Imperial College London

Motor Neurone Disease/ Amyotrophic Lateral Sclerosis I

I Clinical presentation, neuropathology and aetiology
II From genetics to mechanisms of pathogenesis and treatment

Motor disorder resulting in progressive paralysis and death normally from respiratory failure

Clinical presentation, prevalence and neuropathology

Amyotrophic lateral sclerosis (ALS): chronic neurodegenerative condition causing muscle wasting, paralysis and death usually within 3-5 years

- Motor Neurone Disease
- Lou Gehrig's disease
- Charcot's disease

Life time risk of 1 in 500

ALS causes muscle atrophy, wasting and spasticity

- Progressive muscle weakness of limbs, trunk, tongue and respiratory muscles.
- Onset is insidious usually confined to distal muscles of a single limb and progresses to become widespread
- Impaired swallowing and speech ('bulbar signs')
- Respiratory failure
- No impairment of bladder, bowel or sexual function.
- Cognitive, oculomotor, sensory & autonomic function spared

ALS is distinguished from other ***motor neurone diseases**** by the presence of BOTH upper & lower motor neurone signs

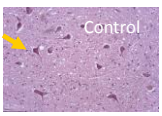
Lower motor neurone (LMN): muscle weakness, wasting, fasciculations, cramps.

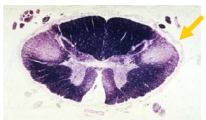
Upper motor neurone (UMN): stiffness and slowness of movement, slow and clumsy speech. Babinski signs are often present.

*e.g. spinal muscular atrophy and hereditary motor neuropathies

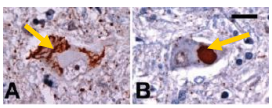
ALS is characterised by:

- Motor neurone loss in spinal cord, brain stem, motor cortex
- Corticospinal tract degeneration

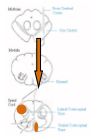




- Ubiquitinated inclusions in neuronal cell bodies and proximal axons



Ubiquitin immunoreactive LMNs with filamentous (A) or compact inclusions (B)



Mackenzie et al 2007 Annals of Neurology

Overlapping syndromes

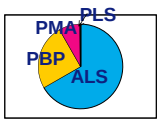
Both Upper and Lower MN signs are present - either may predominate

ALS M>F **UMNs + LMNs** (~66%)

Progressive bulbar palsy PBP F>M **UMNs + LMNs**, older onset, survival 0.5 -3y (~25%)

Progressive muscular atrophy PMA **LMN**s, M:F 5:1
Survival beyond 5y (~8%)

Primary lateral sclerosis PLS
UMNs predominate, spasticity hyperreflexia, survival 20y



What causes cell death – protein aggregates and selective vulnerability of the motor neuron?

What causes ALS?

Environmental factors

Neurofilaments

- Selective vulnerability of MNs high metabolic and functional activity
- Adult-onset
- Gender effect M>F

Genetic factors

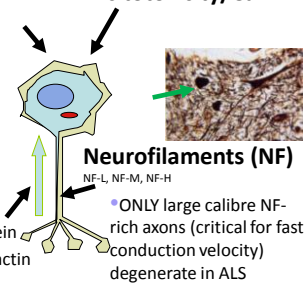
Oxidative stress

Excitotoxicity

High oxidative activity

- Build up of toxic reactive free radicals, $O_2^{\cdot-}$, $OH^{\cdot}$, $ONOO^{\cdot}$ (peroxynitrite) cause oxidative damage: protein carbonyls, 3-nitrotyrosine and 8-hydroxy deoxyguanosine.
- Superoxide-dismutase (SOD1) the first FALS gene

Excitotoxicity/Ca^{2+}

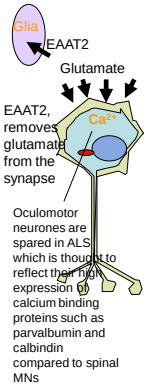


Molecular motors

Retrograde axonal transport of NFs, NTFs, misfolded proteins.

Neurofilaments (NF)
NF-L, NF-M, NF-H

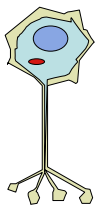
- ONLY large calibre NF-rich axons (critical for fast conduction velocity) degenerate in ALS
- NF accumulations occur in MN proximal axons in ALS



Excitotoxicity hypothesis:

excess excitation by GLUTAMATE is toxic to MNs and causes degeneration in ALS.

- CSF glutamate elevated (40% of cases)
- Glutamate transporter, EAAT-2, decreased in spinal cord in ~ 60% of SALS cases
- EAAT2 is responsible for removal of ~90% of extracellular glutamate
- GluR2 AMPA receptor down-regulated in ALS increasing Ca^{2+} permeability
- Glutamate analogues cause neurodegeneration (e.g.beta methyl amino alanine BMAA and beta oxazole amino alanine BOAA taken in the diet)



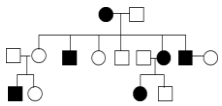
Excitotoxicity hypothesis:

what are the implications for treatment ?

- Riluzole, the currently approved medication for ALS patients which has a modest benefit in ALS (breathing capacity) promoting survival, blocks voltage-gated sodium channels and reduces glutamate release
- Upregulation of EAAT2 expression? Screening of FDA approved drugs for this property yielded candidates e.g. beta lactam antibiotic, ceftriaxone, which is now going through Phase I-III trials in man.

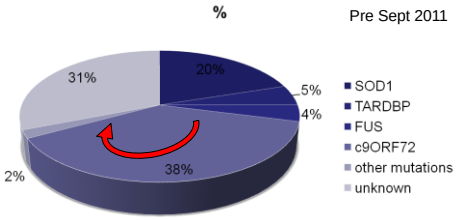
Breakthroughs at the molecular level came with familial ALS gene discoveries and the identification of the key protein in the inclusions

Familial ALS: identification of pathogenic mutations leads the way forward



- ~10% cases
- Feasible outcome
- 208 Families (IC study)
- Abnormal genes highlight mechanisms causing disease
- Mutations found for 70% of FALS cases

Known FALS mutations:



With the majority of the genes identified, the emphasis is on molecular mechanisms and moving closer to treatments

Identification of FALS genes and the characterisation of the Pathways targeted by FALS mutations

Cu/Zn dependent superoxide dismutase (SOD1)

Little advance in 18 years

- 153+ mutations cause FALS
- Overexpression of SOD1 mutations in mice and rats provides a robust model of MND but SOD1 knock-out mice do NOT develop MND
- Gain of function mechanism has been elusive
- Accumulation of ubiquinated protein aggregates suggests impairment or overload of protein quality control (saturation of molecular chaperones). HSP27/22 mutations cause hereditary neuropathy and are abundant in MNs.

Familial ALS: classical ALS, upper and lower motor neuron signs, rapid progression, dominant

c9ORF72	**ALS/FTD**	**~35%**
SOD-1	ALS1	20%
FUS/TLS	ALS6	4%
TARDBP	ALS10	3%
VAPB	ALS8	< 1%
D-amino acid oxidase		< 1%
VCP	ALS14/IBMPFD	< 1%
Ubiquilin 2	X-linked	< 1%
Angiogenin	ALS9	< 1%
Optineurin	ALS12(AR/AD)	< 1%
FIG4	ALS11(CMT4J)	< 1%

Rare forms of ALS/ mnd, atypical presentation, juvenile onset, recessive, normal lifespan

Alsin: ALS2, Juvenile, PLS, AR. Senataxin: ALS4 Juvenile, CMT, dHM, AD

FALS genes
RNA processing
Protein quality control

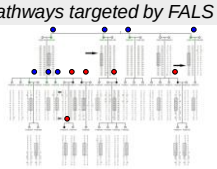
Two major pathways targeted by FALS mutations provide insight into pathogenesis:

RNA processing

Protein quality control

Clues to understanding motor neuron susceptibility and what triggers disease onset

Pathways targeted by FALS mutations *RNA processing*



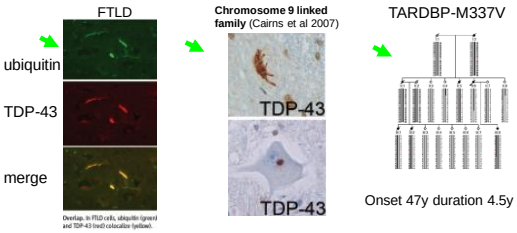
(Morita et al 2006)

Family in which both ALS and Fronto-Temporal Dementia (FTD) were linked to the same **Chr 9p locus**

- Heterogeneous syndrome:
  - semantic dementia,
  - behavioural features
  - progressive non-fluent aphasia
- Neuropathologically defined as Frontotemporal lobar degeneration (FTLD) ±TAU inclusions

RNA processing genes in ALS

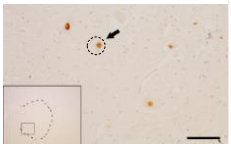
- TDP-43 identified as a major component of ubiquitinated inclusions in FTLD (Arai et al 2006: Neumann et al 2006) and later found in chr 9p linked families and SALS (Cairns et al 2007)
- FALS cases found with TARDBP mutations (Sreedharan et al 2008), mainly ALS not FTLD

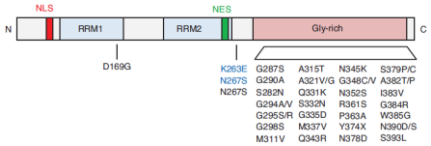


TARDBP

TDP-43, a member of the heterogeneous ribonucleoprotein (hnRNP) family, binds TAR DNA sequences in DNA/RNA acting as a transcriptional repressor, inhibits splicing and regulates mRNA transport/ local translation

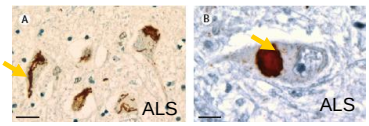
TDP-43-human spinal cord

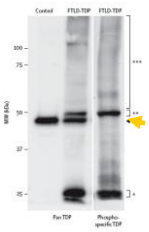




Dormann and Haass 2011
TRENDS in Neurosciences

Effect of TDP-43 mutations





- TDP-43 is cleaved, locates to cytoplasm
- Hyperphosphorylated, ubiquitinated aggregates in cytoplasm, MNs, glia, neurites

Mackenzie et al 2010

Animal models: Neurodegeneration (cortical and spinal neurons) **BUT** without consistent formation of inclusions

- Both wild-type (8) and mutant (4) TDP-43 rodent transgenics produce neurodegeneration and paralysis (threshold for toxicity may vary).

Many unanswered questions

Abnormal TDP-43^{+ve} inclusions*:

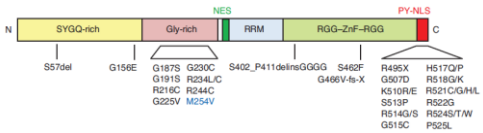
Present predominantly in the **cytosol** of neurons, glia and dystrophic neurites, together with nuclear clearing of TDP-43 occur in a spectrum of disorders.

ALS and ALS-FTLD	(100%)	Spinal cord/ brain
Alzheimer's disease	(33-57%)	Brain
Parkinson's disease	(19%)	Brain
Diffuse Lewy body dementia	(45%)	Brain
Cognitively normal controls >65y	(29%)	Brain
Huntington's disease	(100%)	BG/ inner cortex
Inclusion body myopathy	(100%)	Muscle
Myofibrillar myopathy	(100%)	Muscle

* amorphous, lacking fibrils and do not stain for amyloid (Thioflavin-s)

FUS (fused in sarcoma)/ TLS (translocation in liposarcoma #)

Kwiatkowski et al., 2009 and Vance et al., 2009



FUS-positive inclusions are found in FALS and familial FTLD with FUS mutations, SCA 1-3 and HD

translocated to C-terminal of transcription factors (activation)

Impaired RNA processing

TDP-43		FUS
REPRESSOR binds to TAR DNA HIV-1	**Transcription**	ACTIVATOR nuc hormone receptors, NFkB. Associated with RNA pol II/TFIID complex
Exon skipping Binds to CFTR pre mRNA UG intronic tract	**Splicing**	Part of spliceosome machinery

MicroRNA processing

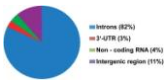
Both associate with Drosha nuclear RNAse III protein involved in miRNA maturation

RNA granules

Both found in RNA transporting granules (stress granules and processing bodies). TDP-43 loss reduces dendritic branching/synapse formation and FUS KO reduces spine formation.

What are the RNA targets?

RNA sequences bound by TDP-43 (Xiao et al 2011)



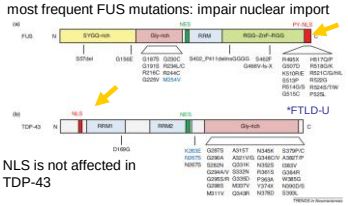
What drives the pathogenic process?

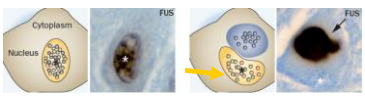
- Impaired RNA processing?
- Is the formation of TDP-43-positive inclusions central to pathogenesis (mere bystanders or do they have a neuroprotective role) ?
- Is the mislocalisation of TDP-43 causal?

What causes TDP-43 mislocalisation?

- Inhibition of FUS nuclear transport protein (transportin) causes FUS accumulation in cytoplasm.
- Similar inhibition of the TDP-43 nuclear transport protein (Importin) causes accumulation of **soluble** TDP-43 in cytoplasm.

Nuclear localisation signal (NLS) is the site of the most frequent FUS mutations: impair nuclear import





Rademakers et al 2012

What is the missing link, what triggers the formation of inclusions?

Effects on nuclear transport proteins are relevant to SALS as these proteins decrease in abundance with age

Cell stress, oxidative stress, heat shock, ER stress,

Formation of stress granules in the cytoplasm containing housekeeping mRNAs that do not require translation during stress also contain FUS and TDP-43

Do these stress granules act as precursors of the large inclusions?

Two-hit hypothesis: protein accumulation in the cytosol cell stress trigger

Chromosome 9-linked ALS/FTD families

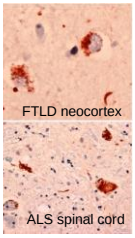
Sequencing the coding region of all genes in the locus: **NO** mutations were identified despite growing number of chromosome 9-linked families being discovered

However by sequencing each gene in full (coding and intervening regions greatly facilitated by Next Generation deep resequencing), an **INTRONIC** mutation was discovered by 2 US groups (DeJesus Hemandez et al 2011: Renton et al 2011)

9p21 ALS/FTD locus (3 genes):

Chromosome 9 open reading frame 72 (**C9ORF72**) mutation
(DeJesus Hernandez et al 2011: Renton et al 2011)

Expanded repeats in intron 1 of C9ORF72 in FTD and ALS



TDP-43 $^{+}$ve inclusions in cytoplasm
Cerebral cortex and spinal cord

DeJesus Hemandez et al 2011

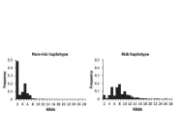
Prevalence of C9ORF72 expansions

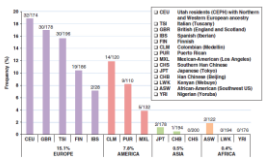
Familial FTD (171)	12%	(DeJesusHernandez et al 2011) 8%GRN/6%MAPT
Sporadic FTD (203)	3%	(DeJesusHernandez et al 2011) 3%GRN/2%MAPT
Familial ALS (268)	38%	(Renton et al 2011) US/GER/ITA
(228)	**39%**	**UK*/Europe (Smith et al 2012)**

Range varies from 20% in Italy to 86% in Belgium
* includes IC cases

Sporadic ALS (195)	4%	(DeJesusHernandez et al 2011) 1%TARDBP/2%FUS
(1048)	**8%**	**UK*/Europe (Smith et al 2012)**
Controls	~0.3 to 0.6%	

- C9ORF72 containing 3 GGGGCC repeats arose in primate evolution: present in human, chimpanzee and gorilla but no other species
- Age of the founder event is thought to be ~ 6,300 years ago (251 generations)
- The Founder risk haplotype has an increased number of repeats (<30) and is more prevalent in Europe than elsewhere.

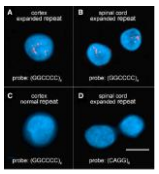




Smith et al 2012

C9ORF72: Function unknown

- Reduced mRNA levels and nuclear RNA granules accumulate
- C9ORF72-specific pathology includes p62 $^{+ve}$ cytoplasmic and nuclear inclusions in hippocampus and cerebellum that are TDP-43^{-ve}



DeJesus Hernandez et al 2011

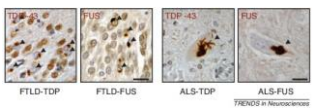
Non-coding repeat expansion:
Myotonic dystrophies (DM1 3'UTR, DM2 Intronic, sequesters RNA binding proteins),
Fragile X (FXTAS 5'UTR),
Spino-cerebellar ataxias (SCA8 5'UTR, SCA10 3'UTR, SCA12 5'UTR)

TARDBP and FUS
A pathology common to an extended family of neurodegenerative conditions

Misfolded TDP-43 and FUS are pathological hallmarks of :

ALS	Most sporadic cases Major proportion of FALS cases (~45 – 55% due to mutations in TARDBP, FUS, C9ORF72, VCP)
FTLD-U	All cases with ubiquitin positive inclusions which are mostly TDP-43 positive (90%) or FUS positive (10%), 50% sporadic and 40% familial (due to mutations in Granulin, TARDBP, FUS (single case), C9ORF72, VCP)

Cytosolic inclusions of nuclear proteins



- ALS occurs with FTD
- FTD cases may have MN degeneration

Proteins involved in protein quality control
"waste disposal system"

Pathways targeted by FALS mutations

Protein quality control

Proteins with a propensity to aggregate (mutant, wild-type, misfolded, damaged, mislocalised) accumulate

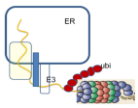
Protein quality control
Molecular chaperones

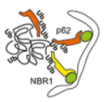
UPR localised in ER

Proteins tagged by ubiquitin for degradation

ER: protein folding, synthesis of secreted proteins, disulphide bridges formation

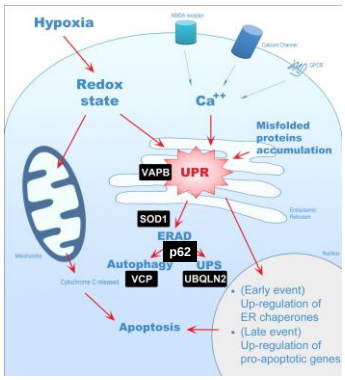
Ubiquitin proteasomal systemUPS





Autophagy

Protein Quality Control



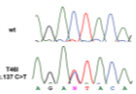
Chen and de Belleroche 2011

Enzymes of ubiquitin transfer

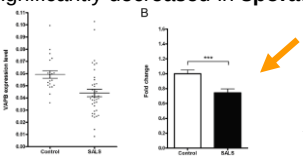
PD (E3 ubiquitin ligase)	Parkin
PD	UCHL1
MJD	Ataxin-3
SCA1	USP7

VAPB (Vesicle associated protein B)

- A VAPB mutation was first described in a Brazilian family linked to 20q13 (Nishimura et al 2004).
- A second UK FALS-associated mutation was found (Chen et al 2010)



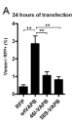
- VAPB is localised in motor neurons and is significantly decreased in **sporadic ALS** spinal cord



Anagnostou et al 2008

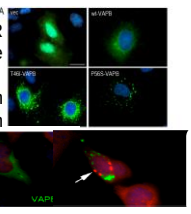
Recapitulate the disease in cell culture

FALS mutations, impair the unfolded protein response

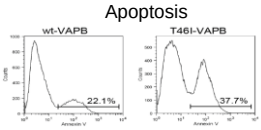


ER structure

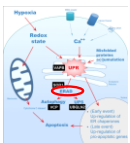
Ubiquitin --ation



Apoptosis

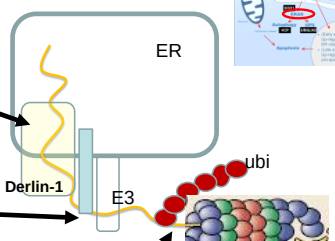


ER protein export to the proteasome: ERAD proteins



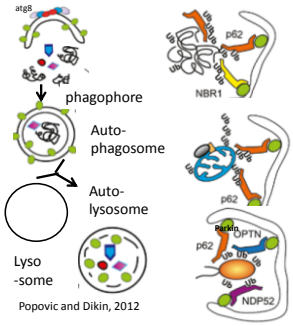
SOD1 : ALS1, binds to Derlin 1

VCP: ALS14 and IBMPFD, ER protein export to the proteasome



Ubiquilin 2: X-linked FALS and SALS, binds to poly-ubi chains and components of the proteasome

Autophagy: aggrephagy of misfolded or aggregated proteins is activated by the failure of proteasomal degradation and molecular chaperones to resolve aggregate build-up.



P62 (Ubiquitin binding protein Sequestosome 1 SQSTM1) Allelic disorder is Pagets disease of bone

OPTN ALS12 slow progressing AR/AD
Allelic disorder is primary open angle glaucoma.

Mitophagy (stress induced)

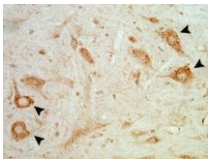
Xenophagy (bacteria)

Pathways targeted by FALS mutations: Clues to understanding motor neuron susceptibility and ***identifying what triggers disease onset***

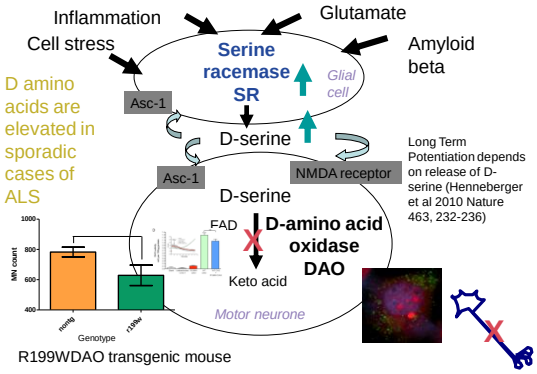
D-amino acid oxidase (DAO)

DAO metabolises ***D-serine***, an essential co-agonist at the NMDA receptor and hence critical in synaptic plasticity

D-serine is elevated in SALS and ^{G93A}SOD1 mouse
^{G93A}SOD1 pathogenicity is potentiated by mutant DAO (Sasabe et al 2012)



Recapitulation of disease from point of initiation to cell death



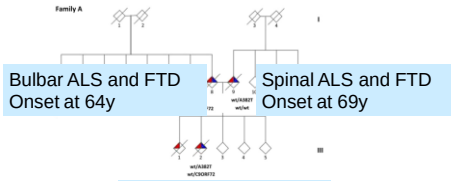
D amino acids are elevated in sporadic cases of ALS

R199WDAO transgenic mouse

Not everyone with a mutation within a family will develop ALS

- 60 – 80% by the age of 85 years
- Age-dependent but age at onset within a family may vary by as much as 20 years or more (unpredictable)
- Other factors play a role: susceptibility genes and risk factors modify disease onset – a new CHALLENGE

In Sardinian, 25% of cases carry a TARDBP (A382T) or C9ORF72 mutation and both mutations have been found in two individuals from separate families (Chio et al 2012)



FALS mutations can co-exist and **may** cause a more severe condition ???

Sporadic ALS

- Generally have a similar pathology to most familial cases with abnormalities in the products of the same genes causing FALS e.g. TDP-43 and VAPB
- Common susceptibility factors – ageing
- 8% of sporadic cases have a c9orf72 expansion (the most common mutation causing FALS)

Treatment perspectives

Drug targets in ALS

With the exception of riluzole* that targets excitotoxicity, drugs tested do not have clinical effectiveness in large trials (e.g. neurotrophic factors, CNTF, BDNF) and may even be detrimental (minocylcine).

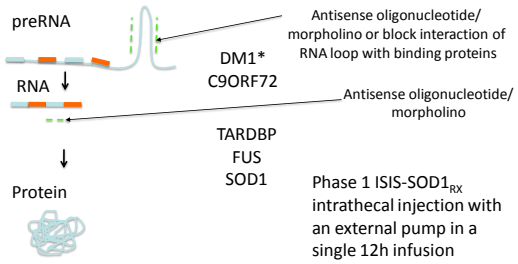
Novel approaches in progress

- ceftriaxone, a beta lactam antibiotic upregulates EAAT2
  Phase III trial in progress
- Treatment by knock-down
  (e.g. Isis-SOD1RX antisense treatment trial Phase I using minipump)
- Stem cells
  Phase I trial of spinal cord derived stem cells for patients with ALS (Emory)

Can we harness endogenous neuroprotective mechanisms?

* Prolonged survival without tracheostomy of **3 months** (100mg/day for 18 months Cochrane review)

Next generation treatment strategies: RNA silencing through RNA interference



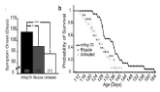
*antisense oligonucleotides rescue DM models leading to reduced RNA foci, normal splicing + reduced myotonia

Potential opportunities for treatment

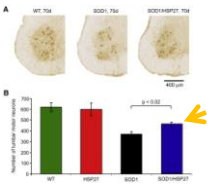
HSPs: recombinant protein or a co-ordinated approach using pharmacological induction of multiple HSPs by HSF1

Recombinant protein

Recombinant HSP70 delays onset in G93A SOD-1 mouse



Gifondorwa et al 2007



HSPB1/HSP2727 delays onset of disease, reducing MN loss, motor unit loss and accumulation of ubiquitinated inclusions (Sharp et al 2008)

HSF-1 induction: In *C.elegans*, accumulation of TDP-43 aggregates is potentiated by HSF-1 reduction by RNAi or HSF-1 loss of function (Zhang et al 2011)

Imperial College London

Conclusions

Advances in research have enabled the definition of fundamental mechanisms of neurodegeneration, providing insights into both Amyotrophic Lateral Sclerosis (ALS) and fronto-temporal dementia (FTD)