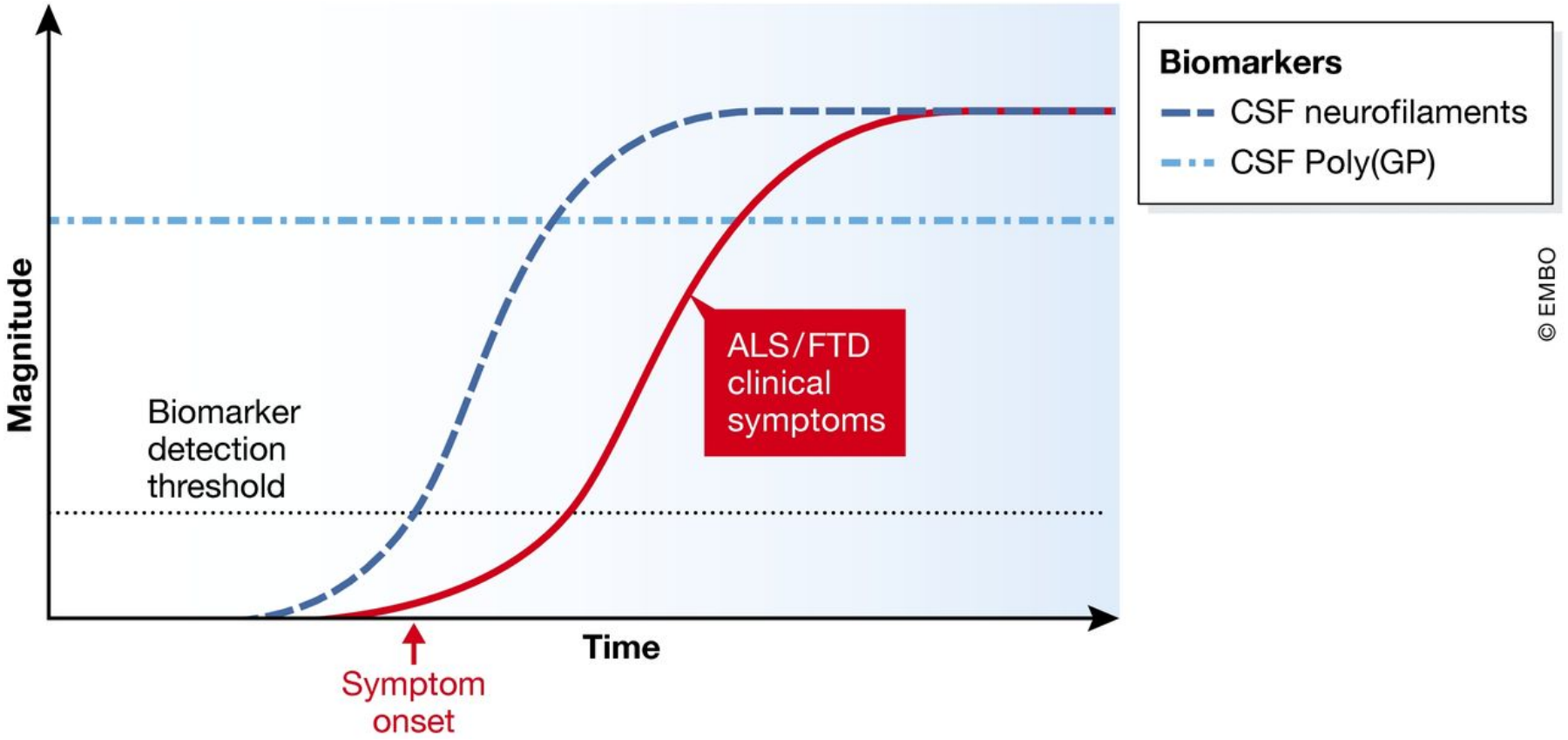
-Tracking poly(GP) proteins in CSF could provide a means to assess target engagement of G4C2 RNA–based therapies in symptomatic C9ORF72 carriers and presymptomatic individuals who are expected to benefit from early therapeutic intervention.

HUMAN

BIIB078 selectively targets expansion-containing C9orf72 transcripts to reduce risk of on-target toxicity



Biomarkers & C9ORF72



BIIB078 in C9ORF72- ALS

Advancing Phase 1 study of BIIB078 in C9orf72 ALS

POPULATION

- > 18 years old
- ALS patients with confirmed expansion in C9orf72
- Slow vital capacity ≥ 50% of predicted value
- Concomitant use of riluzole/edaravone allowed

MAD STUDY

- **Cohort 1:** BIIB078 1st dose
- **Cohort 2:** BIIB078 2nd dose
- **Cohort 3:** BIIB078 3rd dose
- **Cohort 4:** BIIB078 4th dose
- **Cohort 5:** BIIB078 5th dose
- **Cohort 6:** BIIB078 6th dose
- **Placebo**

ENDPOINTS

Primary
Safety and tolerability

Secondary
PK measures of BIIB078

Exploratory endpoints include
ALSFRS-R scores, SVC, HHD megascore, CSF C9orf72-RAN dipeptide protein, CSF pNFH

80 participants total

First patient dosed September 2018

Intrathecal injection (BIIB078 or placebo): 3 loading doses followed by 2 maintenance doses

Patients followed for approximately 8 months

Data expected in 2021

ASOS & future research

doi:10.1093/brain/awz328 BRAIN 2019: 0; 1–23 | 1

BRAIN
A JOURNAL OF NEUROLOGY

REVIEW

Antisense oligonucleotide therapeutics in neurodegenerative diseases: the case of polyglutamine disorders

Ana C. Silva,[1] Diana D. Lobo,[1] Inês M. Martins,[1,2] Sara M. Lopes,[1,2] Carina Henriques,[1,3] Sónia P. Duarte,[1,2] Jean-Cosme Dodart,[4] Rui Jorge Nobre,[1,2,3,*] and Luis Pereira de Almeida[1,3,5,*]

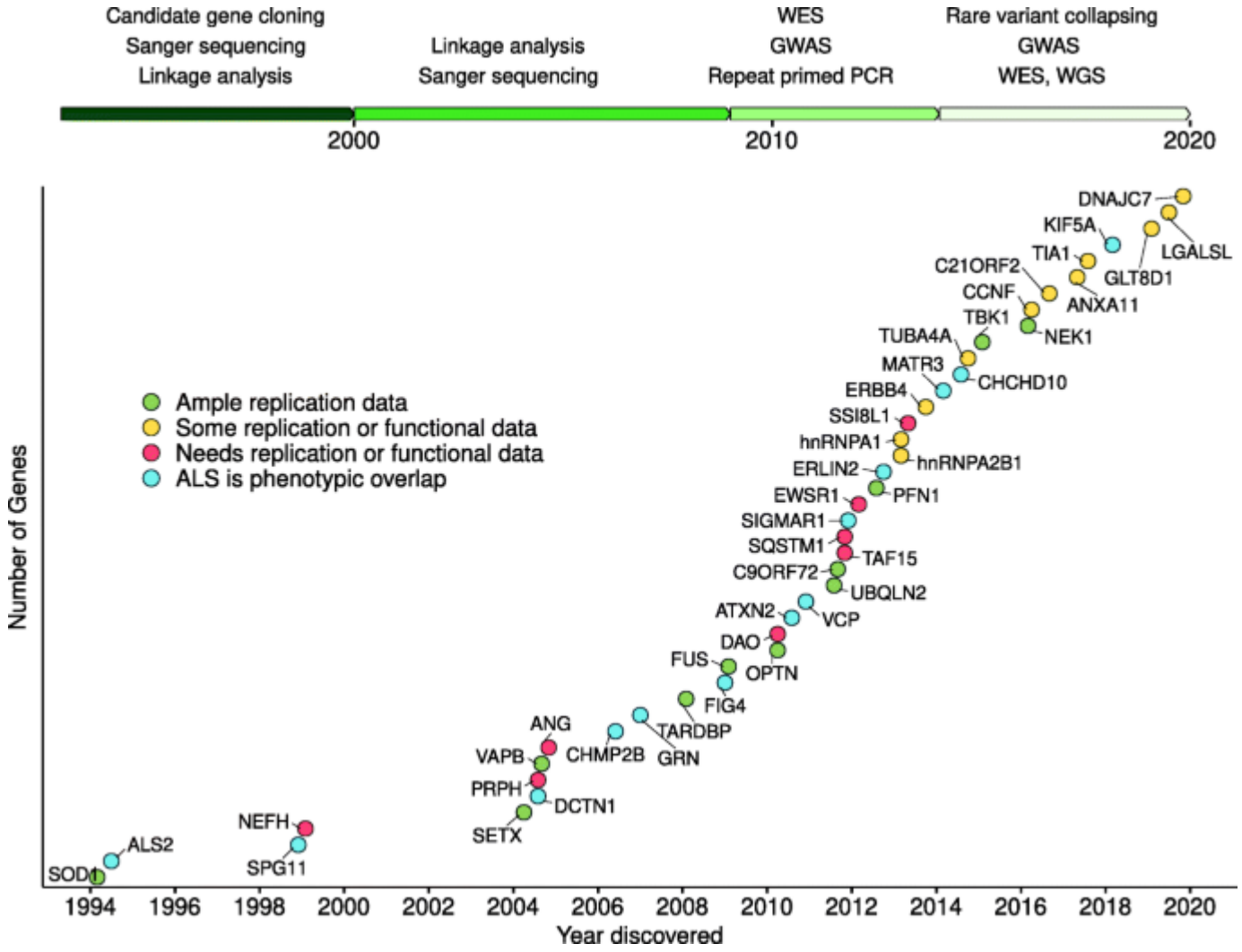
Systemic delivery

There is growing interest in systemic delivery of ASOs to the CNS as this route of administration is significantly less invasive, and therefore a preferable method for clinical applications. Notably, some studies have shown that ASOs can be delivered to the brain in effective doses via the systemic route (Banks *et al.*, 2001; Farr *et al.*, 2014). However, the intravenous dose must be ~100 times higher than the ICV dose (Banks *et al.*, 2001), which greatly increases the risk of toxicity. Therefore, presently this approach does not appear to be an appropriate delivery method of ASOs to the CNS.

Search for alternative delivery systems!

- Exosomes
- Cell penetrating peptides
- Liposomes

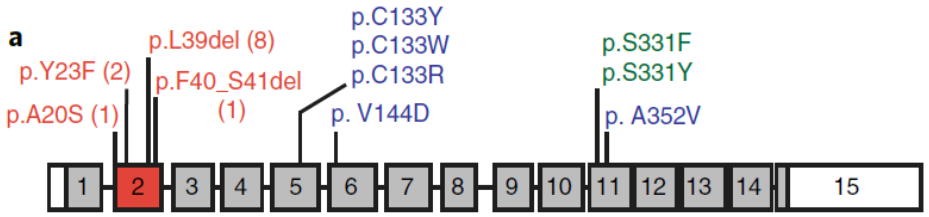
SLA, genetica & medicina di precisione: rilevanza

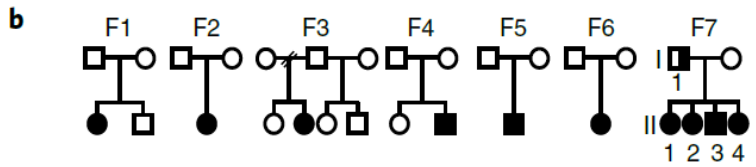


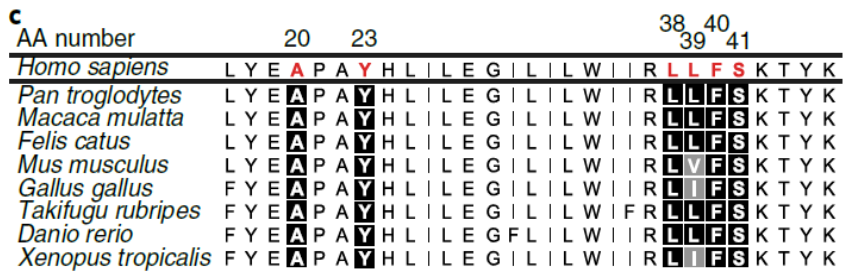
SPTCL1

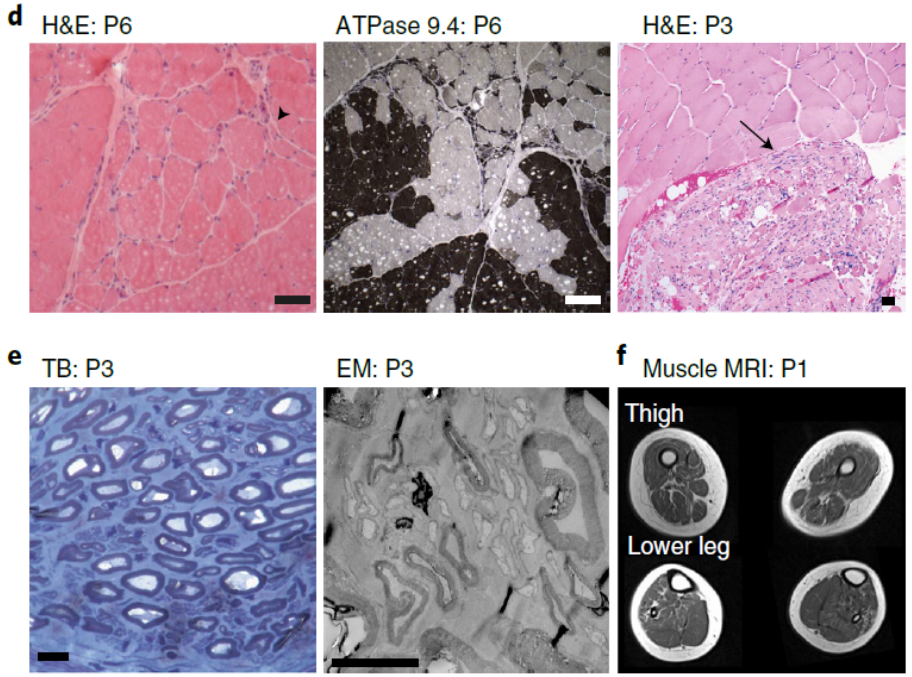
nature medicine

Childhood amyotrophic lateral sclerosis caused by excess sphingolipid synthesis









Early-childhood-onset lower extremity spasticity manifesting as toe walking and gait abnormalities followed by progressive LMN-mediated weakness without sensory symptoms or signs.

The disease was universally progressive and led to loss of independent ambulation and respiratory insufficiency of variable degrees. All 6 individuals UMN and LMN signs and symptoms in the cranial, cervical and lumbar myotomes and thus met the revised EEC-R

SPTCL1

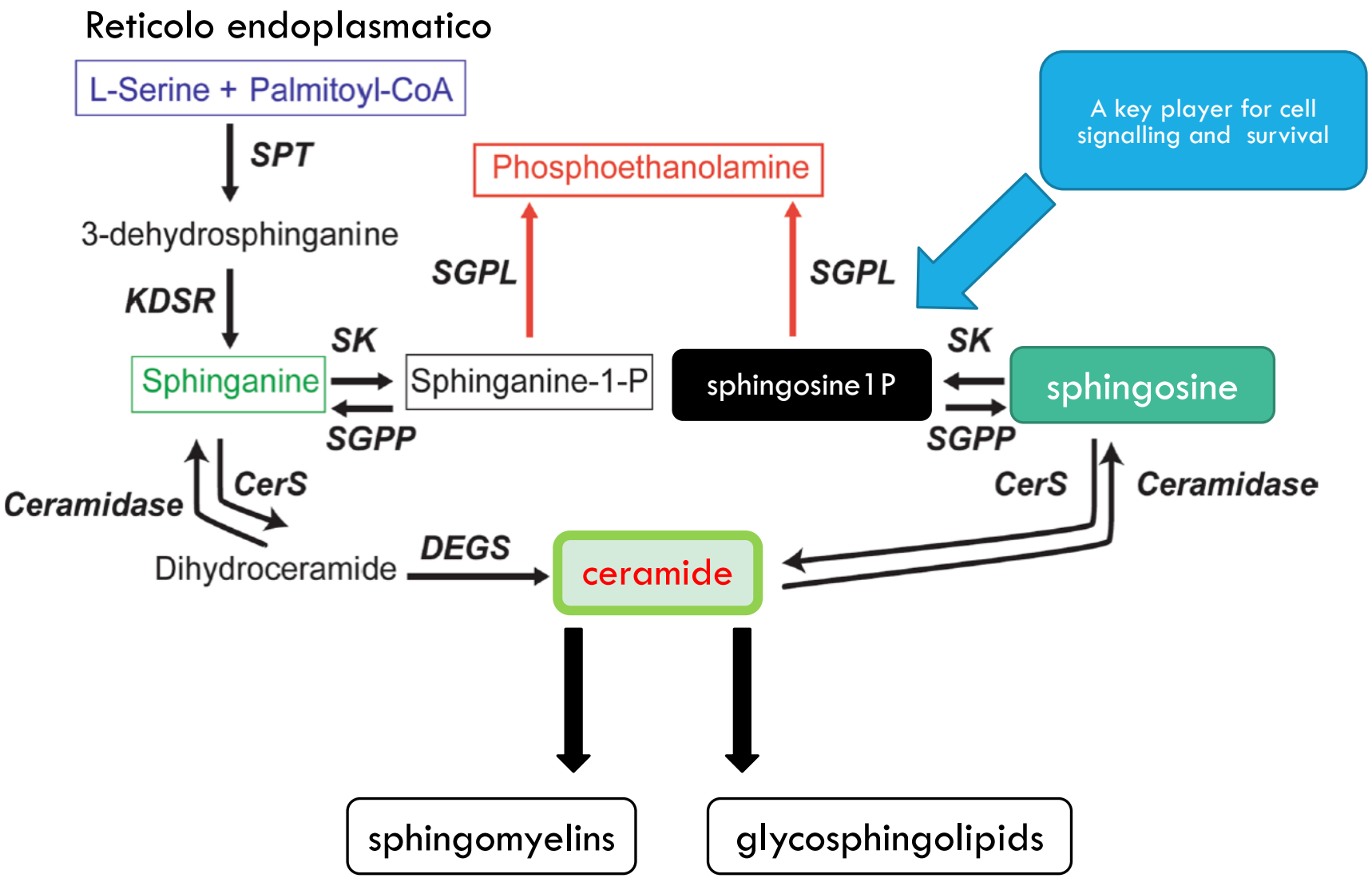
Serine palmitoyltransferase catalyzes the initial step in sphingolipid biosynthesis by condensing l-serine and palmitoyl-CoA to form long-chain bases.

Alterations in SPT activity have been linked to:

- complete loss of SPT function: death
- variants in genes that encode SPTLC1 and SPTLC2, that alter SPT amino acid substrate usage underlie hereditary sensory and autonomic neuropathy type 1 (HSAN1) and macular telangiectasia type 2.

These variants increase SPT usage of l-alanine or glycine rather than l-serine giving deoxysphingolipid synthesis.

Deoxysphingolipids cannot be efficiently degraded by the cell machinery and cause toxicity.



No human disease has been linked to SPT overactivity

SPTLC1

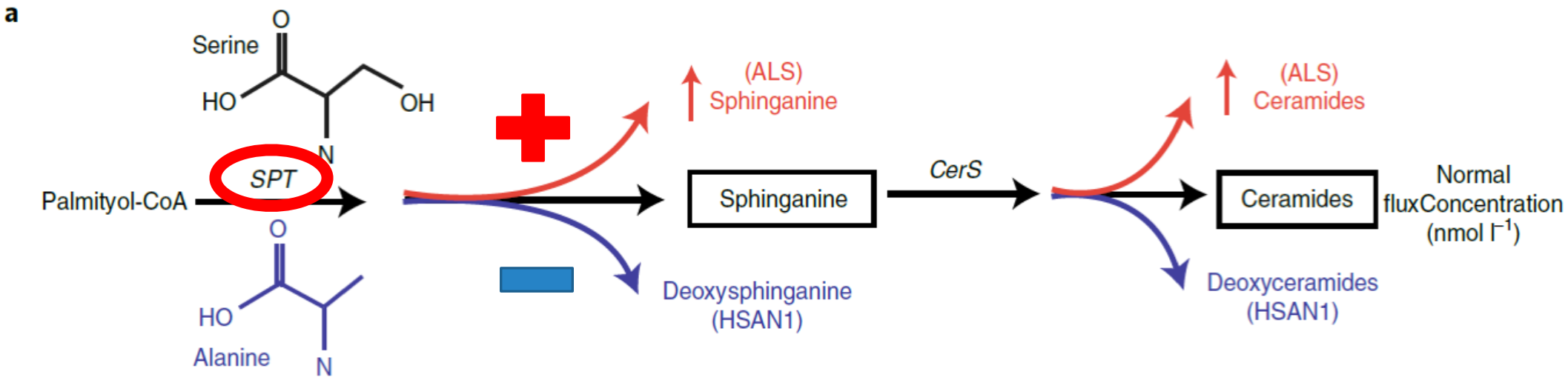
nature medicine

ARTICLES

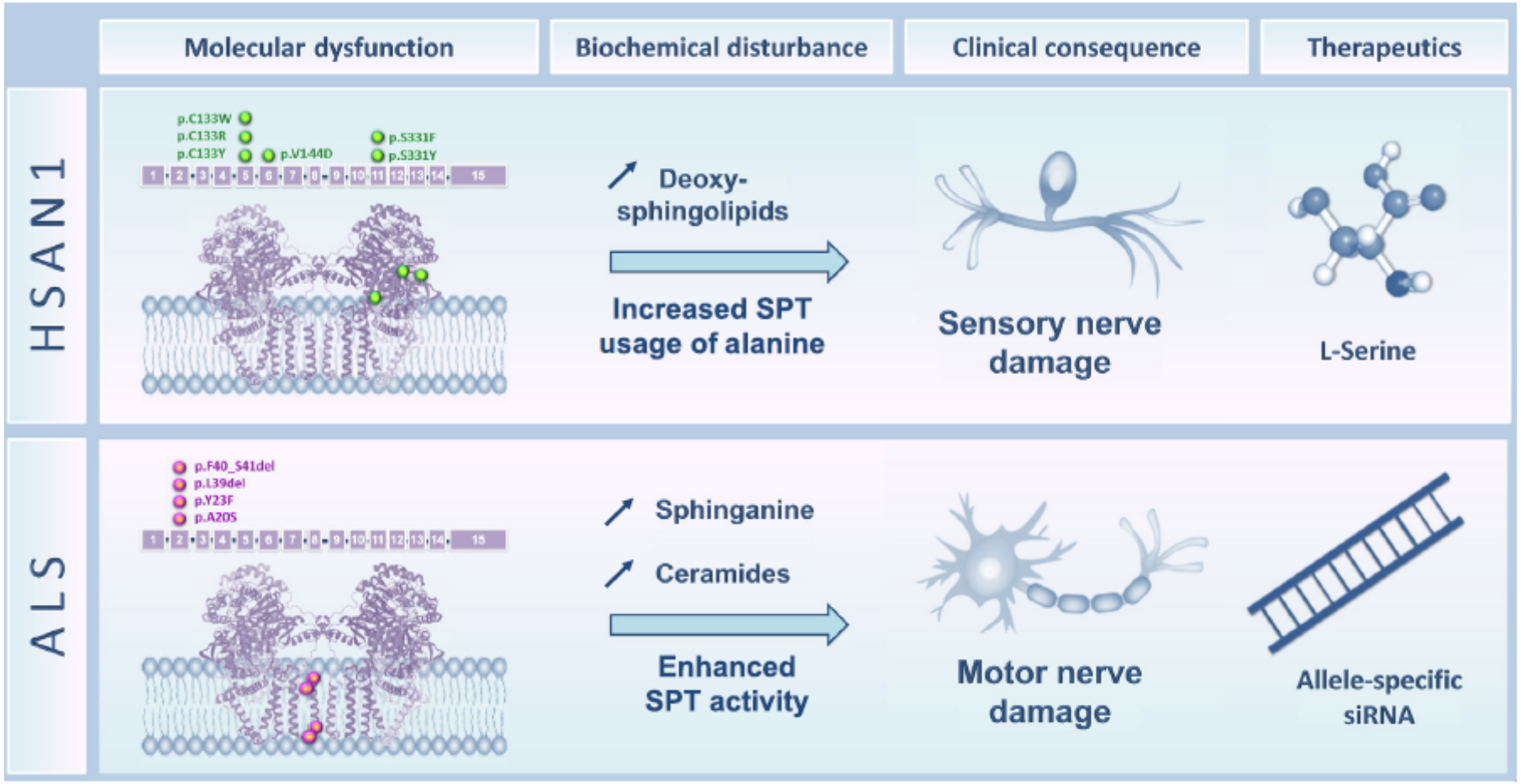
https://doi.org/10.1038/s41591-021-01346-1

Check for updates

Childhood amyotrophic lateral sclerosis caused by excess sphingolipid synthesis

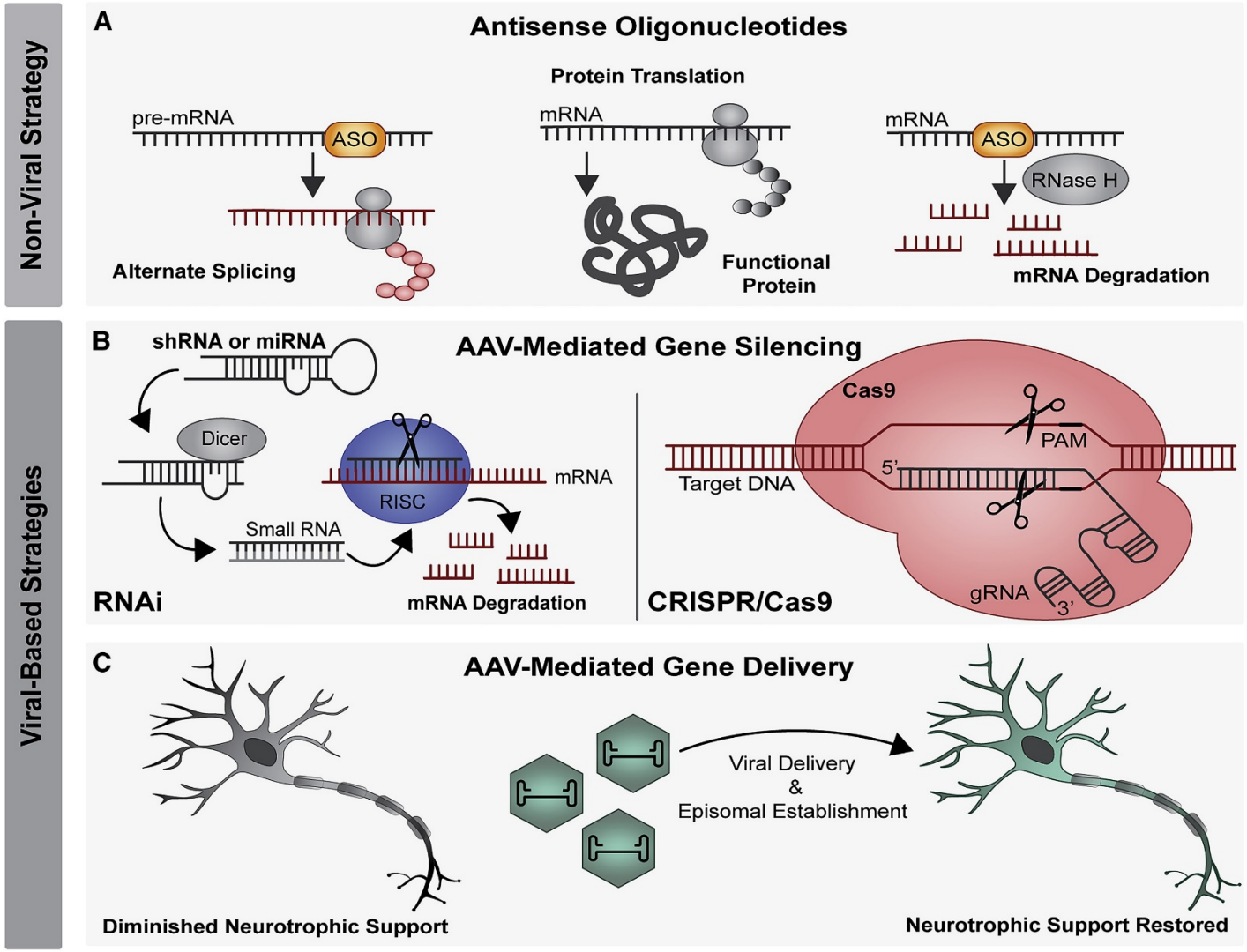


SPTLC1



Overall, this new report provides a proof of concept for a precision medicine approach targeting a newly identified genetic mechanism by siRNA therapy. This echoes current therapeutic developments in ALS based on antisense oligonucleotides (ASOs). After almost 30 years of failure in clinical trials, new hope comes from gene therapy in ALS. Preliminary results of the beneficial effects of ASO (tofersen) therapy in patients with ALS linked to SOD1 mutations appear to be convincing. Because a positive effect was reported, numerous trials are currently being initiated in this field which will change our management of the disease from standardized protocols to personalized therapy based on molecular genetics.

DALLA GENETICA AL TRATTAMENTO: NON SOLO ASOS



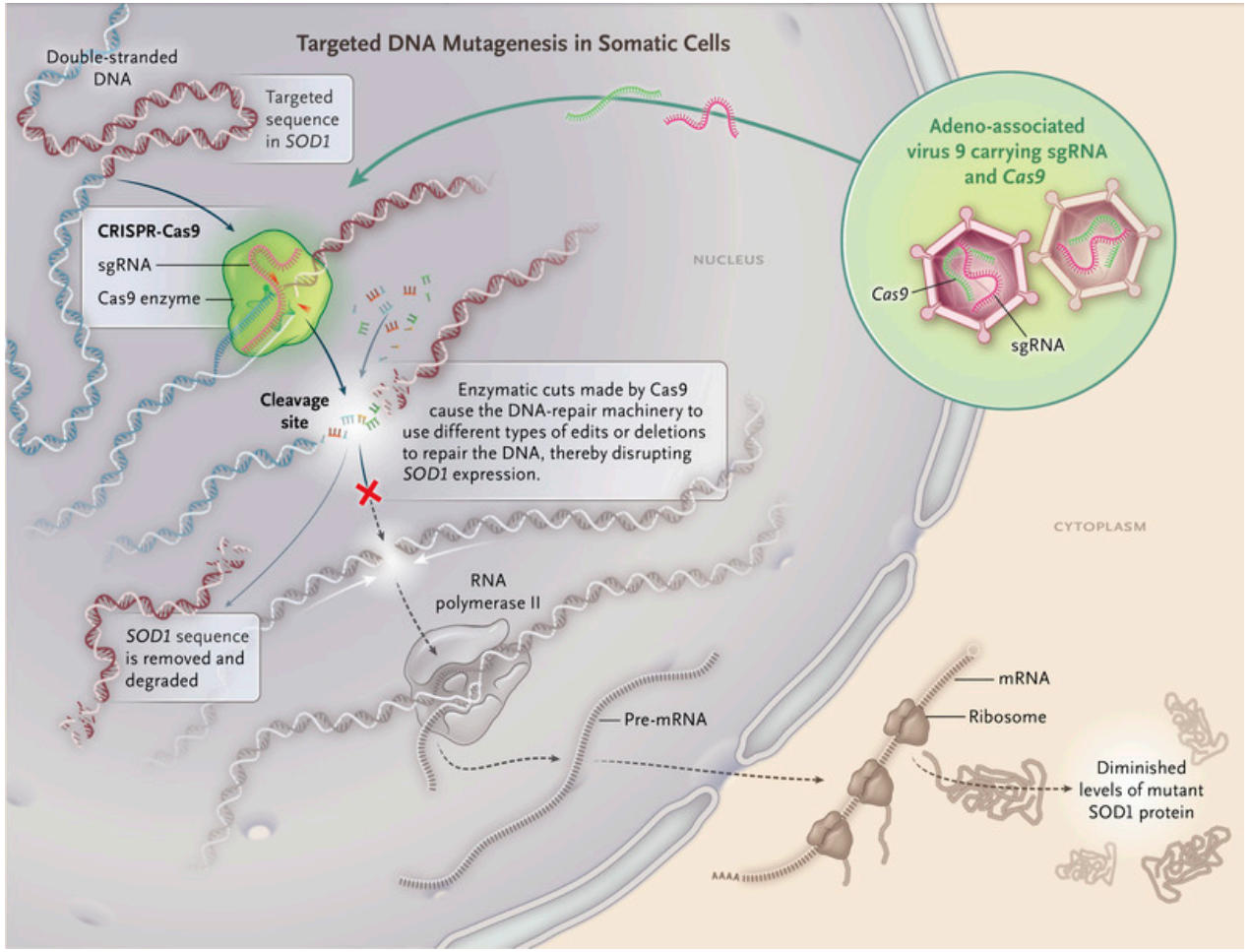
ALS & CRISPR-CAS9

The Greatest Scientific Breakthroughs Of 2020

"Scientists Use A Technique Called CRISPR To Edit A Gene Inside A Patient's Body"

Finding a Treatment for ALS — Will Gene Editing Cut It?

The NEW ENGLAND JOURNAL of MEDICINE

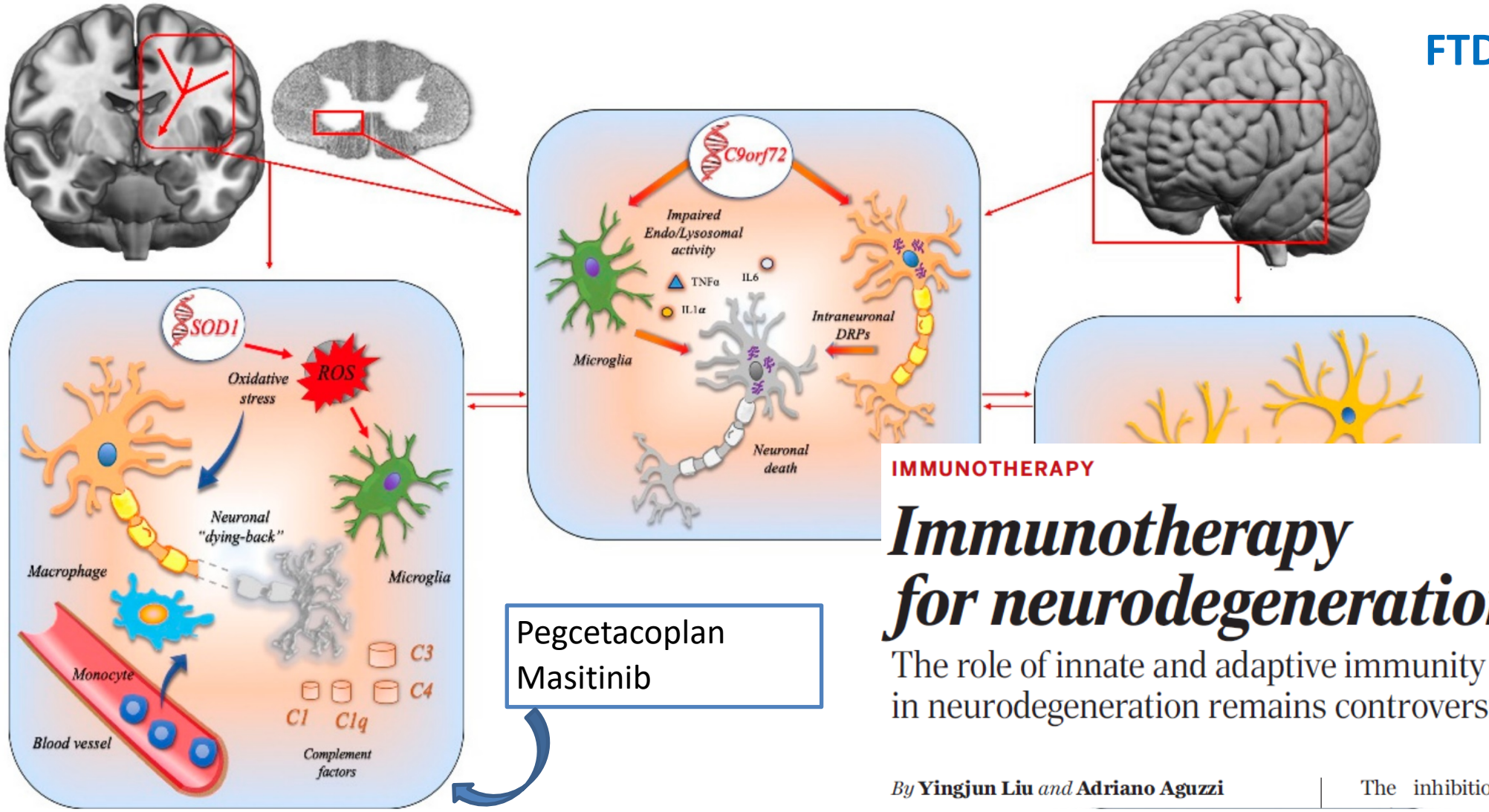


CRISPR-Cas9 mutates DNA by cutting through ds DNA at a specific gene sequence, to which it is directed by a single guide RNA. After the cut is made, the DNA-repair machinery of the cell automatically fix it with different types of edits. Gaj et al. designed a CRISPR-Cas9 system to disrupt expression of human *SOD1* in a transgenic mouse model of ALS; they packaged the CRISPR-Cas9 system into a virus vector and injected it into the facial veins of affected mice, after which levels of mutant protein declined in the lumbar and thoracic spines and the onset of disease was delayed.

Modulare la progressione: il ruolo dell'autoimmunità

ALS

FTD



Pegcetacoplan
Masitinib

IMMUNOTHERAPY

Immunotherapy for neurodegeneration?

The role of innate and adaptive immunity in neurodegeneration remains controversial

By **Yingjun Liu** *and* **Adriano Aguzzi** | The inhibition of i

Immunità e SLA

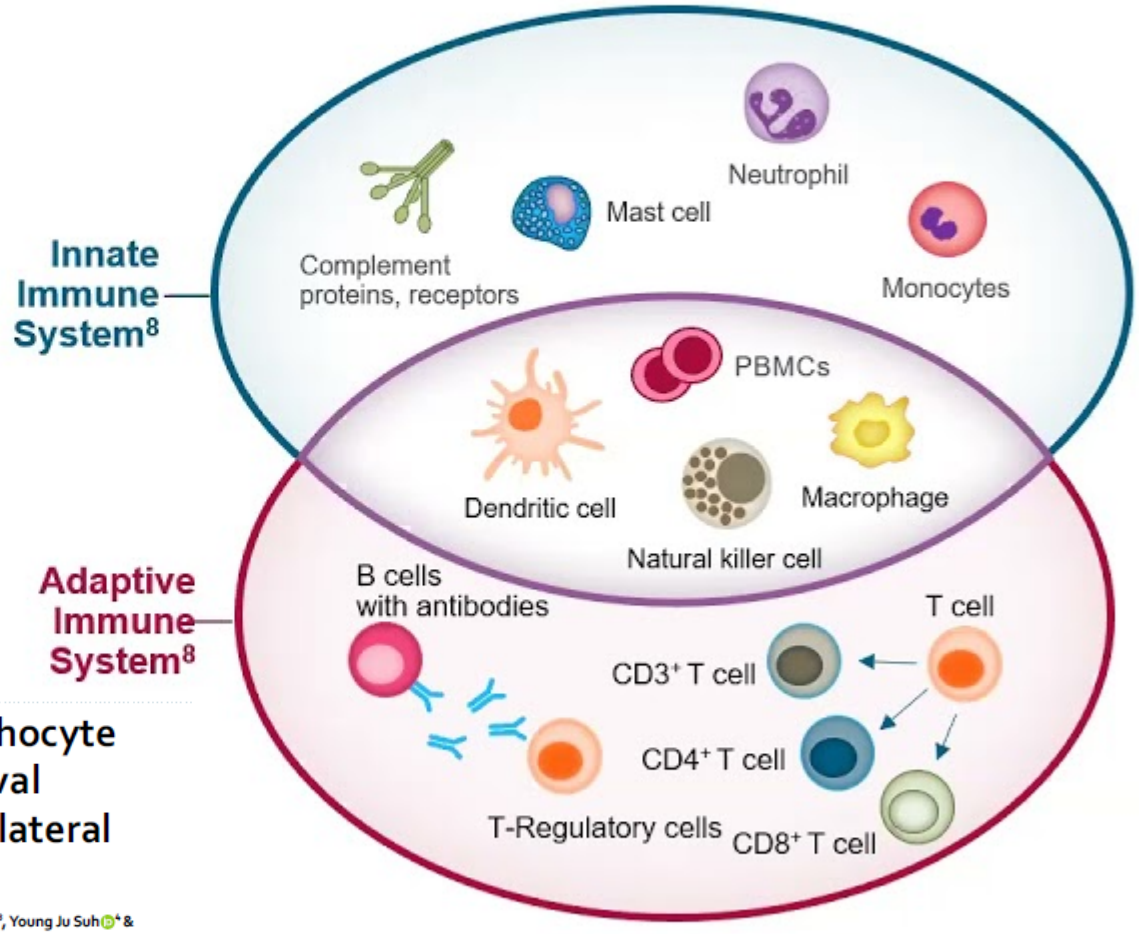
nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-020-15644-8 OPEN

Natural killer cells modulate motor neuron-immune cell cross talk in models of Amyotrophic Lateral Sclerosis

Stefano Garofalo[1✉], Germana Cocozza[2], Alessandra Porzia[3], Maurizio Inghilleri[4], Marcello Raspa[5], Ferdinando Scavizzi[5], Eleonora Aronica[6], Giovanni Bernardini[7], Ling Peng[8], Richard M. Ransohoff[9], Angela Santoni[2,7] & Cristina Limatola[2,10✉]



SCIENTIFIC REPORTS
natureresearch

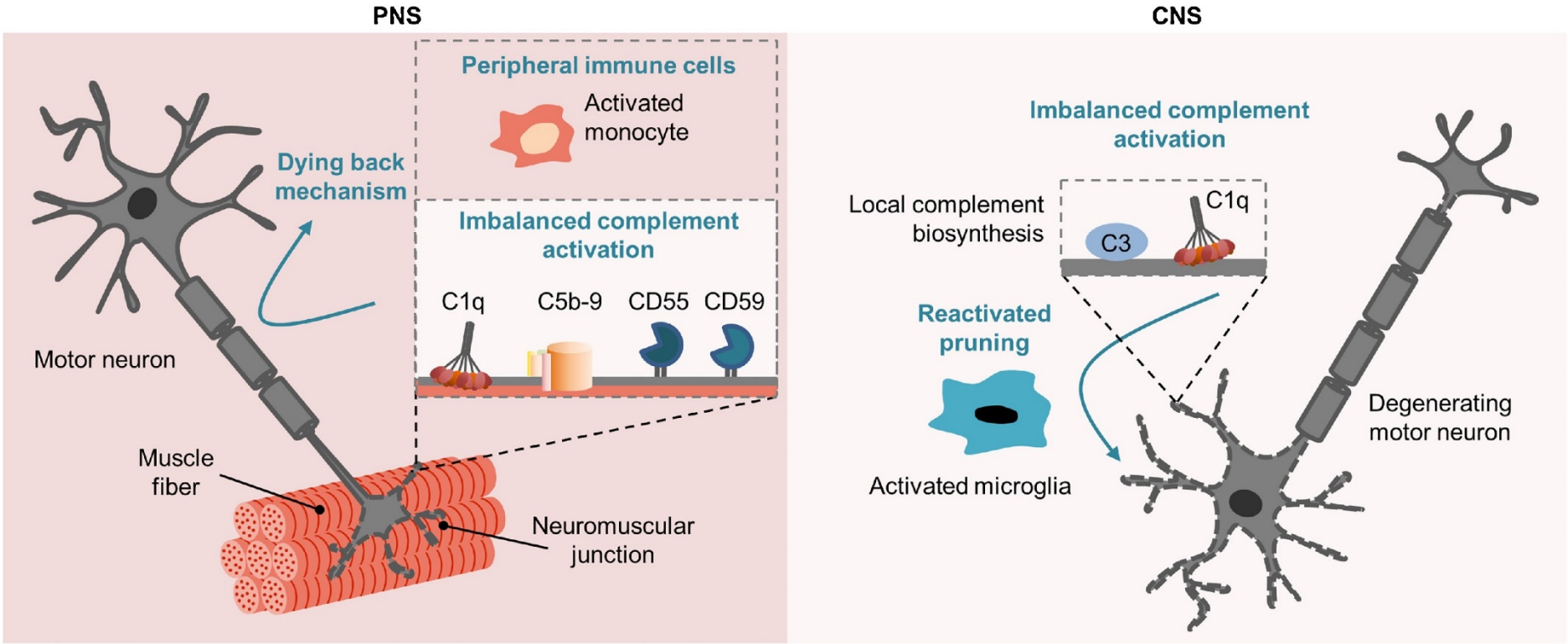
Peripheral proinflammatory Th1/ Th17 immune cell shift is linked to disease severity in amyotrophic lateral sclerosis

Mengmeng Jin[1,2,6], Rene Günther[1,3,6*], Katja Akgün[1,2], Andreas Hermann[1,3,4,5,7] & Tjalf Ziemssen[1,2,7]

High neutrophil-to-lymphocyte ratio predicts short survival duration in amyotrophic lateral sclerosis

Seok-Jin Choi[1], Yoon-Ho Hong[2], Sung-Min Kim[3], Je-Young Shin[3], Young Ju Suh[4] & Jung-Joon Sung[3*]

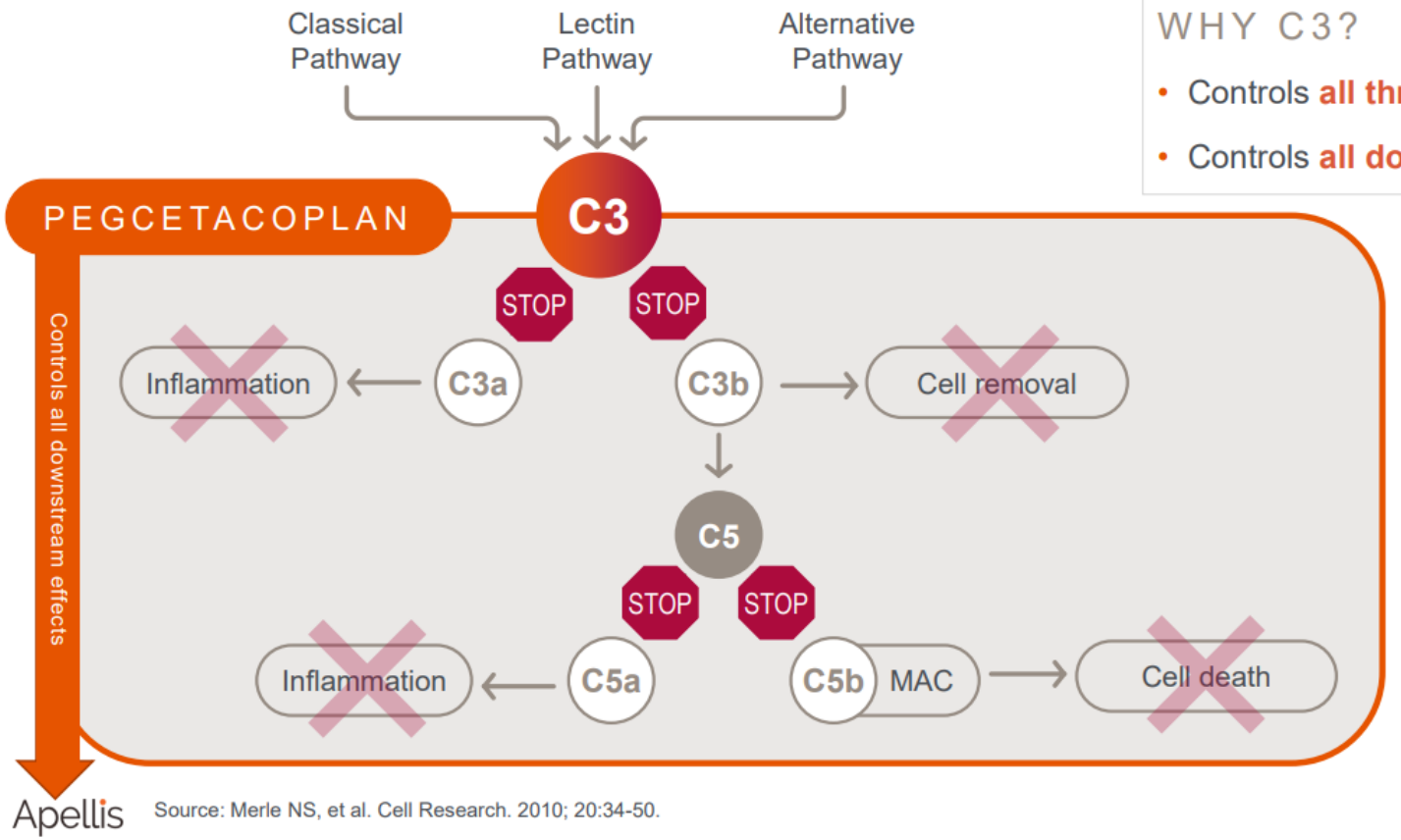
Modulare la progressione della SLA: ruolo del complemento



The dying-back mechanism and the microglial-motor neuronal crosstalk. According to the dying-back mechanism, the degeneration of the motor neuron starts in peripheral nervous system (PNS) in the neuromuscular junctions in the skeletal muscles and then progress to the central nervous system (CNS). Complement proteins of the classical pathway and the terminal pathway have been located in the NMJ as the first sign of pathology which implicates the peripheral innate immune system in the pathophysiology of ALS. In the CNS a crosstalk between microglia and motor neurons mediated by imbalanced complement proteins indicates that the microglia and local complement components contributes to the detrimental spread of destruction during progression of ALS.

Modulare la progressione della SLA: ruolo del complemento

Targeting C3 for Comprehensive Control of Complement



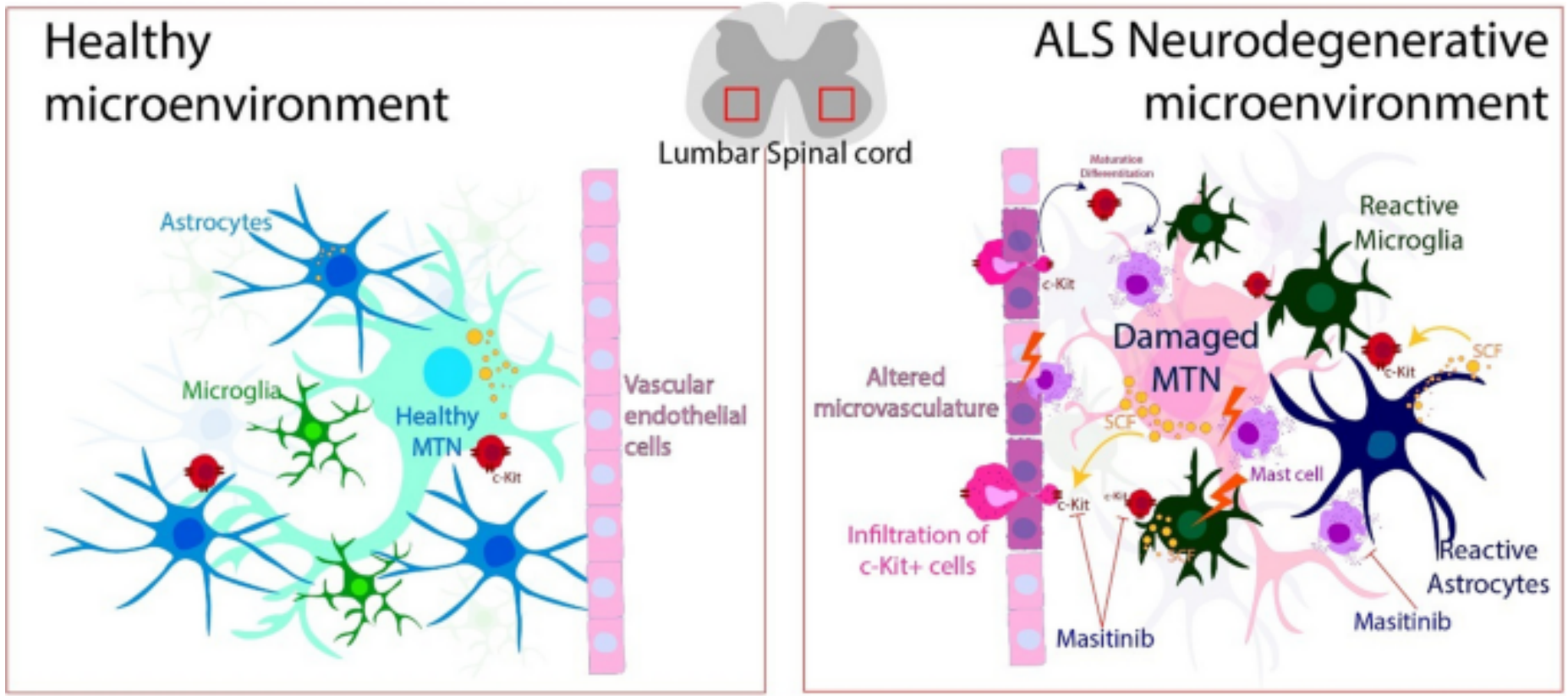
WHY C3?

- Controls **all three** pathways
- Controls **all downstream effects**

Apellis

Source: Merle NS, et al. Cell Research. 2010; 20:34-50.

Modulare la progressione della SLA: ruolo dei mastociti



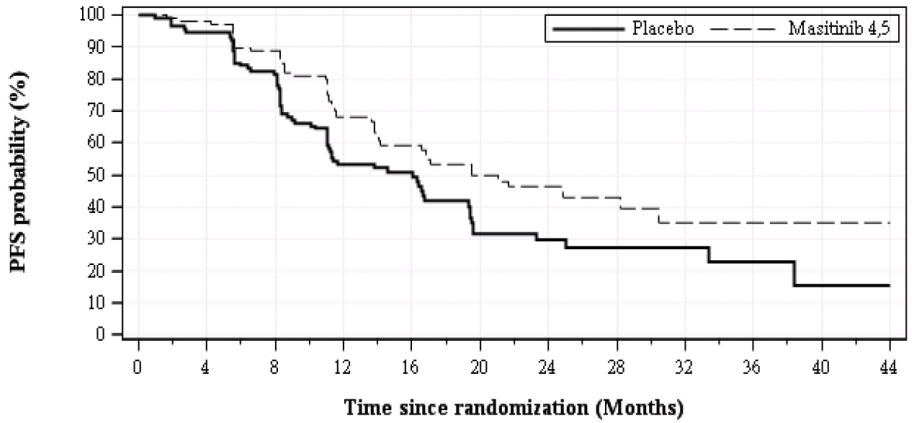
Acta Neuropathologica Communications

RESEARCH Open Access

The pathogenic role of c-Kit+ mast cells in the spinal motor neuron-vascular niche in ALS

Ruolo dei mastociti: Masitinib

Median PFS in NP w/Masitinib 4.5 was 20 months (95% CI [14; 30])
vs 16 months in NP w/Placebo (95% CI [11; 19]), Wilcoxon p = 0.0159



For NP on 4.5 mg/kg/d PFS was 25% longer and statistically significant (p=0.0159)

Change in FVC score
Normal Progressors, Masitinib 4.5 mg/kg/day

TREATMENT GROUP	N	LS Mean	Difference of means [(1-alpha) CI]	p-value
Rule 1				
Placebo + riluzole	102	-33.9	7.5383 [0.7552;14.3214]	0.0296
Masitinib + riluzole	98	-26.45		

Change in ALSAQ-40 score
Normal Progressors, Masitinib 4.5 mg/kg/day

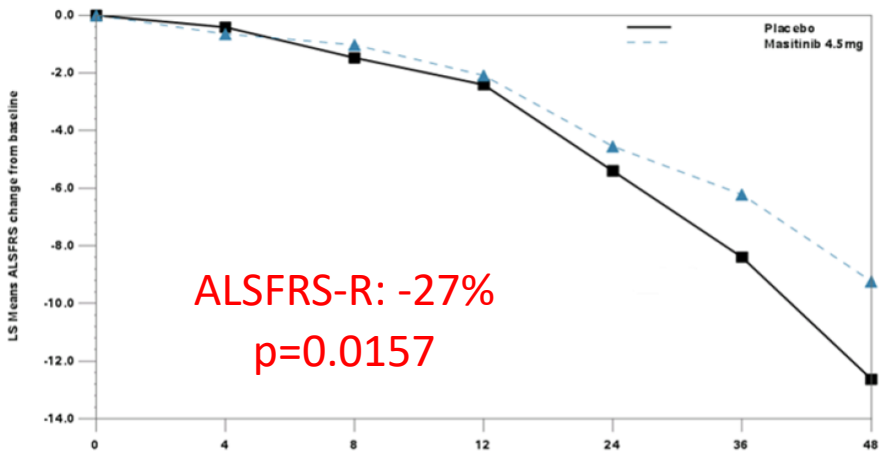
TREATMENT GROUP	N	LS Mean	Difference of means [(1-alpha) CI]	p-value
Rule 1				
Placebo + riluzole	102	27.18	-7.7587 [-13.4543;-2.0631]	0.0078
Masitinib + riluzole	99	19.42		

In a post hoc analysis, a benefit on PFS was observed in the Normal + Fast progressors patients with up to 24 months of disease at entry.

Ruolo dei mastociti: Masitinib

Progressione	placebo	Masitinib 4.5	Masitinib 3
Normale			
N	**114**	**106**	**110**
A 48 sett	75 (66%)	69 (65%)	71 (64%)
Normale + rapida			
N	**133**	**130**	**131**
A 48 sett	81 (61%)	76 (59%)	80 (61%)

ALSFRS-R slope of deterioration
Normal Progressors, Masitinib 4.5 mg/kg/day



Mean change W0-W48:

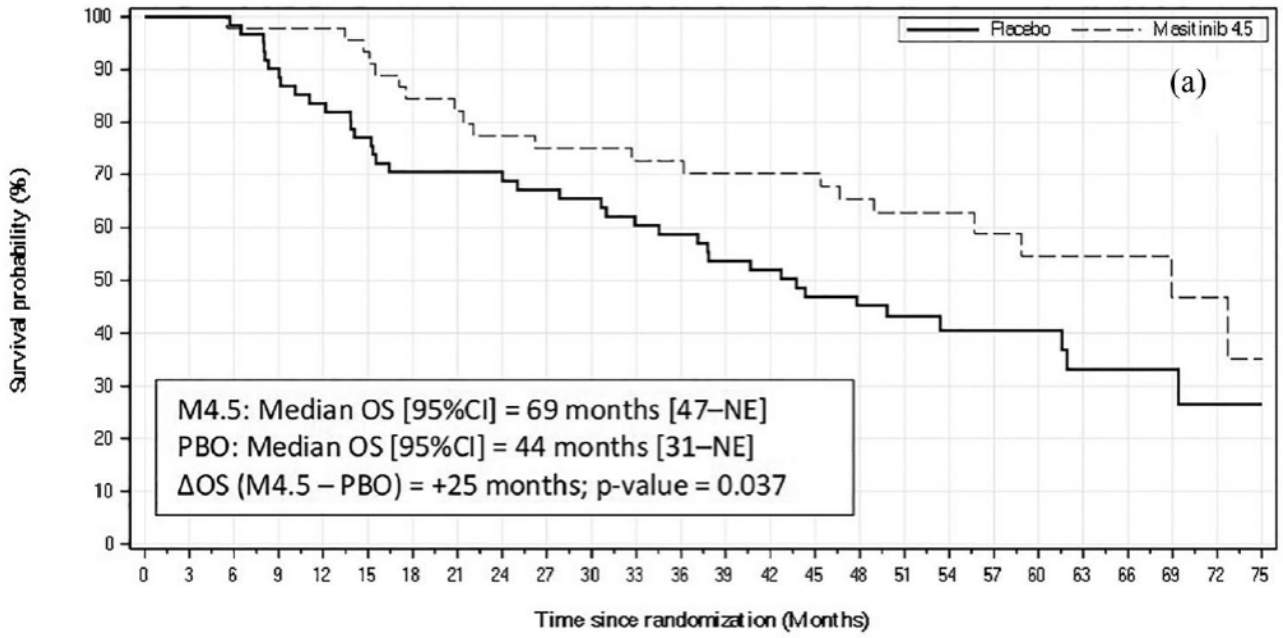
- **-12.6 in placebo arm => slope: -1.05 per month**
- **-9.2 in masitinib 4.5 arm => slope: -0.77 per month**

Ruolo dei mastociti: Masitinib

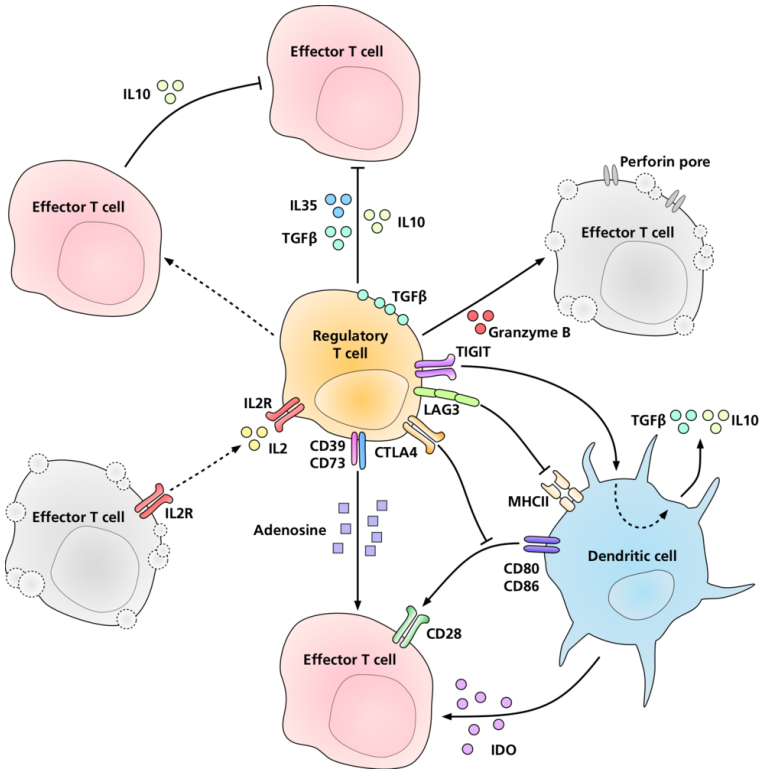
Therapeutic Advances in Neurological Disorders

Long-term survival analysis of masitinib in amyotrophic lateral sclerosis

Jesus S. Mora, Walter G. Bradley, Delia Chaverri, María Hernández-Barral, Javier Mascias, Josep Gamez, Gisella M. Gargiulo-Monachelli, Alain Moussy, Colin D. Mansfield, Olivier Hermine and Albert C. Ludolph

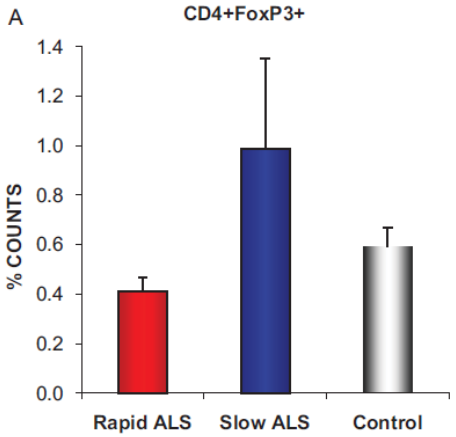


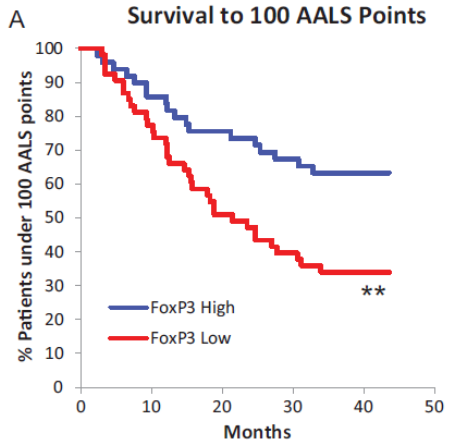
Modulare la progressione della SLA: ruolo dei linfociti T-reg

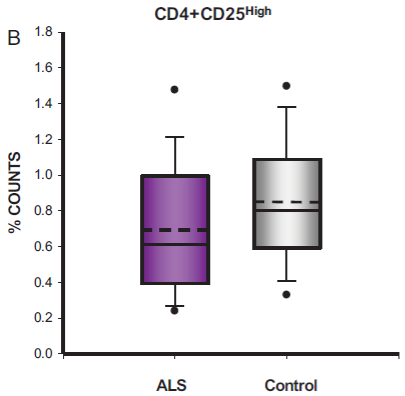


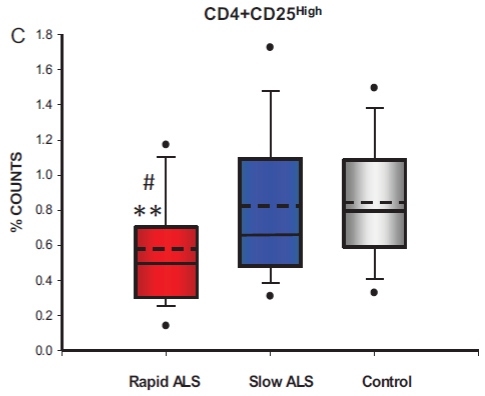
T-reg cells, formerly known as suppressor T cells, are a sub-population of T cells that modulate the immune system, maintain tolerance to self-antigens, and prevent autoimmune disease. Tregs are immunosuppressive and generally suppress or downregulate induction and proliferation of effector T cells.

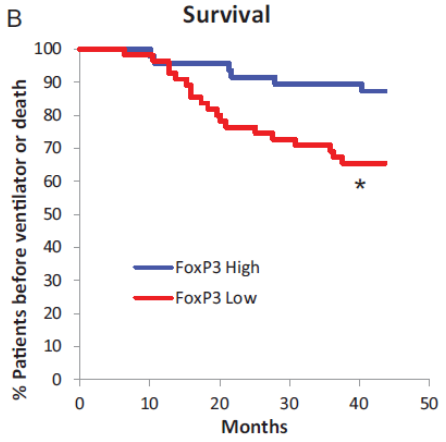
Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival

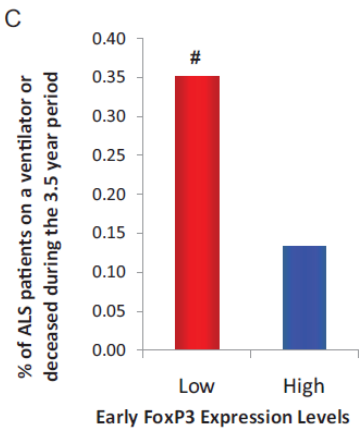








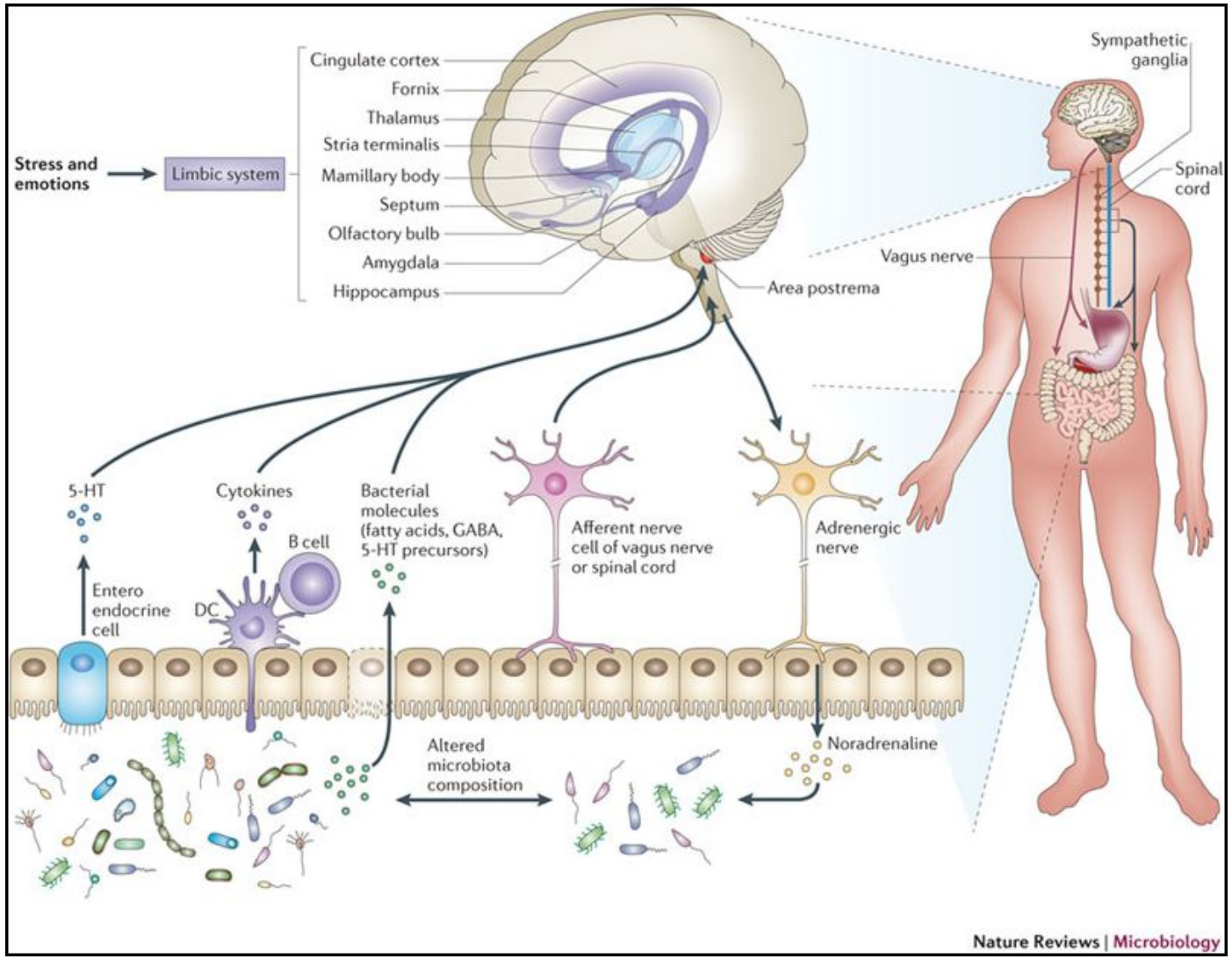


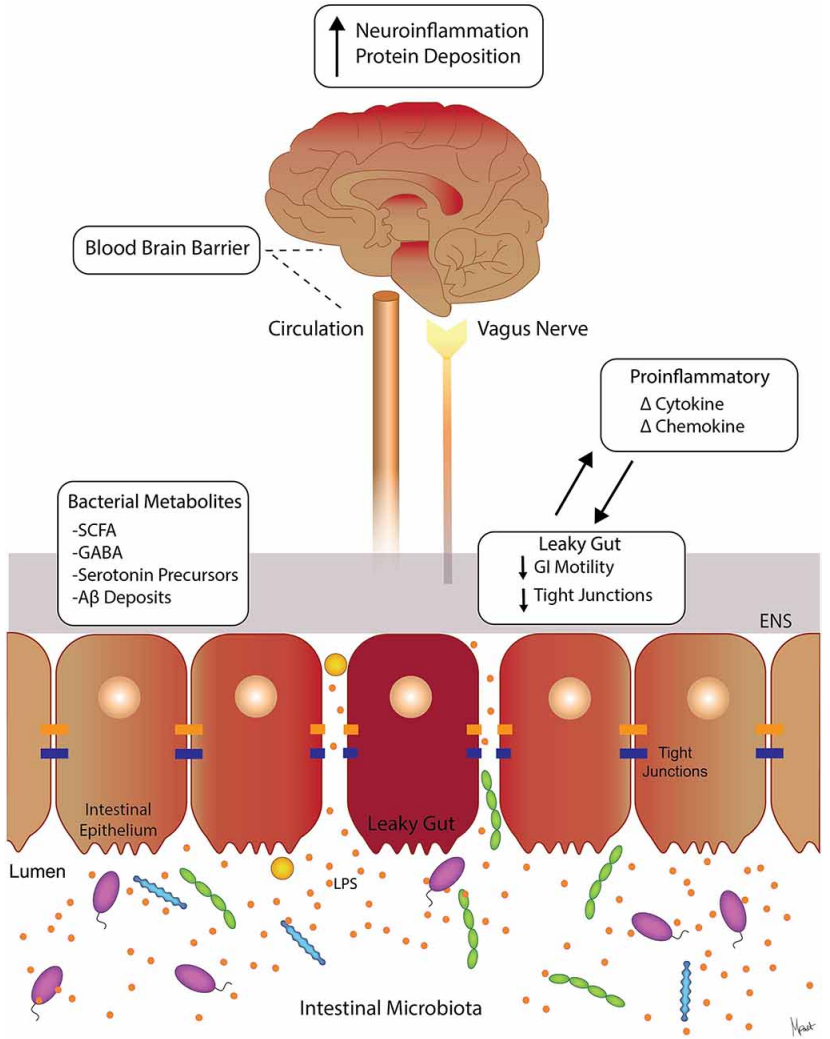


EMBO Molecular Medicine

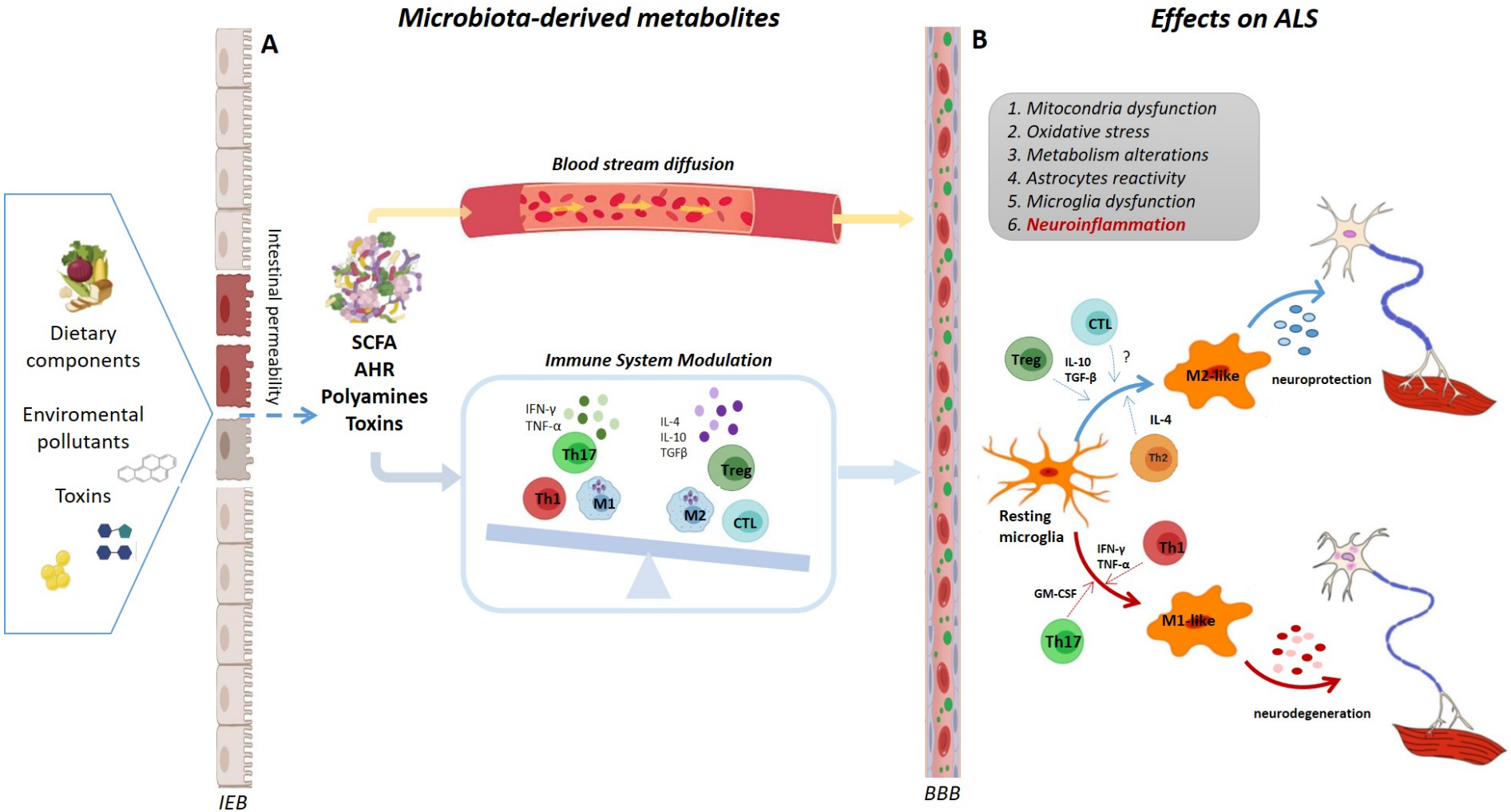
Henkel et al., 2013

Microbiota, immunità e SLA





Microbiota, immunità e SLA



Microbiota e SLA: modelli animali

Potential roles of gut microbiome and metabolites in modulating ALS in mice

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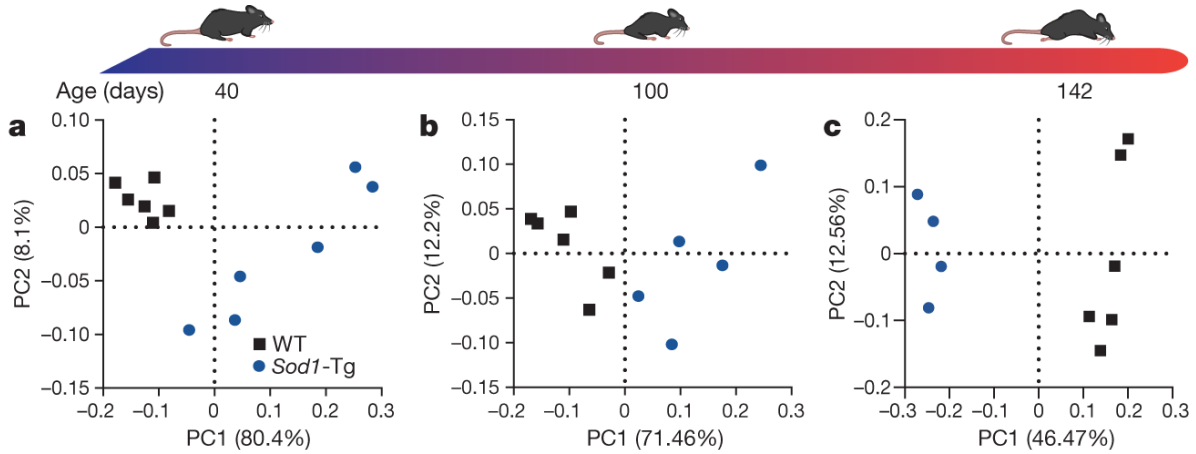

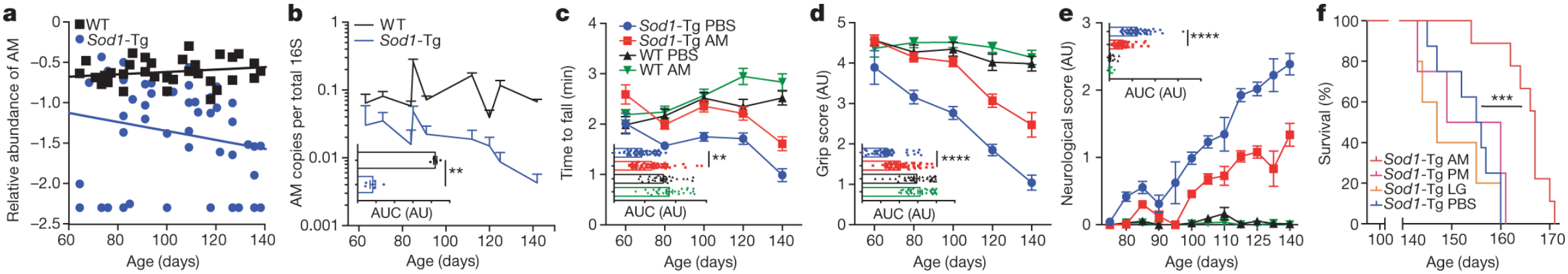

FMT in C9 ALS mouse

C9orf72 suppresses systemic and neural inflammation induced by gut bacteria

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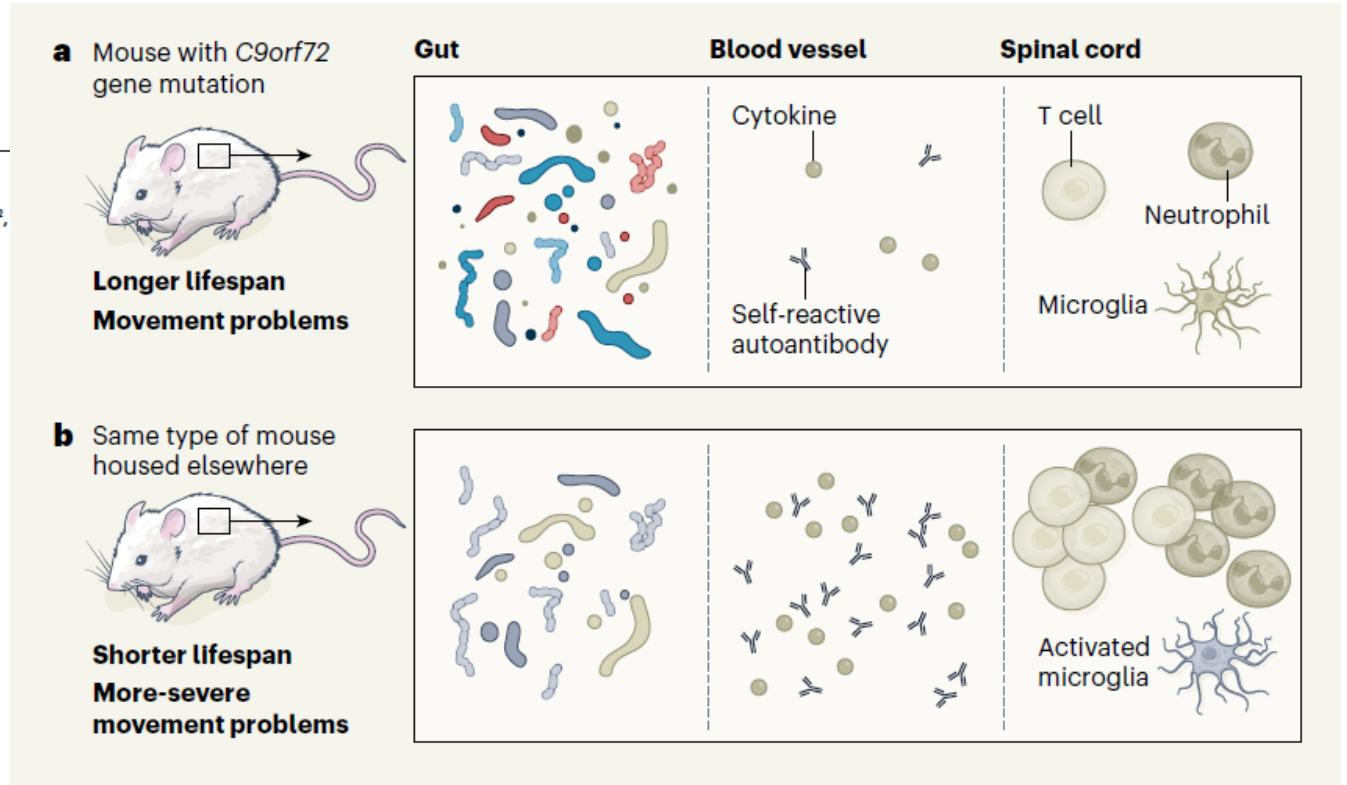

Figure 1 | Gut microbes modulate inflammation and lifespan in a mouse model of neurodegeneration. **a**, **b**, Burberry *et al.*[5] report that mice with a mutation in a gene called *C9orf72*, which is often mutated in the

Microbiota, immunity and ALS

C9orf72 suppresses systemic and neural inflammation induced by gut bacteria

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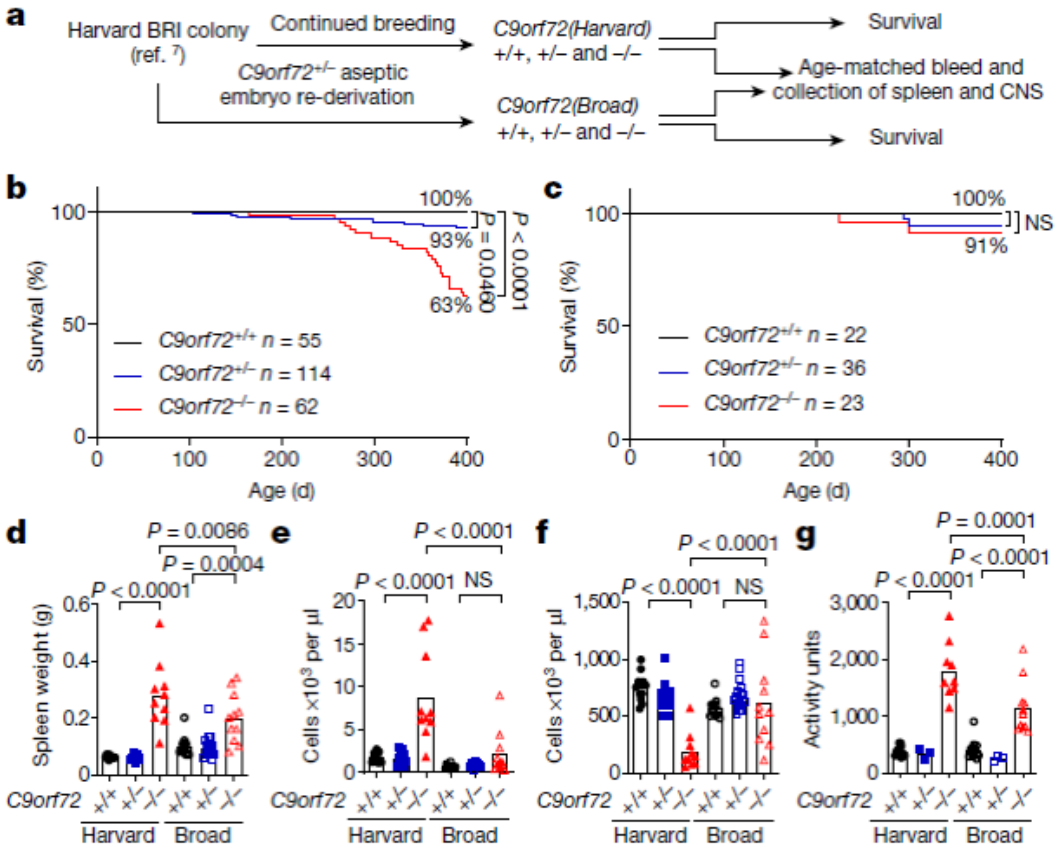
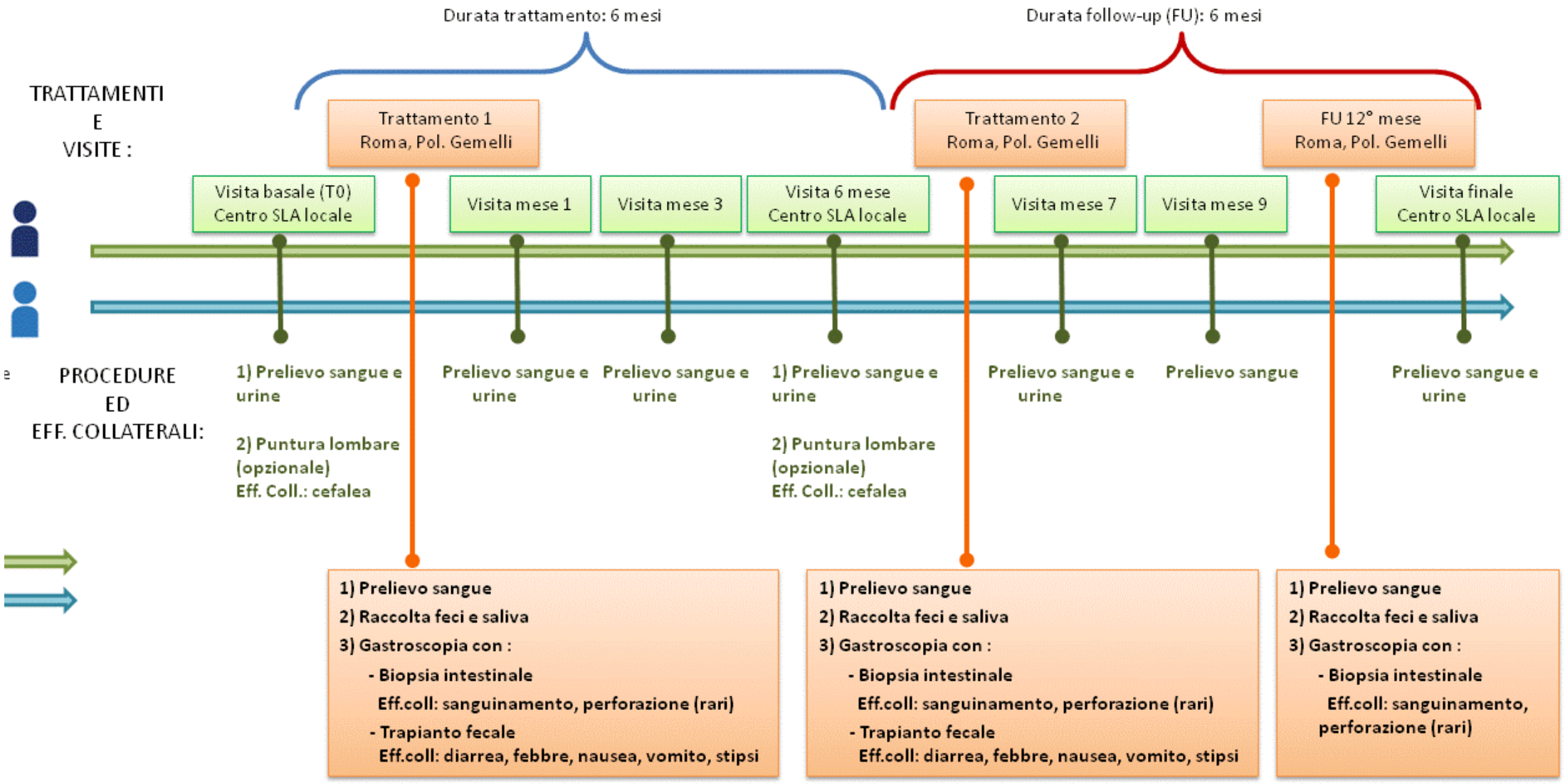


- C9orf72 mice had different survival in different condition.
- Harvard phenotype (worse) exhibit more inflammation and autoimmune phenotype
- They had an environment that improved survival also ameliorated the underlying inflammatory and autoimmune disease found in *C9orf72* mutant mice.

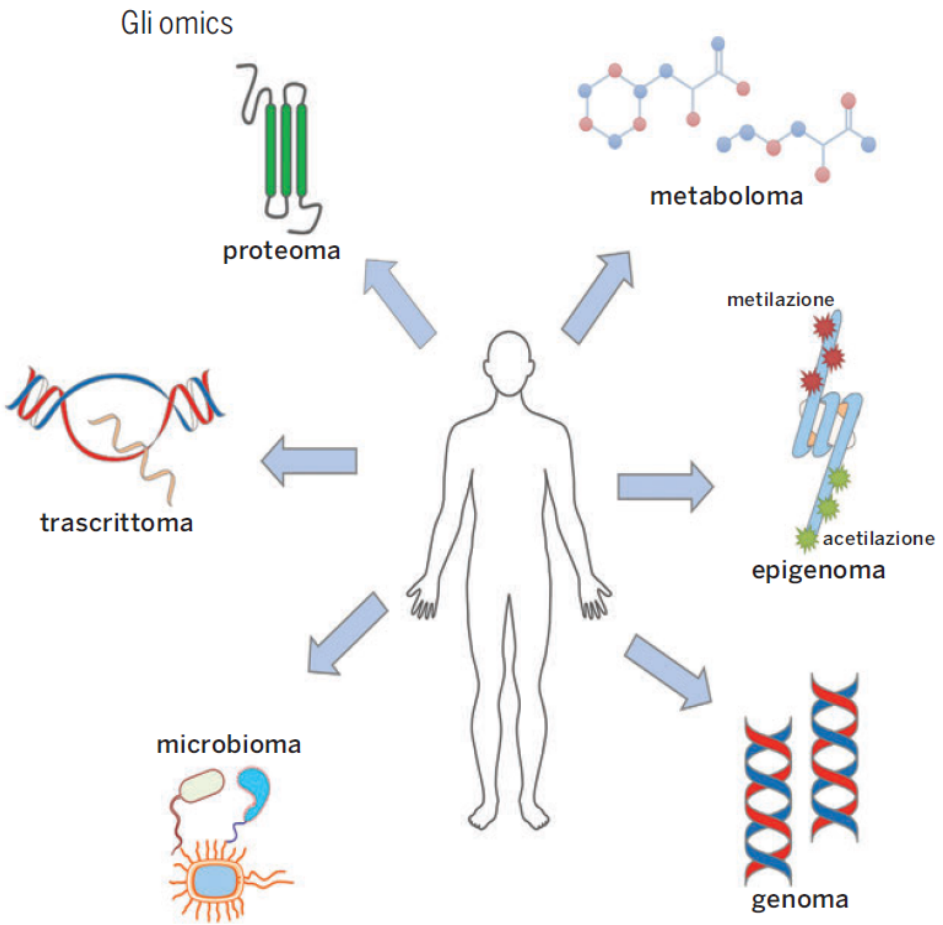
- Different microbial species in the two phenotypes (without pathogens)
- Broad antibiotic treatment did not alter Broad phenotype, whereas reduced autoimmune pattern in Harvard phenotype.

- Transplantation of pro-survival gut microflora significantly improved each of the inflammatory and autoimmune phenotypes
- Therefore, the inflammatory and autoimmune disease that underlies premature mortality in *C9orf72(Harvard)* mutant mice can be therapeutically prevented, and that signals from particular gut microbiota help tomaintain this disease.

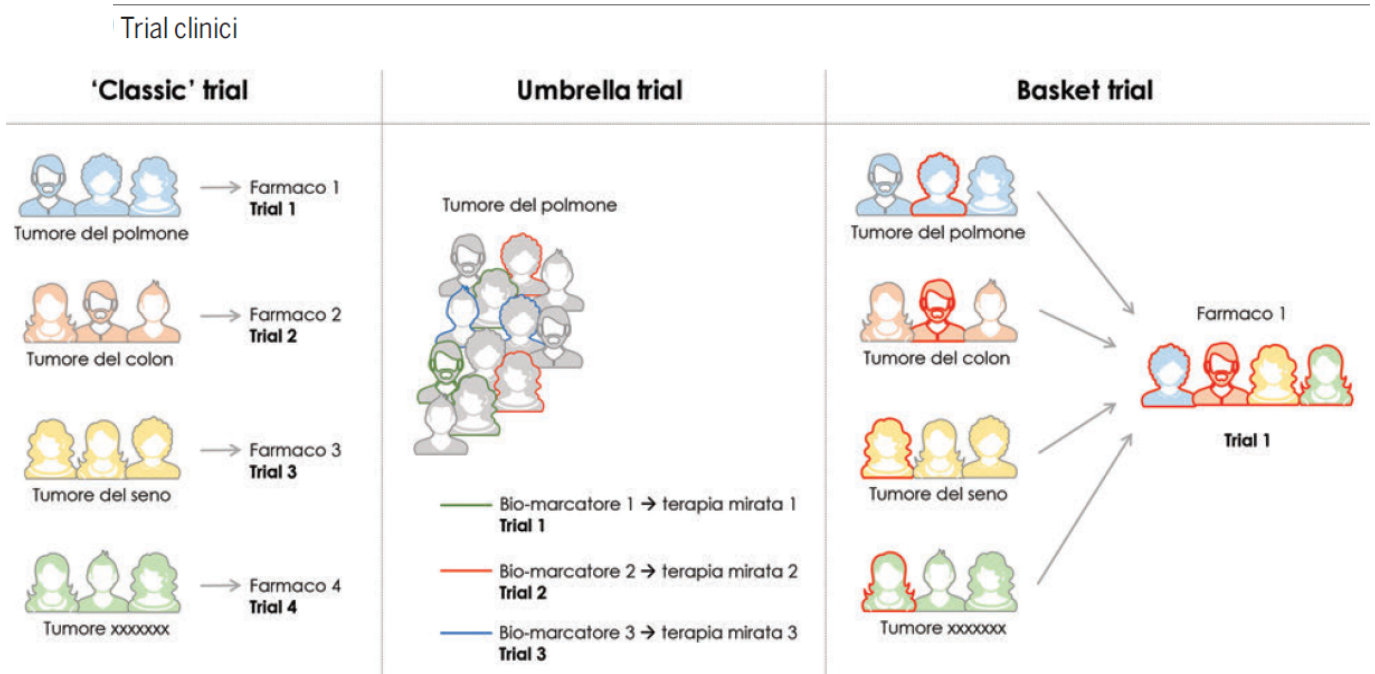
FETR-ALS flow chart



La strada verso interventi personalizzati



Sono rappresentate le varie tecniche di indagine (omics) che meglio permettono una comprensione totale del singolo individuo. Non basta studiare i geni, ma occorre ottenere informazioni sul funzionamento dell'organismo nella sua integrità, tenendo anche in considerazione gli eventi esterni.



"Classic" trial: la classificazione e l'inclusione dei pazienti all'interno del trial dipendono dall'analisi istologica del tipo di tumore. "Umbrella trial": l'analisi istologica definisce il campione di pazienti, che però viene ulteriormente suddiviso sulla base delle caratteristiche molecolari del tumore (per quale biomarcatore il tumore risulta positivo). "Basket trial": non viene considerata l'analisi istologica del tumore, ma si considerano solo le caratteristiche molecolari e si testa il farmaco su un campione eterogeneo di pazienti.

Grazie!

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