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The effect of TDP-43 antibodies on TDP-43 aggregation and toxicity

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List of abbreviations

AD – Alzheimer's disease

ALS – Amyotrophic lateral sclerosis

BCA – Bicinchoninic acid

CTF – C-terminal fragment (of TDP-43)

DMEM – Dulbecco's modified eagle medium

DNA – Deoxyribonucleic acid

DPR – Dipeptide repeat proteins

EMA – European medicines agency

EV – Empty vector transfection

fALS – familiar Amyotrophic lateral sclerosis

FCS – Fetal calf serum

FDA – US food and drugs administration

FTLD – Frontotemporal lobar degeneration

FUS – Fused in sarcoma

HBS – 2-ethensulfonic acid buffered saline

HEK-293 – Human embryonic kidney cells 293

HSP – Heat shock proteins

hNFL – Low molecular weight Neurofilament

hnRNP – Heterogenous nuclear ribonucleoproteins

HRP – Horseradish peroxidase

LDH – Lactate dehydrogenase

LLPS – Liquid-liquid phase separation

LMN – Lower motor neuron

MFN2 – Mitofusin 2

MTT – 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid

NES – Nuclear export sequence

NLS – Nuclear localization signal

Opti-MEM – Optimised minimal essential medium

PD – Parkinson's disease

PDL – Poly-d-lysine

PHB2 – Prohibitin 2

pTDP-43 – Phosphorylated TDP-43

PVDF – Polyvinylidenfluorid

RBP – RNA binding proteins

RIPA buffer – Radioimmunoprecipitation assay buffer

RLU – Relative luminescence units

RNA – Ribonucleic acid

ROS – Reactive oxygen species

RRM – RNA recognition motive

sALS – sporadic Amyotrophic lateral sclerosis

SEM – Standard error of the mean

SG – Stress Granula

SOD1 – Superoxide dismutase 1

TDP-43 – Transactive response DNA binding protein 43 kDa

UMN – Upper motor neuron

1. Introduction

1.1 Amyotrophic lateral sclerosis

1.1.1 History and Epidemiology

First described by Charcot in 1869[47], amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease[225]. It is characterized by selective loss of upper and lower motor neurons. Charcot also already described the disease as progressive, with patients showing signs of a continuous spread of the disease throughout the nervous system[47]. It is an always fatal disease with death occurring within 3-4 years after onset most of the time by respiratory failure[225]. The incidence of ALS is estimated around 1-3 cases per 100.000 people per year but has a relatively low prevalence at 6/100.000 due to the short survival of patients[225]. New studies showed a slightly higher incidence of ALS in western countries compared to Asia[155] with few exceptions e.g. Guam or the Kii peninsula in Japan. The average onset of ALS is between 58 and 60 years of age, with men being affected slightly more than women (~1.5:1)[121, 155]. More than 90% of ALS cases do not seem to have a clear familiar background and are most commonly referred to as sporadic ALS (sALS). Cases where relatives are also affected with ALS are referred to as familiar ALS (fALS). Up to 70% of all fALS cases are caused by mutations in one of 4 genes: *C9orf72*, *TARDBP*, *SOD1* and *FUS*[51, 181]. Even though disease onset is earlier in fALS cases, both groups are mostly indistinguishable from a clinical perspective[51, 111], suggesting similar patho-mechanisms.

1.1.2 Clinical presentation and diagnostics

Selective loss of motor neurons leads to progressive deterioration of motor cortex, brain stem and spinal cord in ALS patients, but early symptoms vary greatly between individuals. Most patients, around 70%, present with asymmetric symptoms of the extremities (limb-onset). A second group presents with problems in swallowing, chewing or dysarthria (bulbar onset, ~25% of patients) and the smallest group shows initial involvement of the trunk (~5% of patients)[239]. Loss of upper motor neurons (UMNs are located in the cerebral cortex and brainstem) and loss of lower motor neurons (LMNs are located downstream of the UMNs,

e.g. in the spinal cord, and directly signalling muscles to contract or relax) also present differently, especially early in the course of the disease. Patients with impaired upper motor neurons characteristically show signs of spasticity, finally leading to muscular weakness. In the case of initial lower motor neuron loss typically fasciculations, wasting of muscles, hyporeflexia and hypotonia can be observed as first symptoms[77, 190]. The disease progresses and spreads fast until at last also the muscles of breathing and swallowing are affected, making respiratory treatment a necessity and ultimately resulting in respiratory complications and failure, the main cause of death in ALS patients[92, 126]. On top of the characteristic motor symptoms, up to 50% of patients also develop cognitive and/or behavioural deficits[170, 183], up to 15% also fulfilling the criteria for frontotemporal lobar degeneration (FTLD) the main cause for dementia in patients younger than 60 years[130]. Both of these diseases are now recognised as the extremes of a disease spectrum[24, 130, 141, 170].

With many possible differential diagnoses and greatly varying early presentation, diagnosis is often complicated. Although there are first promising studies for potential biomarkers being published[1, 76, 146, 221], none are established yet, making it essential to have a standardized clinical diagnostic tool for ALS. In 1990, the World federation of neurology published the 'El Escorial criteria' which have since then been repeatedly modified. The latest update was the introduction of electromyography resulting in the 'Awaji-Shima-Criteria' of 2008 (Table 1) and the very similar criteria of the Chinese medical association in 2012. Both resulting in increased sensitivity and specificity [44, 62, 202, 228]. However, ALS is still diagnosed approximately 18 month after disease onset, resulting in late or inappropriate therapy for patients and a delayed enrolment in clinical studies[6, 154].

Table 1: Awaji-Shima-Criteria (2008)

staging	definition
clinically possible ALS	Clinical or electrophysiological evidence of upper motor neuron (UMN) and lower motor neuron (LMN) signs in two or more regions, or LMN signs rostral to UMN signs.
clinically probable ALS	Clinical or Electrophysiological evidence of UMN and LMN signs in at least two regions, with some UMN signs rostral to LMN signs.
clinically definite ALS	Clinical or electrophysiological evidence of UMN and LMN signs in the bulbar region and at least two spinal regions, or UMN and LMN signs in three spinal regions.

1.1.3 Causes of ALS

ALS is considered a multifactorial disease thought to at least in parts caused by a combination of environmental factors as well as genetic susceptibility or distinct mutations[92]. A recent study on post-mortem brains observed three major groups with differing main causes: oxidative stress (~60%), TDP-43 and retrotransposon activity (~20%) and glia activation and stress (~19%)[226]. All these groups may also share underlying patho-mechanisms ranging from protein misfolding and aggregation, altered RNA metabolism, DNA damage repair, protein homeostasis, mitochondrial damage, altered neurofilaments and axonal transport to non-cell autonomous mechanisms and neuro-inflammation. The most relevant pathological hallmarks are described in more detail below.

1.1.3.1 Genetics

In 1993, the first gene directly associated with ALS was *SOD1*[196]. *SOD1* codes for the superoxide dismutase 1 which plays a vital role in oxidative stress and is mutated in 12-20% of all fALS cases[66, 193]. As of today, more than 25 ALS genes are known which are responsible for 2/3 of all familial ALS cases and ~11% of sALS cases[193]. Many of these genes can be grouped together, e.g. mutations in genes linked to protein homeostasis *(e.g. SQSTM1*[74]*, UCP*[244]*, UBQLN2*[70] *and OPTN*[156]*)*, RNA processing *(e.g. TARDBP*[52, 113, 220]*, FUS*[127, 238]*, hnRNPA1*[122]*, C9orf72*[54, 69, 160]*)* and cytoskeletal dysfunction *(e.g. DCTN1*[162, 168]*, PFN1*[162, 251, 257]*, TUBA4A*[162, 215]*)*. *C9orf72* is the most frequently mutated gene and accounts for ~40% of fALS and ~7% of sALS cases[69, 191, 193]. On the contrary mutations in the *TARDBP* gene coding for TDP-43 only account for ~5% of fALS and ~1% of sALS cases despite TDP-43 aggregates being present in ~97% of all ALS patients[52, 113, 193]. Furthermore, ongoing research still reveals new genes, genetic risk factors and new mutations in already known ALS genes to this day.

1.1.3.2 Neuropathology

Next to the afore mentioned loss in spinal and cortical motor neurons and their axons, gliosis and microglia infiltration as well as altered mitochondria and protein aggregates are present in motor neurons of ALS patients[92, 115]. The most prominent hallmark of ALS is the accumulation of ubiquitinated and phosphorylated proteins in motor neurons which has also been observed in many other neurodegenerative diseases and is still part of extensive research[92, 161, 170, 218]. These aggregates vary from patient to patient and with differing genetic background. Depending on the composition of these aggregates, ALS may be clustered into different subtypes (Table 2). Around 97% of patients show signs of TDP-43 proteinopathy[170]. That includes nuclear depletion of TDP-43 and ubiquitination and phosphorylation of cytoplasmic aggregates suggesting a loss of TDP-43 function in addition to aggregation related effects in these patients [38, 205, 226]. Accumulation of dipeptide repeat proteins (DPR) as well as TDP-43 aggregates are seen in another group, most often patients carrying *C9orf72* repeat expansions[181]. These aggregates are produced by repeat-associated non-AUG translation of hexanucleotide repeat containing RNA[160, 266]. Additionally, *C9orf72* patients accumulate RNA foci[130]. Differing in appearance are two significantly smaller groups which show aggregates of either *SOD1* (~2%) or *FUS* (~0.5%)[31, 119, 150, 238] but no TDP-43 aggregation. *FUS* has similar functions to TDP-43 and is also thought to act through a loss or gain of function mechanism, whereas SOD1 is closely related to oxidative stress.

Table 2: Protein inclusions in patients with amyotrophic lateral sclerosis (ALS) *(based on Laferriere/Polymenidou, 2015 and Feiler et al., 2015)*

protein component	frequency in patients	type of ALS	means of pathology
TDP-43	>97%	*sporadic ALS (sALS):* nearly all cases *familiar ALS (fALS):* TDP-43; *C9orf72*	loss of function / toxic aggregation effects
DPR and RNA foci	each ~ 10%	*fALS: C9orf72*	gain of toxicity/ loss of *C9orf72* or RNA binding protein function
SOD1	~2%	*fALS:* SOD1	gain of toxicity
FUS	< 1%	*fALS:* FUS	gain of toxicity

1.1.3.3 Alterations in RNA and DNA metabolism

The fact that many of the known mutations are in genes encoding for RNA binding proteins (RBP) like FUS, TDP-43 and hnRNPA1[69, 113, 122, 127], altered RNA metabolism seems to play an important role in ALS pathogenesis. Additionally, a recent study found altered RNA metabolism together with retrotransposon silencing to be the leading characteristic in a group of 20% of ALS post-mortem samples[226]. RBPs are involved in many different processes such as RNA processing, alternative splicing and micro-RNA silencing. Upon nuclear depletion of e.g. TDP-43, many of these functions are lost and up to 1/3 of the transcriptome was shown to be altered in disease models[5, 11, 230]. Additionally, TDP-43 is involved in DNA double strand repair [163] and retrotransposon silencing[123] and will be discussed in more detail later (see 1.2.3). FUS mutations also lead to impaired RNA metabolism and DNA damage repair[264], in similar loss/gain of function mechanisms[130]. Lastly, toxicity due to mutations in the most common ALS-associated gene, *C9orf72*, are largely associated with impaired RNA functions. The afore mentioned pathogenic repeat expansions in C9orf72 lead to a characteristic accumulation of RNA. In these RNA foci, RNA binding proteins are sequestered, resulting in the dysregulation of many different transcripts in C9orf72 patients[60, 130, 136, 266]. Together, these findings suggest a crucial role of RBPs and malfunctioning of RNA and DNA metabolism in the pathogenesis of ALS.

1.1.3.4 Oxidative stress and stress granules

Ever since the antioxidant *SOD1* has been discovered to be a genetic cause of ALS, oxidative stress has been a point of focus in ALS research. Oxidative stress is defined as an imbalance between the production and removal of reactive oxygen species (ROS) that ultimately accumulate in a cell. It is closely linked to mitochondrial damage, which has been observed in ALS as well[34, 65]. ROS accumulation also leads to structural damage to the cell and DNA and RNA dysmetabolism[138, 153] which has already been discussed as an important patho-mechanism in ALS. In this regard, oxidative stress markers and oxidative damage are both shown to be elevated in ALS[66, 79, 208].The role of oxidative stress is further underlined by Tam *et al.* who found clear signs of oxidative and proteolytic stress in post mortem brain samples of 60% of all the patients included in this study[226].

Furthermore, oxidative stress is linked to other pathological pathways involved in ALS-like protein aggregation[31, 33, 137], excitotoxicity[92] and stress granule (SG) formation[27, 34, 56] most likely impacting the disease not only directly but also by aggravating other pathways. Stress granules are formed upon cellular stress and act as a cellular defence mechanism, selectively inactivating RNAs[7, 8, 14, 56]. While SGs are supposed to be protective to the cells[34, 94], under conditions of prolonged stress they may undergo phase separation resulting in the formation of stable aggregates[14, 71, 94]. Two proteins closely associated with ALS, TDP-43 and FUS, are also found in SGs, further suggesting important interactions between RNA metabolism, oxidative stress and the resulting formation of SGs[27, 56, 71] (see 1.2.3 for further details).

1.1.3.5 Misfolding, Aggregation and Seeding

Protein aggregates are a hallmark of ALS, that have been observed in dying neurons, microglia and astrocytes of patients as well as rodent models[61, 81, 170, 234]. Despite the fact that aggregates have been accepted as a hallmark of ALS pathology for years, the role of these aggregates in disease is still a highly debated topic. While enhanced aggregation is shown to be toxic to cells[25, 33, 108, 109, 246], different studies have also shown that toxicity may occur even before visible aggregates can be detected[11, 234, 245]. Of critical importance may be that aggregation almost always is accompanied by nuclear depletion of the respective protein resulting in a loss of this protein's function[15, 19, 132, 134, 175, 218]. Another indication that aggregation may be toxic is that many disease associated mutations impair autophagy and protein degradation pathways which are essential to sequester aggregates (see 1.1.3.1). Additionally, enhanced clearance of aggregates and an induction of autophagy both reduce toxicity in disease models[21, 141, 204].

Next to intracellular toxicity, the spread of ALS through the nervous system is what ultimately makes the disease as fatal as it is. First mentioned by Charcot Braak *et al.* later found a correlation to the continuous spread of the disease in post mortem brain samples of patients[35, 37, 47]. In this regard, many of the proteins involved in ALS, e.g. TDP-43 and SOD1, have been shown to be released from one cell and taken up by adjacent cells [102, 175, 185] (also see 1.1.3.4) or may be transmitted across axon terminals[75]. There, they have the ability to act as seeds thus propagating pathology from cell to cell as can be observed in a mouse model with TDP-43 pathology mimicking disease progression [72, 187,

213]. In these pathologic aggregates proteins are readily misfolded, cleaved or post translationally modified, thereby increasing their pathologic properties e.g. their seeding ability[10, 145, 170, 205, 218]. Additionally, it has been suggested that not only large aggregates first observed by Neumann *et al.*[170] are to be considered the toxic species, but rather smaller, oligomeric aggregates that can also be transmitted from cell to cell much more easily[73, 92, 188, 205, 209]. All of the above mentioned has led to the hypothesis that ALS, similar to other neurodegenerative diseases like Parkinson's and Alzheimer's, may be considered as a prion-like disease and that inhibiting protein propagation may be a valuable therapeutic option[92, 152, 161, 174].

1.1.4 Treatment

As of today, there is no effective cure for ALS. Best results are achieved in a multidisciplinary approach involving neurologists as well as other disciples e.g. psychiatrists, nutritionists and physio-therapists[28, 92, 195, 233]. Next to symptomatic medical treatments like anti-depressants, opioids and such the US Food and Drug Administration (FDA) has two drugs approved for ALS: Riluzole and Edavarone. Riluzole was approved in 1995 and increases survival by ~3 month in the original trial[26, 128]. Although it has been shown to act as a glutamate antagonist, other glutamate antagonists did not show positive results[9], suggesting a different, unknown, mechanism of action. On the other hand, Edavarone, which is supposed to act as an antioxidant, only has an effect in a highly selected cohort (early onset, fast progression) but is not approved as an ALS drug by the European Medicines Agency (EMA)[93]. Many more potential therapeutics have since then been tested in rodents but none have showed sufficient effects in clinical trials[18, 63, 92, 131]. Still new therapies are being explored, such as a stem cell based approach currently in a phase II clinical study [182] and many studies show promising effects e.g. modulating aggregates[39, 133, 149] among other approaches [18, 21, 59, 227].

1.2 Transactive response DNA binding protein 43 kDa (TDP-43)

1.2.1 Physiological function

TDP-43 was first described in 1995 as a transcriptional activator of the HIV-1 gene[178] and is encoded by the *TARDBP* gene. It is a ubiquitously expressed member of heterogenous nuclear ribonucleoproteins (hnRNP)-family and is involved in RNA processing[43, 82] . TDP-43 can bind RNA and influence its turnover and stability[29, 64, 206]. Additionally, it can interact with the Drosha/Dicer complex and is hence involved in the biogenesis and processing of microRNAs[42, 117, 142, 262]. Around 30% of the entire transcriptome are considered potential targets of TDP-43[186] including low molecular weight neurofilament (hNFL) and other proteins involved in neuronal development[186, 222, 230]. TDP-43 also has the ability to bind to its own mRNA, resulting in a feedback loop strongly regulating its own expression levels[15].

To unfold its molecular function, TDP-43 can shuttle between cytoplasm and nucleus[16]. A process that might be disturbed by TDP-43 aggregation via two separate mechanisms. Either by leading to reduced expression levels due to high protein levels in the cytoplasm and ensuing nuclear depletion and loss of TDP-43 function or a loss of autoregulation which would result in elevated TDP-43 expression and more aggregates[38, 134, 141]. TDP-43 also associates with RNA to form granules that can transport mRNAs across longer distances in a cell , e.g. along axons,[4]. It is also recruited to SGs, which form reversibly upon cellular stress in eukaryotic cells to safely store or selectively sequester mRNAs[14, 27, 56, 71, 94, 118].

TDP-43 is also able to interact with DNA directly. Recently it was found that TDP-43 plays an important role in DNA double strand repair by recruiting classical non-homologous end joining repair factors to the break sites[163]. Lastly, TDP-43 is also able to interact directly with other proteins. Many of these binding partners are RNA/DNA binding proteins themselves[32, 82], others are involved in proteostasis like HSP 40/70 [179, 236] or are associated with mitochondria such as Prohibitin-2 (PHB2) and Mitofusin-2 (MFN2)[55, 67, 250].

1.2.2 TDP-43 structure

TDP-43 is highly conserved in eukaryotic species[240]. It is a 414 amino acid long and 43 kDa heavy protein that is ubiquitously expressed. It has a nuclear localization sequence (NLS) and a nuclear export sequence (NES) as well as two RNA recognition motives (RRM1&2) and a c-terminal low complexity domain (Fig. 1). While TDP-43 is predominantly a nuclear protein, the NES and passive diffusion towards the cytosol as well as the NLS allow nucleocytoplasmic shuttling[16, 184]. Consequently, mutations in the NLS lead to enhanced cytoplasmic aggregation[22, 205, 249]. Under physiological conditions, TDP-43 is in a monomer-dimer equilibrium[209] mediated by the first ten residues of the N-terminus[259]. While dimerization is a necessary step for its interaction with RNAs it may also play a role in aggregation[2, 106, 199, 259].

Next to their ability to bind RNAs (see 1.2.1), the RRM1 and RRM2 are also involved in SG formation and autoregulation [214] as well as TDP-43 dimerization, respectively[125, 188, 243]. However, most protein-protein interactions are mediated by the c-terminal domain of TDP-43. This glycine rich domain is highly prone to aggregation and self -interaction[109, 124, 161, 198, 205] and contains a prion-like domain. Additionally, it is able to induce conformational changes of native TDP-43[48, 161] and forms amyloid like fibrils *in vitro*[90, 105], either as full length TDP-43 or just as a c-terminal fragment[72, 105, 161, 261]. The c-terminal region is also involved in SG formation having the ability to undergo liquid-liquid phase separation (LLPS)[58, 180]. Interestingly, most ALS-associated mutations in TDP-43 are clustered in this domain[214].

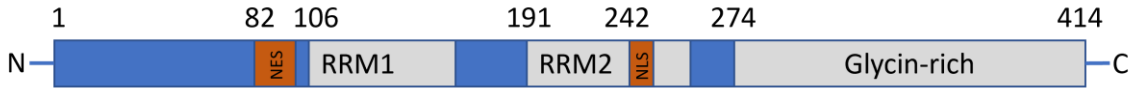

Figure 1: TDP-43 and its different regions
RRM: RNA recognition motive. NES: nuclear export sequence. NLS: nuclear localisation sequence

1.2.3 TDP-43 in ALS

As mentioned before, over 97% of ALS patients display aggregates containing TDP-43. That, as well as the fact that mutations in TARDBP are linked to ALS, suggest a high relevance of TDP-43 in ALS pathology[188]. As described previously (see 1.2.1), TDP-43 is closely linked to a multitude of functions inside living cells. Unsurprisingly, numerous different pathways have been associated to be affected by TDP-43 so far (Fig. 2).

TDP-43 is closely involved in protein homeostasis which seems to be mainly attributed to an abnormal turnover due to aggregation and mis-localization[40, 189]. TDP-43 itself is prone to aggregate[98, 174, 249] and as mentioned previously TDP-43 aggregates are one of the most prominent hallmarks of ALS pathology. But while disease associated mutations may induce or enhance aggregation, self-seeding and cross-seeding[49, 89, 107] studies have reported TDP-43 associated toxicity without aggregate formation[11, 99, 188], shifting the focus away from big, microscopically visible aggregates and towards smaller oligomeric aggregates as the main toxic species[73, 188, 209]. ALS linked mutations in UBQLN2 result in impaired autophagy and increased TDP-43 aggregation[78, 177]. Vice versa, enhanced autophagy reduces toxicity in a TDP-43 mouse model and neurons[21, 23, 205]. Furthermore, high TDP-43 levels seem to inhibit endocytosis in ALS patients and disease models[144] probably by altering the number of recycling endosomes in neurons[203]. Enhancing endocytosis also reduced the amount of TDP-43 aggregates[144].

Another characteristic is the post-translational modification, misfolding and cleavage of TDP-43 inside of aggregates and oligomers. Phosphorylation and ubiquitination are the two most prominent post-translational modifications observed in ALS patients[10, 170]. The most prominent phosphorylation site is s409/410[169, 188]. Phosphorylated TDP-43 has an increased half-life and is associated with misfolding, aggregation, increased insolubility and toxicity[53, 174, 205]. Ubiquitination has been reported to be both, beneficial and harmful[91, 95, 134] and was shown to facilitate TDP-43 accumulation[95, 207]. Cleavage of TDP-43 into c-terminal fragments (CTF's) and n-terminal fragments has been observed as early as 2006[170, 246, 261] and may be one of the most prominent toxic factors[41, 72, 98, 263]. Especially CTF's have been observed to enhance aggregation, impair degradation of TDP-43 and ultimately increase cellular toxicity[114, 261].

As TDP-43 assembles physiologically in SGs, it has been suggested that altered LLPS, which is closely linked to SG formation[164, 192], may lead to TDP-43 aggregates[211].

Especially upon a higher protein to RNA ratio, TDP-43 has been shown to undergo liquid-solid state transition[151] leading to the hypothesis that prolonged stress in cells may ultimately lead to stable aggregate formation[14, 71]. Possible resulting cytosolic aggregates are also linked closely to nuclear depletion, which typically occurs alongside aggregation[10, 170]. However, it is suggested that nuclear depletion already occurs prior to aggregate formation[134].

TDP-43 is not only involved in the (oxidative) stress response of cells but may contribute to oxidative stress and toxicity as well. A key origin of ROS and oxidative stress in general are mitochondria. Mitochondrial damage has been reported to be especially involved in neuronal vulnerability[200] with neurons relying mainly on oxidative phosphorylation to ensure the high ATP supply needed to maintain ion gradients and Ca^{2+} homeostasis[216]. ALS patients, as well as many disease models, show multiple signs of mitochondrial dysfunction[224, 241, 242]. A yeast model with TDP-43 aggregation shows increased oxidative stress, toxicity and ROS formation that can be rescued upon adding GSH monoethyl ester[50]. The exact mechanistic flow is still under investigation but it is now recognized that TDP-43 colocalizes within mitochondria and that mutations of TDP-43 can lead to damage of mitochondria[97]. TDP-43's toxic gain of function can be reduced by impairing mitochondrial localization of TDP-43[242]. Additionally, mutant TDP-43 disrupts the mitochondrion-ER connection and reduces Ca^{2+} uptake[223].Oxidative stress on the other hand may lead to a reduced nucleic acid binding ability of TDP-43 and subsequent aggregation[45], resulting in a vicious cycle of increasing oxidative damage.

Next to cellular toxicity, TDP-43 may also play a vital role in progression of ALS as TDP-43 is being attributed with prion-like properties much like α-synuclein or Amyloid-ß in Parkinson's disease (PD) and Alzheimer's disease (AD), respectively[96, 152, 161]. TDP-43 aggregates and fibrils have been shown to induce aggregation in recipient cells nearly a decade ago[84, 175] and especially c-terminal TDP-43 fragments and the RRM2 of TDP-43 are able to form skein-like structures that form fibrils *in vitro*[3, 89, 129, 229]. Furthermore it was shown that TDP-43 is transferred from cell to cell in co-culture and seeding assays and can even propagate via axon terminals[75, 129, 258]. In mice, TDP-43 pathology can be induced by injecting TDP-43 isolated from patients' brains[129, 187]. This pathology spreads, much alike to what is known from patients, throughout the brain of these mice[102, 187, 210, 213]. Therefore it is now recognized, that TDP-43 propagation does most likely

play a vital role in disease progression, and blocking its uptake, propagation or seeding potential may be a potential therapeutic option[18, 161, 188].

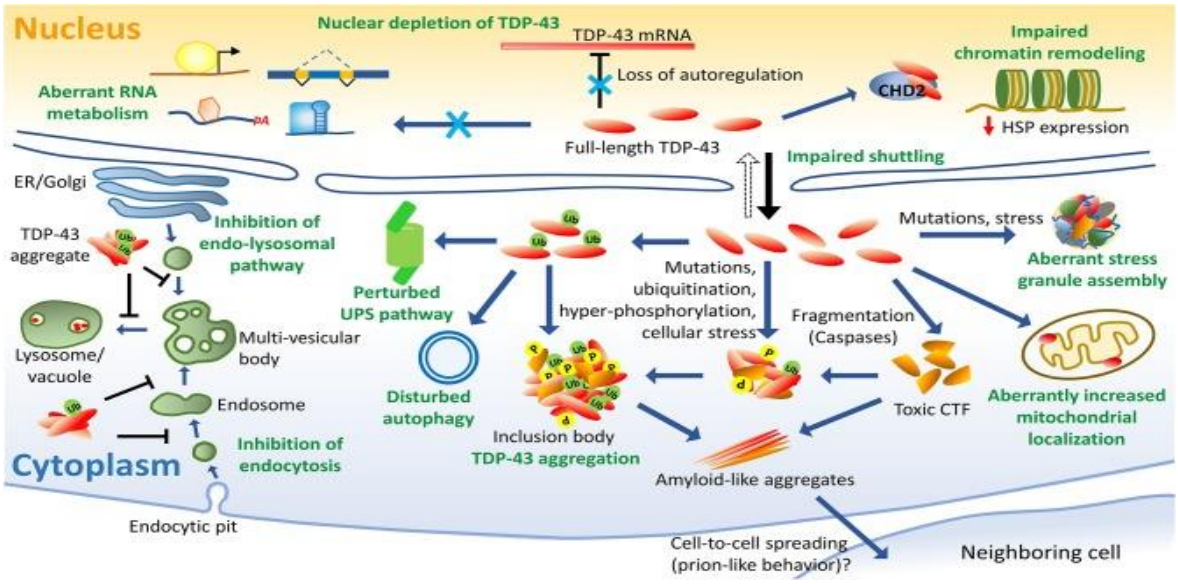

Figure 2: Overview of TDP-43 induced pathology (As published by Prasad et al., 2019[188])
Illustration of the main aspects of TDP-43 associated functional impairment, interactions and means of toxicity (written in bold green). Loss of the primarily nuclear protein results in an array of mis-regulated nuclear functions like impaired RNA/DNA metabolism. A key event in pathology is impairment of nuclear shuttling, resulting in TDP-43 accumulation in the cytoplasm.

1.3 Immunization in neurodegenerative disease

In neurodegenerative diseases a common trait is the aggregation of key proteins. These can be used as targets for active immunization that results in the formation of antibodies targeted against said protein, similar to how surface proteins of viruses are used in common vaccines. In 1999, the first immunization studies were conducted in an AD mouse model yielding positive results on outcome and disease progression[104, 165, 201]. Shortly after, active immunization also showed prolonged life in mouse models of prion disease and PD[157, 212]. Over time, immunization approaches became more specific using antibodies against phospho-tau, mutant SOD1 and human α-synuclein yielding positive results in different rodent models[13, 197, 237]. Tau immunization has since been further developed[30]

resulting in a so far successful phase II clinical trial for one tau vaccine in patients with mild AD[116, 176]. However, in the last few years severe side effects such as encephalitis and neuroinflammatory responses have been reported in patients treated with active immunization strategies, even resulting in the end of a clinical study on A-ß antibodies[17, 86, 172, 194].

Therefore, passive immunization has been suggested to yield a better risk to benefit ratio. Hereby antibodies against a desired protein or epitope are directly administered to the patient. For this strategy to be effective, antibodies have to be able to penetrate the blood brain barrier and enter neurons in concentration levels sufficient to have an impact. By now multiple studies have shown that intraperitoneally administered antibodies do in fact enter the central nervous system (CNS)[17, 20, 158, 232, 255] and are taken up by neurons, most likely via the Fcγ-receptor[57], opening up the possibility of passive immunization in neurodegenerative diseases. The effects of passive immunization in neurodegenerative diseases, most prominently PD and AD, have been extensively studied. For AD, positive effects have even been attributed to antibodies against tau as well as Amyloid-ß peptide. Tau antibodies were shown to block aggregate formation and propagation [120, 256] and both, Amyloid-ß and tau antibodies were also able to induce aggregate clearance and reduce pathology in mouse models[20, 247, 255].

Since then multiple clinical trials for Amyloid-ß and tau specific approaches have started and Amyloid-ß targeted antibodies already entered in phase III clinical trials[116]. In PD, α-synuclein antibodies have shown first success in early studies. These antibodies reduce aggregates and propagation[17, 157, 194] and improve the phenotype of mice injected with preformed α-synuclein fibrils[232]. So far, one antibody has shown slightly reduced α-synuclein levels in a phase Ib clinical study[103], and has been approved for phase II.

Interestingly, the means by which antibodies are influencing a disease differ depending on the antibody but one antibody may also work in different, potentially additive, ways. Different tau and α-synuclein antibodies have been shown to inhibit neuronal propagation *in vitro* and *in vivo*[120, 171, 232, 255, 256]. Microglial uptake has also been shown to be hampered by tau antibodies depending on the antibody and aggregate size[83]. Closely associated to the propagation is the seeding ability and aggregate formation of these proteins, which may also be altered by different antibodies as has been shown in *in vitro* and *in vivo* models[158, 171, 256]. Li *et al.* recently discovered that α-synuclein antibodies derived from

PD patients are also able to block α-synuclein seeding *in vitro* and co-stain with Lewy pathology in patients' brains[139].

Finally, antibodies are also suggested to be involved in the intracellular clearance of existing aggregates via autophagy or lysosomal degradation. Many studies recognised a reduction in aggregate load combined with enhanced autophagy[20, 57, 116, 139, 158, 232]. A recent study emphasized this by linking an intrabody specifically binding to misfolded TDP-43 to a proteasome sequence, thereby further enhancing degradation[227]. Taken together, these findings further emphasize passive immunization against TDP-43 as a viable therapeutic strategy.

1.4 Antibodies used in this thesis

To cover a broad spectrum of binding sites we decided to use four different, commercially available antibodies detecting human TDP-43. The first antibody was purchased from Proteintech (catalogue number: '12892-1-AP') that binds to the c-terminal region of TDP-43 (amino acids (AA) 288-414; see Fig. 3) and is able to detect CTF's[87]. It is in the following referred to as the 'c-terminal' TDP-43 antibody. As discussed previously, both the c-terminal region and CTF's appear to play a central role in aggregation, seeding and cellular transmission. With the n-terminal region getting more attention and being important in self-assembly, we decided to use another antibody from Proteintech (catalogue number: '10782-2-AP') that targets n-terminal residues of TDP-43 (raised against AA 1-260; see Fig. 3) which will be referred to as the 'n-terminal' TDP-43 antibody[87]. The third antibody we used is a monoclonal antibody from Abnova (Catalogue number: 2E2-D3/ H00023435-M01) and was raised against the RRM2 of TDP-43 (AA: 192-262; see Fig. 3) and will be referred to as 'RRM2'. The RRM2 has been reported to form amyloid like skeins and may play a role in aggregation[87, 161, 229]. Lastly, we used an antibody specifically binding phosphorylated TDP-43 (pTDP-43; catalogue number: 22309-1-AP from Proteintech) since pTDP-43 is a key feature of pathological TDP-43 aggregates. It is targeted against phosphorylated Serine residues 409 and 410 (pTDP-s409/410; see Fig. 3), two characteristic phosphorylation sites of TDP-43[87, 169, 213]. As controls we used an unconjugated polyclonal rabbit IgG and a heat denaturated TDP-43 antibody.

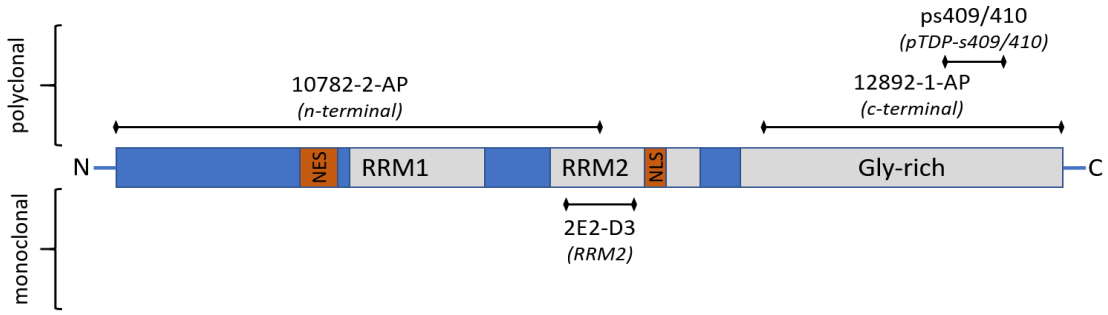

Figure 3: TDP-43 and binding sites of the antibodies used in this thesis
RRM: RNA recognition motive. NES: nuclear export sequence. NLS: nuclear localisation sequence

1.5 Aim of the thesis

Until now, there is no cure for amyotrophic lateral sclerosis and the available therapeutics show minimal, if any, effect on reducing severity or progression of this fatal disease. The role of TDP-43 as a major component of ALS pathology has been established over the last couple of years. Cumulating evidence also suggests a prion-like behaviour of TDP-43, very similar to key proteins in other neurodegenerative disease like tau in Alzheimer's or α-synuclein in Parkinson's. In both diseases antibody mediated therapies look promising, not only in disease models but also in first clinical trials. These antibodies are targeted against key proteins and seem to have a positive impact on aggregation and propagation of pathology. Even though TDP-43 antibodies have been suggested as a promising therapeutic strategy, little data has been published on their influence on TDP-43 aggregation and propagation.

To address this topic, we used established antibodies targeting different parts of TDP-43 and investigated their effect on TDP-43 aggregation and solubility in a cellular model. To quantify aggregate formation in cells, we intended to use a highly sensitive protein complementation assay for TDP-43 to detect differences in oligomer formation. Keeping in mind a potential therapeutic use of TDP-43 antibodies, another key question to answer was whether these antibodies had any influence, on cellular toxicity. Lastly, we used the same complementation assay to assess whether TDP-43 antibodies were capable of interfering with cell-to-cell spreading of TDP-43 as this would open up another potential therapeutic use in slowing down disease progression.

2. Material and Methods

2.1 Materials

2.1.1 Antibodies

Epitope	Origin	Catalog number	Manufacturer
c-terminal TDP-43	rabbit/ polyclonal	12892-1-AP	proteintech
n-terminal TDP-43	rabbit/ polyclonal	12892-2-AP	proteintech
TDP-43, AA 205-222	mouse/ monoclonal	H00023435-M01	Abnova
phospho-TDP (s409/410)	rabbit/polyclonal	22309-1-AP	proteintech
unconjugated IgG	rabbit/ polyclonal	910801	BioLegend
Alexa Fluor 568 anti-rat	goat	A-11011	life technologies
Alexa Fluor 568 anti-mouse	goat	A-11004	life technologies
Alexa Fluor 647 anti-rabbit	goat	A-27040	life technologies
Anti-HA high affinity	rabbit	GR3245707-1	Abcam
Anti-HA high affinity	rat	11867423001	Roche
GAPDH	10494-1-AP	rabbit	proteintech
anti-rabbit IgG, HRP conjugated	W401B	goat	Promega

2.1.2 Chemicals and reagents

Chemical or Reagent	Catalog number	Manufacturer	Location
Ampicillin Sodium Salt BioChem	A0839	AppliChem GmbH	Darmstadt, Germany
Bromphenol blue	A512.1	Carl Roth GmbH	Karlsruhe, Germany
Bovine Serum Albumin	K41-001	GE Healthcare	Chicago, IL, USA
Calcium Chloride ($CaCl_2$)	223506	Sigma-Aldrich GmbH	Steinheim, Germany
Coelenterazine	102172	PJK GmbH	Kleinbittersdorf, Germany

Dextrose	G7528	Sigma-Aldrich	St. Louis, MO, USA
Dimethyl sulfoxide (DMSO)	D8418	Sigma-Aldrich	St. Louis, MO, USA
Disodium phosphate (Na_2HPO_4)	P030.2	Carl Roth GmbH	Karlsruhe, Germany
Dulbecco's Modified Eagle Medium (DMEM)	41965-039	Gibco, life technologies	Carlsbad, CA, USA
Dulbecco's Modified Eagle Medium (DMEM) + GlutaMAX™ -I	31966-021	Gibco, life technologies	Carlsbad, CA, USA
Dulbecco's Phosphate Buffered Saline (PBS)	14190/094	Gibco Invitrogen	Carlsbad, CA, USA
Ethanol	32205	Sigma-Aldrich	St. Louis, MO, USA
Ethylenediaminetetraacidic acid (EDTA)	A3553	AppliChem GmbH	Darmstadt, Germany
Fetal Calf Serum (FCS) gold plus	GP0500	Bio&Sell GmbH	Nürnberg, Germany
Glycin	33226	Sigma-Aldrich	St. Louis, MO, USA
HEPES	H3375	Sigma-Aldrich	ST. Louis, MO, USA
Hoechst 33342, Trihydrochloride, Trihydrate in H_20	H3570	Thermo Fisher Scientific	Rockford, IL, USA
TrypLE™ Express Enzyme	12604013	Thermo Fisher Scientific	Rockford, IL, USA
Isopropanol	20842.330	VWR International	Radnor, PA, USA
LB-Medium	X968.2	Carl Roth GmbH	Karlsruhe, Germany
Methanol	32213	Sigma-Aldrich	St. Louis, MO, USA
N, N, N′, N′-Tetramethylethylendiamin (TEMED)	2367.3	Carl Roth GmbH	Karlsruhe, Germany
Nonidet® P40 (NP-40)	A1694	AppliChem GmbH	Darmstadt, Germany
Opti-MEM®	11058-021	Gibco, life technologies	Carlsbad, CA, USA
Pen/Strep	P11-010	GE Healthcare	Chicago, IL, USA

4% PFA phosphate buffered	PF101	FD NeuroTechnologies, Inc.	Columbia, MD, USA
Poly-D-Lysin	P6407	Sigma-Aldrich	St. Louis, MO, USA
Ponceau S	5938.2	Carl Roth GmbH	Karlsruhe, Germany
Potassium chloride (KCL)	P9541	Sigma-Aldrich	St. Louis, MO, USA
ProlongGold antifade reagent with DAPI	P36931	Invitrogen	Carlsbad, CA, USA
su®-Block (10x)	A151.2	Carl Roth GmbH	Karlsruhe, Germany
Rotiphorese® Gel 30 (37,5:1)	3029.2	Carl Roth GmbH	Karlsruhe, Germany
Sodium Chloride (NaCl)	A3597	AppliChem GmbH	Darmstadt, Germany
Sodium Deoxycholate	A1531	AppliChem GmbH	Darmstadt, Germany
Sodium dodecylsulfate (SDS)	A1502	AppliChem GmbH	Darmstadt, Germany
Sorbitol	S1876	Sigma-Aldrich	St. Louis, MO, USA
Trichloroacetic acid (TCA) 20%	7437.1	Carl Roth GmbH	Karlsruhe, Germany
Tris	93362	Sigma-Aldrich	St. Louis, MO, USA
Tris-HCL	9090.3	Carl Roth GmbH	Karlsruhe, Germany
TritonX-100	T8787	Sigma-Aldrich	St. Louis, MO, USA
Tween-20	BP337-500	Thermo Fisher Scientific	Rockford, IL, USA
Urea	33247	Sigma-Aldrich	St. Louis, MO, USA

2.1.3 Consumables

Consumable	Catalog number	Manufacturer	Location
96-well, clear	655098	Greiner bio-one	Gremsmünster, Österreich
96-well, white	655073	Greiner bio-one	Gremsmünnster, Österreich
24-well	3526	Corning, Inc.	Corning, NY, USA
6-well	83.3920	Sarstedt AG & Co.KG	Nümbrecht, Germany
T75cm^2 flask	83.3911.002	Sarstedt AG & Co.KG	Nümbrecht, Germany
T175cm^2 flask	83.3912.002	Sarstedt AG & Co.KG	Nümbrecht, Germany
Biosphere Filter Tips (2; 20; 200; 1100 µl)	70.1130.212; 70.760.213; 70.760.211; 70.1186.210	Sarstedt AG & Co.KG	Nümbrecht, Germany
Pipet Tips (10; 200 µl)	70.1115; 70.760.002	Sarstedt AG & Co. KG	Nümbrecht, Germany
Pipet Tips (1000 µl)	83007-376	VWR International	Radnor, PA, USA
Multiflex round tips 1-200 µl	28480	Sorensen Bioscience, Inc.	Salt Lake City, UT, USA
Serological pipettes (5; 10; 25 ml)	86.1253.001; 86.1254.001; 86.1685.001	Sarstedt AG & Co. KG	Nümbrecht, Germany
Cassettes 1.0 mm	NC2010	Novex® by life technologies	Carlsbad, CA, USA
Cassettes 1.5 mm	NC2015	Novex® by life technologies	Carlsbad, CA, USA
Blotting Filter Paper 2.5mm thick	LC2008	Invitrogen	Carlsbad, CA, USA
Amershan Protran 0.2µm Nitrocellulose membrane	10600006	GE Healthcare	Chicago, IL, USA
Filtrapur S 0.2 µm	83.1826.001	Sarstedt AG & Co. KG	Nümbrecht, Germany
BD Plastipak^{TM} 20 ml	300629	Beckton, Dickinson and Company	Franklin Lakes, NJ, USA

Tube 50 ml, PP	62.559.001	Sarstedt AG & Co. KG	Nümbrecht, Germany
Cellstar tubes 15 ml, PP	188271	Greiner bio-one	Gremsmünster, Österreich
Reaction tube 2.0 ml, SafeSeal	72.695.400	Sarstedt AG & Co. KG	Nümbrecht, Germany
Reaction tube 1.5 ml, Safe Seal	72.706.400	Sarstedt AG & Co. KG	Nümbrecht, Germany
PCR single cap 8er Soft Strips, 0.2 ml, clear	710970	Biozym Scientific GmbH	Hessisch Oldendorf, Germany
Microfuge Tube 1.5 ml, PP	357448	Beckman Coulter	Brea, CA, USA
Microscope slides	631-1553	VWR Collection	Darmstadt, Germany
Cover slips	631-0148	VWR Collection	Darmstadt, Germany

2.1.4 Buffers and Solutions:

2.1.4.1 Microbiology

Bacteria Cultures	Buffer/Solution	Ingredients
	LB-medium	25g in 800 ml H_2O > autoclave, store at 4°C

2.1.4.2 Cell culture

Media	Buffer/Solution	Ingredients
	Culture medium HEK293	DMEM + 10% FCS
	Freezing medium	DMEM + 10% DMSO
	Stress Medium	Opti-MEM + 400 mM Sorbitol

Coating	Buffer/Solution	Ingredients
	Poly-D-Lysine	5 mg diluted in 5 ml H_2O > 1 mg/ml (frozen as stock)

Transfection	Buffer/Solution	Ingredients
	2x HBS	280 mM NaCl +10 mM KCl +1.5 mM Na_2HPO_4 +12 mM Dextrose + 50 mM HEPES > pH 7.04, sterile filtered; stored at -20°C in H_2O
	2.5 M $CaCl_2$	> sterile filtered; stored at -20°C

2.1.4.3 Biochemical

Centrifugal fractionation	Buffer/Solution	Ingredients
	RIPA-Buffer	50 mM Tris + 150 mM NaCl + 0.25% Sodium-deoxycholate + 1% NP-40 + 1 mM EDTA > pH 7.4
	UREA-Buffer	10 mM Tris + 8 M Urea > pH 8

SDS-Page	Buffer/Solution	Ingredients
	Solution A	Rotiphorese® Gel 30 > storage in the dark
	Solution B	1.5 M Tris > pH 8.8
	Solution D	0.5 M Tris > pH 6.8
	10 % (w/v) APS	APS in H_2O > storage at 4°C
	4x LDS	106 mM Tris-HCl 141 mM Trisbaseq 2% LDS 10% Glycerol 0,51 mM EDTA 0,22 mM Serva Blue G250 0,175 mM Phenol Red > dilute in H_2O and store at 4°C
	DTT	100 mM in H_2O

	SDS running buffer	3.03 g/l Tris + 14.4 g/l Glycin + 1 g/l SDS > no pH adjustment

Western Blot	Buffer/Solution	Ingredients
	Blot buffer	3.03 g/l Tris + 14.4 g/l Glycin + 20% Methanol > no pH adjustment needed
	1x TBS-T	150 mM NaCl + 10 mM Tris + 0.1% (v/v) Tween® 20 > pH 7.4
	Ponceau S	0.2% (w/v) Ponceau S + 0.3% (v/v) trichloroacetic acid
Immunocytochemistry	**Buffer/Solution**	**Ingredients**
	4% PFA	4% PFA in PBS
	Blocking solution	PBS +10% NGS +0.1% TritonX
	Permeabilization solution	PBS +0.1% TritonX-100

2.1.5 Technical equipment

Technical Equipment	Product	Manufacturer	Location
Bacteria shaking incubator	GFL 3033	GFL – Gesellschaft für Labortechnik GmbH	Burgwedel, Germany
Bench	HERA safe	Thermo Fisher Scientific	Rockford, IL, USA
Cell incubator	HERA cell	Thermo Fisher Scientific	Rockford, IL, USA
Centrifuges	MIKRO 22 R	Hettich Zentrifugen	Tuttlingen, Germany
	MICRO STAR 17R	VWR, Part of Avantor	Radnor, PA, USA

	Heraeus Fresco 21 Centrifuge	Thermo Fisher Scientific	Rockford, IL, USA
	MiniStar Silverline	VWR, Part of Avantor	Radnor, PA, USA
	Allegra® 25 R	Beckman Coulter	Brea, CA, USA
	Optima XPN-80 Ultracentrifuge + 70.1 Ti Rotor	Beckman Coulter	Brea, CA, USA
Confocal microscope	Leica TCS SP5 + Leica x63 lens	Leica Microsystems GmbH	Wetzlar, Germany
Haemocytometer	Neubaur Improved bright-line, 0.1 mm	Paul Marienfeld GmbH & Co. KG	Lauda-Königshofen, Germany
Microscope	Axio Observer.A1 Microscope	Carl Zeiss Microscopy GmbH	Oberkochen, Germany
	Leica DM IL	Leica Microsystems GmbH	Wetzlar, Germany
Pipetting aid	Pipetus®	Hirschmann Laborgeräte GmbH & Co. KG	Eberstadt, Germany
pH Meter	Multical®	WTW Wissenschaftlich-Technische Werkstätten GmbH	Welheim, Germany
Photometer	NanoDrop-2000 Spectrophotometer	Thermo Fisher Scientific	Rockford, IL, USA
Plate Reader	SpectroStar Nano	BMG Labtech	Ortenberg, Germany
	2030 Multilabel Reader Viktor™ X3	Perkin Elmer, Inc	Waltham, MA, USA
Shaker	KS-15	Edmund Bühler GmbH	Bodelshausen, Germany
Sonicator	SonoPuls GM 70 + MS 72 Sonotrode	Bandelin	Berlin, Germany
Scales	Scout II	Ohaus Corporation	Parsippany, NJ, USA
	Adventurer™ AR0640	Ohaus Corporation	Parsippany, NJ, USA

Thermomixer	MixingBlock MB-12	BIOER	Hangzhou, China
Ultrapure water system	Arium® pro VF	Sartorius Stedim biotech	Göttingen, Germany
Western Blot Development	Fusion Solo 4 S	Vilber Lourmat	Collégien, France
Western Blot Equipment	NuPAGE Gel System	Thermo Fisher Scientific	Rockford, IL, USA
	XCell II™ Blot Module	Thermo Fisher Scientific	Rockford, IL, USA
	XCell SureLock™ Blot Module	Thermo Fisher Scientific	Rockford, IL, USA
Western Blot Power Supply	PeqPOWER 250 V	Peqlab Biotechnologie GmbH	Erlangen, Germany

2.1.6 Kits

Kit	Catalog number	Manufacturer	Location
Pierce™ BCA Protein Assay Kit	23225	Thermo Fisher Scientific	Rockford, IL, USA
CytoTox 96® Non-Radioactive Cytotoxicity Assay	G1780	Promega	Madison, WI, USA
Lipofectamine™ LTX and PLUS™	15338030	Thermo Fisher Scientific	Rockford, IL, USA
Vybrant® MTT Cell Proliferation Assay	V13154	Thermo Fisher Scientific	Rockford, IL, USA
QIAGEN Plasmid Mini Kit	27106	Qiagen	Hilden, Germany
QIAGEN Plasmid Maxi Kit	12163	Qiagen	Hilden, Germany
SuperSignal® West Pico Chemiluminescent Substrate	34577	Pierce/Thermo Fisher	Rockford, IL, USA

2.1.7 Proteins:

Protein	Catalog number	Manufacturer	Location
PageRuler^{TM} Prestained Protein Ladder	26616	Thermo Fisher Scientific	Rockford, IL, USA

2.1.8 Software:

Name	Version	Provider	Location
MARS Data Analysis Software		BMG Labtech GmbH	Ortenberg, Germany
GraphPad Prism		GraphPad Software, Inc	San Diego, CA, USA
Fusion X		Vilber Lourmat	Collégien, France

2.2 Methods

2.2.1 Cell culture

2.2.1.1 Culturing of cells

DMEM supplemented with 10% FCS was used as a growth medium for human embryonic kidney 293 cells (HEK-293). Cells were cultivated under standard conditions at 37°C and 5% CO_2. To ensure optimal growth conditions, growth medium was changed every 3-4 days. Therefore, the medium was discharged completely and replaced by 8 ml or 16 ml of fresh growth medium for 75 cm^2 and 175 cm^2 flasks, respectively.

2.2.1.2 Passaging of cells

To keep the cells from growing in multiple layers and maintain healthy, cells were passaged regularly. To do so, cells were split when they were at approximately 75% confluency. The old media was discharged completely and 1-2 ml of Trypsin (TrypLE^{TM} Express, Thermo Fisher Scientific, Rockford, IL, USA) were added and spread out evenly across the cells. After 3-5 minutes incubation in the incubator, cells were diluted with growth medium, added to stop trypsination. Depending on their growth rate and the number of cells needed for subsequent experiments, they were split 1:5 or 1:10.

2.2.1.3 Preparation of plates prior to seeding

To ensure optimal adhesion and cell growth, wells were coated with a final concentration of 15µg/ml Poly-D-Lysine (PDL) diluted in water prior to seeding. PDL was added to each well and incubated for 1hour at 37°C. Afterwards wells were washed three times prior to use. If coverslips were used, they were added to relevant wells prior to coating. The respective volumes for coating and washing are stated in Table 3.

2.2.1.4 Seeding cells

Culture plates were prepared as described above. Afterwards a set number of cells were added according to Table 3, 24 hours prior to experiments:

Table 3: HEK293 cell numbers, growth area and respective culture volumes

Plate	Approx. surface area [cm^2]	Seeding density	Culture medium [ml]
96 well	0.32	$2*10^4$	0.1
24 well	1.9	$3*10^4$	0.5
6 well	9.6	$2*10^5$	1
100 mm	56.7	$3*10^6$	10

2.2.1.5 Transient transfection of HEK-293 cells

2.2.1.5.1 Calcium-phosphate mediated transfection

HEK-293 cells were transfected using the calcium phosphate method. Hereby, DNA and precipitated crystals of calcium phosphate form complexes that are then taken up by the cells [110].

Cells were transiently transfected with the respective plasmids needed for the experiments 24 hours after seeding. Using the respective volumes shown in Table 4, water, DNA, 2.5M $CaCl_2$ and 2x HEPES Buffered saline (HBS) were incubated for one minute at RT. Subsequently, DMEM + 2% FCS was added to the transfection mixture, old media was removed, and the transfection mix was added to the cells. 12 hours after transfection, cells were washed once with PBS double the volume used for transfection before adding fresh culture medium. If the cells were to be used for luciferase detection, colourless Opti-MEM was used instead of regular DMEM.

Table 4: Reaction mixes for calcium phosphate transfection

Plate	DNA [µg]	H_2O [µl]	2.5 M $CaCl_2$ [µl]	2x HBS [µl]	DMEM + 2% FCS [µl]
96 well	0.2	5.5	0.4	4	80
6 well	3.0	100	11.5	115	1800
100 mm	15	450	50	500	9000

2.2.1.5.2 Lipofectamine™ LTX and PLUSTM Reagent-based transfection

For ICC experiments, Lipofectamine™ LTX with PLUS™ Reagent (Thermo Fisher Scientific, Rockford, IL, USA) was used as a transfection agent for HEK-293 cells according to the manufacturer's protocol. In short, for each well of a 24 well plate with cover slips 0.5 µg of the respective plasmid were mixed with PLUS™ Reagent and Opti-MEM and

incubated for 20 minutes at RT. Afterwards, LTX was diluted in Opti-MEM and added to the DNA mix. After incubating for 30 minutes at RT 100µl of the transfection mix were added to each well. Here, a medium change was not necessary.

2.2.1.6 Induction of cellular stress

Cells were stressed to form aggregates and stress granules, features regularly observed in ALS and other TDP-43 associated diseases [[36, 181]].

Sorbitol is known to induce oxidative stress in cells and hence lead to stress granule formation and protein aggregates with properties of TDP-43 pathology [[71]]. Therefore, HEK-293 cells were incubated with 0.4M D(-)-Sorbitol dissolved in Opti-MEM for 2 hours at 37°C and 5% CO_2.

2.2.2 Biochemical methods

2.2.2.1 Quantification of Protein concentrations

2.2.2.1.1 BCA Protein Assay

The Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL, USA) was used to determine protein concentration of cell lysates. It uses a protein's ability to reduce Cu^{2+} to Cu^{1+} in the so-called biuret reaction[88]. In this assay, the biuret reaction is coupled to the colourimetrically detectable reagent bicinchoninic acid (BCA) that changes its colour from green to purple upon chelation with Cu^{1+} [217]). The purple-coloured reaction product exhibits an absorbance maximum at 562 nm that is linear with increasing protein concentration. According to manufacturer's description, working solution was prepared by mixing BCA Reagent A with BCA Reagent B in a ratio of 50:1. As standard, BSA was serially diluted in H_2O with concentrations ranging from 0-2000 µg/ml (0; 125; 250; 500; 750; 1000; 1500; 2000). 20 µl of the standards as well as the samples were pipetted in duplicates into a clear 96-well plate. 200 µl of working solution were added per well and the entire plate was incubated at 37°C for 30 minutes. Using a plate reader, the absorbance at 562 nm was measured and protein concentration evaluated using the BSA standard curve and MARS software (BMG Labtech, Ortenberg, Germany).

2.2.2.2 Cytotoxicity assays

2.2.2.2.1 MTT-assay

Viable cells contain NADPH-dependent oxidoreductase enzymes. These enzymes are utilised to reduce the tetrazolium salt MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to insoluble purple formazan crystals. By measuring the amount of formazan produced, one is able to quantify cellular metabolic activity and hence also toxicity [166].

We used the Vybrant® MTT Cell Proliferation Assay (Thermo Fisher Scientific, Rockord, IL) as instructed by the manufacturer. In short, MTT solution was added to the respective wells and incubated for 4 hours at 37° C. To dissolve the generated formazan crystals, 100 µl of an SDS-HCL solution, consisting of 10 ml of 0.01M HCL and 1g SDS, was added to the wells, thoroughly mixed using a pipette and incubated for 4 hours at 37° C. Each sample was mixed again using a pipette and the absorbance at 570 nm was measured using a plate reader.

2.2.2.2.2 CytoTox® assay

To measure cytotoxicity after antibody treatment, we used the Promega CytoTox®-96 assay (Promega, Madison, WI, USA) which was performed according to manufacturer's protocol. It is based on the enzyme lactate dehydrogenase (LDH) that is ubiquitously expressed in human cells and is released by dying cells. In this assay, the LDH released into culture medium is measured via a coupled enzymatic assay resulting in a red formazan reaction product whose amount is proportional to the number of cells with impaired cellular membrane integrity [68].

Here, we used 50 µl of culture medium and transferred it to clear 96-well plate. 50 µl of freshly made CytoTox® reagent were added to each well and incubated in the dark at RT for 30 minutes. Stop solution was added and the absorbance at 490 nm was measured using a plate reader.

2.2.2.3 Centrifugal fractionation

A RIPA/Urea based fractionation assay was used to analyse the effect of antibody treatment on TDP-43 solubility. Therefore, HEK-293 cells were seeded in 6-well plates, transfected and treated as described in 1.5. After 48 hours of incubation with different antibodies, cells were washed with 100 µl of PBS and lysed in 200µl RIPA buffer. Next, protein concentrations were measured using a BCA assay (see 2.1.1) and all samples were diluted in RIPA to a final concentration of 0.5 mg/ml. Then, samples were centrifuged at 4° C with 100,000x g for 30 minutes and supernatant was collected as the RIPA soluble fraction. The pellet was washed with 100 µl RIPA to prevent contamination. To do so, RIPA was added to the pellet and samples were briefly sonicated and re-centrifuged. The supernatant was now discharged, and Urea buffer was added to the pellet. Again, all samples were sonicated and vortexed to completely dissolve the pellet. RIPA and Urea soluble fractions were further analysed by Western blotting.

2.2.2.4 SDS-PAGE

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used to separate protein mixtures based on their molecular weight. SDS is hereby binding to proteins proportional to their size and masking their intrinsic charges while being negatively charged itself. Therefore, in the presence of a constant electric field, all proteins migrate towards the anode, each with differing speed depending on its mass.

Tris-glycine gels were prepared according to Table 5 and stored at 4° C until usage.

Depending on the sample, 15 µg protein were mixed with 4x LDS and 10% DTT. Prior to use samples were cooked at 95°C for 5 minutes to denaturate the proteins. The NuPAGE® Gel system (Thermo Fisher Scientific, Rockford, IL, USA) was used according to manufacturer's instructions. The appropriate sample volume as well as 5 µl PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific, Rockford, IL, USA) were placed into the wells and the gel was run for 2-3 hours at 120 V in SDS running buffer (0,025 M Tris, 0,192 M Glycin, 0,0035 M SDS).

Table 5: Solutions for preparing tris-glycine gels for SDS-PAGE

	Separation gel 12%	**Stacking gel 5%**
Rotiphorese® Gel 30	3.75 ml	1.5 ml
1.5 M Tris, pH 8.8	5 ml	-
0.5 M Tris, pH 6.8	-	2.55 ml
20% SDS	50 µl	50 µl
H_2O	1.05 ml	5.85 ml
10% APS	100 µl	100 µl
TEMED	10 µl	10 µl

2.2.2.5 Western Blot

Due to its high sensitivity, one of the most commonly used techniques to transfer proteins from a gel onto a nitrocellulose or PVDF membrane is Western blotting. Proteins are transferred onto a membrane using electroblotting and a primary antibody against the protein of interest is added. After washing off excess antibody, an HRP-conjugated secondary antibody is added that specifically binds the primary antibody. Upon addition of a substrate, a chemiluminescent reaction is induced, and emitted light can be detected.

Proteins that had previously been separated via SDS-PAGE were transferred to a nitrocellulose membrane (GE Healthcare, Chicago, IL, USA) using the XCell II™ Blot Module (Thermo Fisher Scientific, Rockford, IL, USA) in combination with NuPAGE® Gel Systems (Thermo Fisher Scientific, Rockford, IL, USA). Proteins were transferred for 2 hours at 25 V. Membranes were then covered with Ponceau S and incubated for 15 minutes to stain immobilized proteins and check whether transfer was successful and uniform. To avoid unspecific binding of antibodies, membranes were then blocked using Roti-Block (Carl Roth GmbH, Karlsruhe, Germany) for 1 hour at room temperature. After 3 washes with 1x TBST for 15 minutes each, the respective primary antibody was added over night at 4° C (for dilutions see Table 6). Again, membranes were washed 3 times with 1x TBST for 15 minutes each before an HRP-coupled secondary antibody was added and incubated for 1hour at RT. After another 3 washes with 1x TBST for 15 minutes each, the chemiluminescence was detected using SuperSignal® Chemiluminescent substrate (Pierce/Thermo Fisher, Rockford, IL, USA) according to the manufacturer's protocols. Images were acquired using the Fusion Solo 4 S Imager and analysed using the FusionX software.

Table 6: Antibodies used for western blotting

Antibody	Manufacturer	Species	Dilution	Diluted in
12892-1-AP (c-terminal TDP-43)	Proteintech	rabbit	1:1000	Roti-Block
10494-1-AP (GAPDH)	Proteintech	rabbit	1:20.000	Roti-Block
Anti-rabbit, HRP conjugate	Promega	goat	1:10.000	Roti-Block

2.2.2.6 Immunocytochemistry

Immunocytochemistry was used to visualize specific proteins within cells. Hereby the target protein is indirectly detected by using a primary antibody against the protein of interest, that is then visualised by using a secondary antibody conjugated to a fluorophore that emits light when stimulated at a specific wavelength under a microscope.

HEK-293 cells were seeded in 24-well plates on PDL coated coverslips (for PDL coating see 1.3). After 24 hours, cells were transfected, and the respective antibody was added 16 hours later. After 48 hours of antibody treatment, medium was discharged, and cells were fixed with 0.3 ml 4% PFA. After incubating for 30 minutes at RT the cells were washed with 0.3 ml PBS per well and permeabilized with 0.1% TritonX in PBS for 10 minutes at RT. After blocking with 0.3 ml 10% NGS and 0.1% TritonX in PBS for 1 hour at room temperature, the primary antibody was applied (diluted in 10% NGS + 0.1% TritonX in PBS, TDP-43 antibody 1:200, every other antibody 1:1000) to the cells and the cells were incubated for another 90 minutes. The cells were then washed 3 times with 0.5 ml PBS + 0.03% TritonX for 10 minutes per wash. Afterwards, secondary antibodies conjugated with different Alexa Fluor® Dyes (1:1000, diluted in 10% NGS + 0.1% TritonX in PBS) were applied to the cells and incubated for 1 hour at room temperature. For nuclear staining, Hoechst 33342 was added to the cells at a final dilution of 1:5000 for 1 minute, before the cells were washed twice with PBS + 0.03% TritonX for 10 minutes per wash. Finally, the cover slips were mounted using Fluoromount-GTM mounting medium and analysed the by confocal microscopy the following day.

2.2.2.7 Split-luciferase assay

HEK-293 cells were seeded and transfected in a 96-well plate as described previously (see 1.4 and 1.5.1). Prior to performing the assay, HEK-293 cells were washed once with 100 µl PBS and 100 µl Opti-MEM were added per well. 25 minutes before each measurement, Coelenterazine (stock solution: 1 mg/ml in methanol, stored at -80° C) was diluted in Opti-MEM at a final concentration of 17 µg/ml. The Viktor™ X3 (Perkin Elmer, Waltham, MA, USA) system was used to dispense 100 µl of substrate mix per well and read the ensuing luciferase mediated flash reaction 1 millisecond later.

2.2.3 Molecular biological methods

2.2.3.1 Determining concentrations and purity of Nucleic Acids

Nucleic Acid concentrations and purity were measured using a spectrophotometer (NanoDrop™, Thermo Fisher Scientific, Rockford, IL, USA). To do so, a modified Lambert-Beer-Equation is used to calculate the concentrations of DNA and RNA that absorb light at 260 nm. Additionally, the ratios of sample's absorbance at 260 nm and the wavelength at 230 or 280 nm can be used to identify contaminations with solvents or proteins, respectively.

We performed concentration testing as per manufacturer's protocols. Hereby we blanked the system using 2 µl of the dilution media before 2 µl of the sample were measured 3 times. The means out of all 3 measurements were then considered the concentration and purity of the sample.

2.2.3.2 Liquid bacterial cultures

The amount of LB-medium required for the DNA extraction Kit used (see 2.2.3.3) was added to a sterile flask and Ampicillin was added at a final concentration of 1µg/ml. Chemically competent bacteria (Z.competent DH5a E. coli) transformed with the plasmid of interest were transferred into the media from a frozen glycerol stock. Cultures were incubated at 37° C und shaking. After an overnight incubation, cultures were centrifuged at 8000xg for 10 minutes and pellets were used for plasmid extraction (see 2.2.3.3).

2.2.3.3 Extraction of Plasmid DNA from bacterial cultures

Plasmid DNA used to transfect cells was isolated and prepared using a QIAGEN Plasmid Kit according to the manufacturer's protocol. Depending on the amount of DNA necessary, either a Mini- or Maxi-Kit was used.

For a Mini-Preparation, 5 ml of bacterial culture were prepared overnight as described in 2.2.3.2. After purification, DNA was eluted in 50µl nuclease free water.

For a Maxi-Kit, 300 ml of bacterial culture were prepared overnight. After purification the DNA was air-dried after washing it with ethanol and then resolved in 200 µl nuclease free water.

In both cases the resulting DNA concentration was determined as described in 2.2.3.1 and stored at 4° C.

2.2.4 Cell culture experiments

2.2.4.1 Antibody co-incubation

To investigate the effects of TDP-43 targeting antibodies on TDP-43 aggregation and cellular viability we co-incubated the antibody with HEK-293 cells with the respective antibodies. As has been shown in brains of rodents, antibodies can cross the blood brain barrier, are taken up by cells out of their surrounding media, may impact ongoing aggregation and may also have an impact on cellular viability[13, 20, 57, 227].

Therefore, HEK-293 cells were seeded and transfected with the respective plasmids as described above (see 2.2.1.5). When changing the medium following transfection, antibodies of interest were added at a final concentration of 2 µg/ml if not stated otherwise. 72 hours after the medium change, cells were either processed directly or stressed with sorbitol for 2 hours at 37°C and 5% CO_2 (see 2.2.1.6). To quantify aggregate formation and cellular viability, we performed luciferase assay (see 2.2.2.7) and cytotoxicity assays (see 2.2.2.2), respectively.

2.2.4.2 Biochemical fractionation

To determine whether the antibody treatments had a direct effect on TDP-43 aggregate formation and solubility of TDP-43, we co-incubated HEK-293 cells as described before (see 2.2.4.1). Cells were seeded in a 6-well plate and were transfected with human wildtype TDP-43 c-terminally tagged with a myc-tag 24 hours later. Antibody treatment was performed as described in 2.2.4.1. Fractions were separated as described under 2.2.2.3 and analysed via western blotting. When quantifying the samples only blots with a Urea/RIPA ratio higher than 0.5 for the negative control were analysed further to ensure comparable stress levels in all samples. Both the bands for exogenous as well as endogenous TDP-43 were analysed together to ensure a more complete picture of TDP-43 solubility in the cells.

2.2.4.3 Propagation of TDP-43

To investigate whether our antibodies of interest have an effect on the cell to cell propagation of TDP-43 as described by Feiler et al [75], we first generated conditioned medium containing TDP-43 tagged with full length Gaussia luciferase. Therefore, HEK-293 cells were seeded in 100 mm^2 dishes and transfected with the respective plasmids as described above (see 2.2.1.5). Following transfection, medium was changed to Opti-MEM. After 72 hours of incubation, conditioned medium was measured via a luciferase assay and adjusted to 1,000,000 counts per 100 µl medium. We then added our antibodies of interest at a final concentration of 2 µg/ml before adding the respective mixes to naïve HEK-293 cells that had been seeded 24 hours prior. After incubating the cells for 72 hours, media was discharged, and luciferase activity was measured in the recipient cells (see 2.2.2.7).

2.2.5 Statistical analysis

Statistical analysis was carried out using GraphPad Prism 8 Software. To test statistical significances an unpaired two-tailed student's t-test was performed. Graphs were generated using the GraphPad Prism 8 Software and are presented as means ± standard error of the mean (SEM) if not stated otherwise.

3. Results

Antibodies are used as a rapidly emerging therapeutic strategy to target aggregation in many different neurodegenerative diseases like Alzheimer's or Parkinson's. In ALS, antibodies against TDP-43 appear to be a possible therapeutic option to interfere with its aggregation and prion like spreading. With rarely any studies performed so far, I was interested if a treatment with these antibodies had effects *in vitro* to further underline a possible therapeutic relevance. Therefore, commercially available antibodies against different regions of TDP-43 were used to address TDP-43 aggregation utilizing an established protein complementation assay.

3.1 Co-incubated antibodies can enter HEK-293 cells

While it is known that antibodies can enter the central nervous system when peripherally administered[20, 116, 248], they also have to permeate cellular membranes to affect intracellular aggregation. To check if antibodies related to our study are able to penetrate the cell membranes, we treated HEK-293 cells 16 hours after transfection with an empty vector (EV) or HA-tagged TDP-43 and performed immunocytochemistry and confocal microscopy after 48 hours of co-culturing (see Fig. 4B, 4C respectively). For treatment, we selected an antibody targeting the c-terminus of TDP-43 as well as an IBA-1 antibody, that served as a control. Both tested antibodies were able to enter cells, suggesting that co-incubated antibodies regardless of their target can enter HEK-293 cells. While the IBA-1 antibody control was readily found in cells overexpressing HA-TDP-43, it did not show co-localization. The TDP-43 specific antibody on the other hand could not be detected in cells overexpressing HA-TDP-43 (Fig. 4).

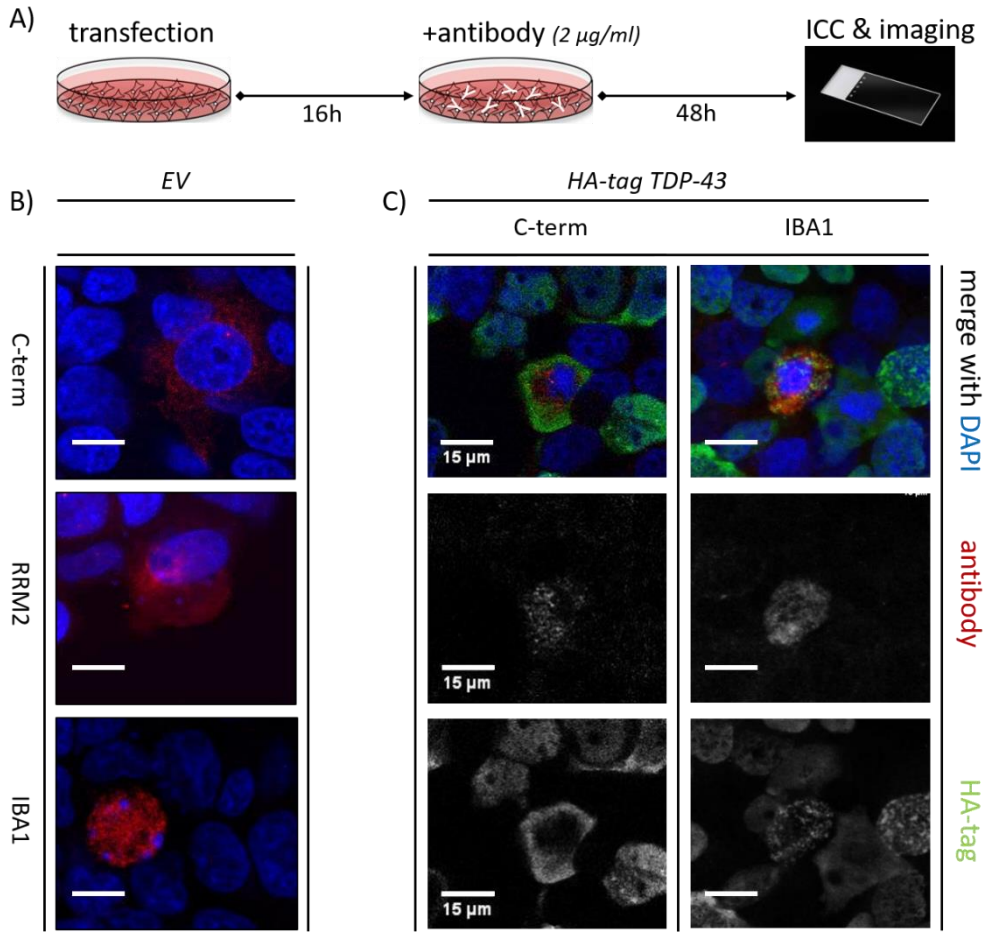

Figure 4: Antibodies can enter cells and do not show co-localization with TDP-43 aggregates

A) Experimental setup for immunocytochemistry (ICC) experiments with antibody co-incubation.
B) Representative pictures from empty vector (EV) transfected human embryonic kidney cells 293 (HEK-293 cells) co-incubated with the respective antibodies. C) Representative pictures from HA-tagged (hemagglutinin-tagged) TDP-43 (green) transfected HEK-293 cells co-incubated with the respective antibodies (red). Scale bar = 15µm.

3.2 Titration of optimal antibody concentration

For further experiments I wanted to find the highest possible antibody concentration that showed the lowest toxicity level to increase the likelihood of detecting any antibody mediated effects. Using a similar setup for co-incubation with the antibody as described before (see 3.1 and Methods 2.2.4.1), we tested an IgG control in addition to an antibody specifically targeting the c-terminus of TDP-43 in HEK-293 cells transfected with and empty vector (EV) and wild type TDP-43, respectively. Toxicity levels were determined using an MTT assay that utilizes NADPH-dependent oxidoreductases present in viable cells to

determine cell viability. We could see that increasing antibody concentrations lead to a significant reduction in cell viability in cells overexpressing TDP-43 (Fig. 5). While we could see a clear trend that concentrations of 5 µg/ml and upwards were reducing cell viability, a concentration of 2.5 µg/ml only reduced cell viability slightly for the TDP-43 antibody but not significantly for the IgG control antibody. Therefore, we decided to continue with an antibody concentration of 2µg/ml for all further experiments to minimise antibody toxicity while keeping the concentration as high as possible to maximise possible effects of our antibodies.

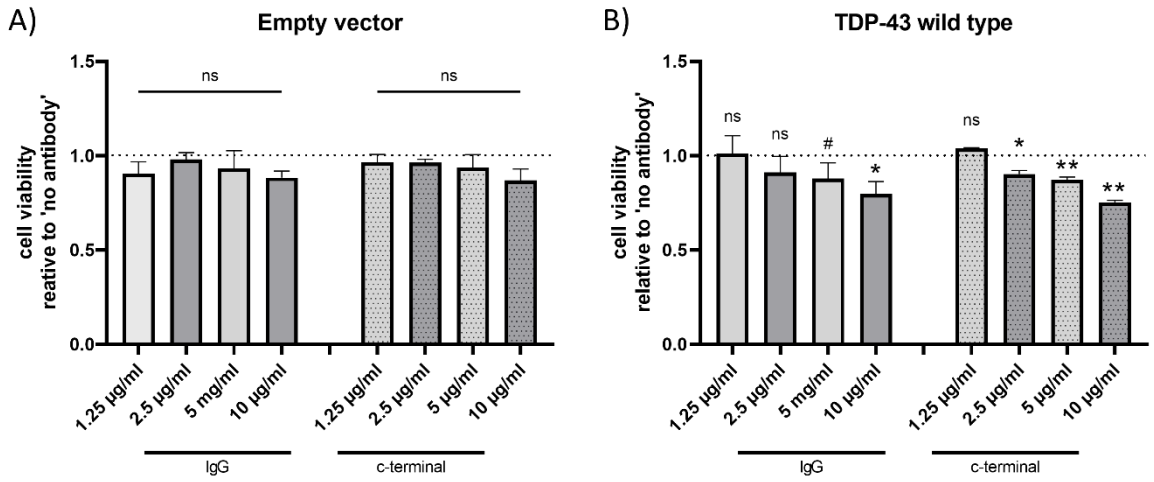

Figure 5: Changes in cellular viability with increasing antibody concentration

Antibodies were added 12 hours after transfection of human embryonic kidney cells 293 (HEK-293 cells) at the stated concentrations. Cell viability was determined using an MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid-assay) that was performed after 48 hours of antibody incubation. HEK-293 transfected with empty vector. B) HEK-293 cells transfected with wild type TDP-43.

Bars indicate mean ± SEM (n=4 per group, unpaired t-test against 'no antibody': $^{\#}p \leq 0.1$; $*p \leq 0.05$; $**p \leq 0.01$; ns = not significant.)

3.3 Influence on TDP-43 aggregation and solubility

To quantify aggregate formation we used a split protein complementation assay that was established for TDP-43 by *Feiler et al.* in our laboratory in 2015[75]. The system uses a split Gaussia luciferase with each half bound to a TDP-43 molecule. Once the two halves meet, the luciferase is reconstituted and, in the presence of a substrate, emits light that is

proportional to the amount of dimerized TDP-43. To induce TDP-43 aggregation and stress granule formation, as is present in TDP-43 associated disease, cells were treated with Sorbitol[36, 71, 181].

3.3.1 Co-incubation of TDP-43 antibodies shows positive effects on aggregation but has limited influence on toxicity under unstressed conditions

First, we wanted to evaluate the effects of our antibodies on intracellular aggregation under unstressed conditions. Therefore, we added the antibody of interest 16 hours after transfecting the cells with both parts of our TDP-43-split-luciferase constructs and performed analysis 48 hours post antibody treatment (Fig. 6A). As negative control we used an unconjugated IgG antibody.

We found that the polyclonal antibodies with broader, less specific, binding sites (c-terminal and n-terminal antibody) were also the ones with the greatest effects on aggregation. Both significantly reduced the dimerized TDP-43 levels by about 10% compared to control conditions (Fig. 6B). Furthermore, the addition of the pTDP-43-s409/410 antibody resulted in a trend ($p=0.08$), reducing the levels of dimerized TDP-43 by about 7% compared to the control group without antibody treatment. No effect was found for the RRM2-antibody, appearing similar to both control groups.

Additionally, we used the same setup to look at the different antibodies and their impact on cellular toxicity. To do so, we performed a lactate dehydrogenase (LDH) release assay with the media obtained from the cells used in the luciferase assay described above. While both antibodies with the best effects on aggregation (c-terminal and n-terminal antibody) had no significant change in toxicity compared to the untreated cells (Fig. 6C), the pTDP-s409/410 treated cells showed significantly less toxicity, reducing LDH release by more than 10%. Surprisingly not only the pTDP-s409/410 antibody but also our unconjugated IgG antibody had a positive, although less significant, effect on cellular toxicity reducing LDH release by roughly 8%.

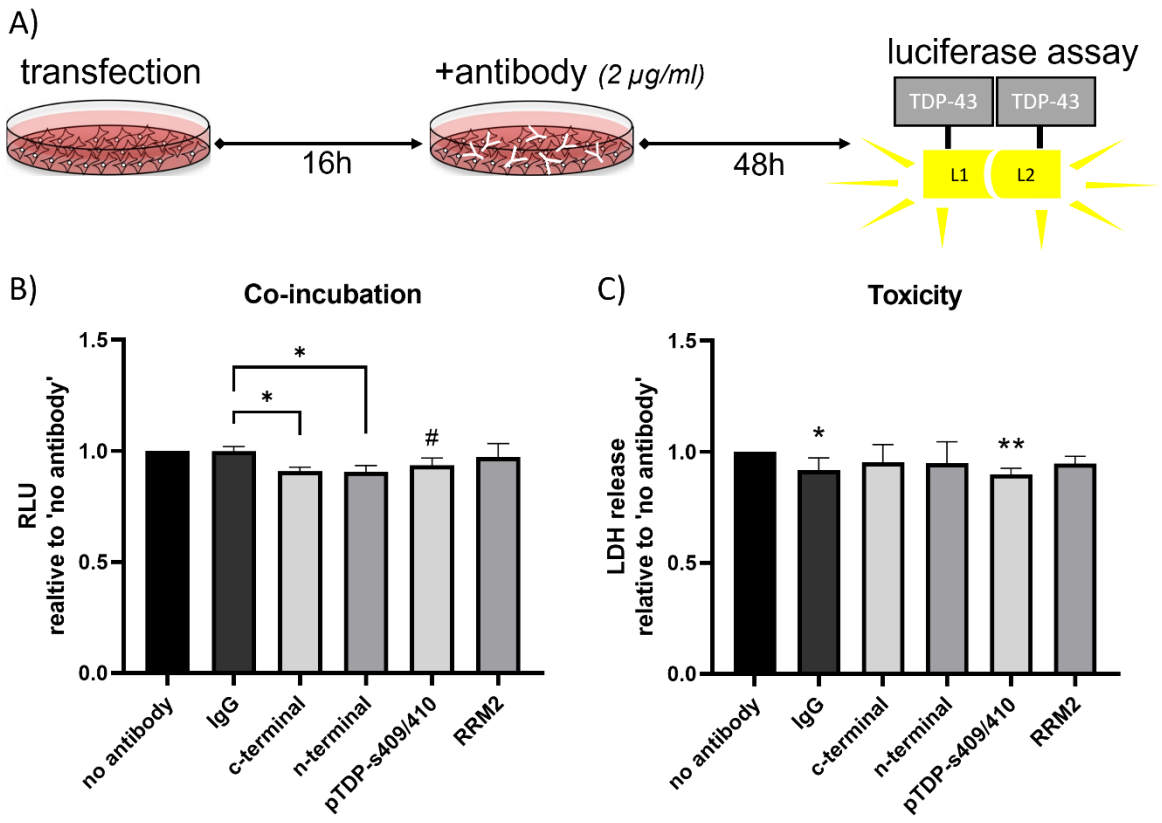

Figure 6: Effects of TDP-43 antibodies on TDP-43 aggregation and toxicity under unstressed conditions

The effects of antibody treatment on TDP-43 dimerization in transfected human embryonic kidney cells 293 (HEK-293 cells) were tested using a split-luciferase assay measuring relative luminescence units (RLU). Media from these experiments were further used to measure how antibody treatment effected cellular toxicity.

A) Experimental design of the co-incubation experiments without stress. B) Luciferase count normalised per n to the 'no antibody' group when co-incubating different antibodies. n=4. C) Growth media of the cells used in 'B' was used to perform a lactate dehydrogenase (LDH) release assay. LDH release was normalised per n to 'no antibody'. n=3.

Bars indicate mean ± SEM (unpaired t-test against 'no antibody' if not stated otherwise: $^{\#}p\leq0.1$; $^{*}p\leq0.05$; $^{**}p\leq0.01$)

3.3.2 Co-incubation of TDP-43 antibodies show minor positive effects on aggregation while toxicity remains unaffected under conditions of oxidative stress

Cellular stress is considered to be an important contributor in aggregate formation and ALS pathology. To mimic cellular stress, we transfected HEK-293 cells with the TDP-43 split luciferase constructs before applying 0.4 M sorbitol for two hours (Fig. 7A). Sorbitol is a well-known stressor and reported to induce aggregate formation in cellular models such as HEK-293[71, 75]. Upon addition of sorbitol, luciferase signals increased significantly indicating TDP-43 oligomerization under cellular stress as it has been observed before[75] (Fig. 7B).

To exclude an unspecific effect of the antibody treatment on the luciferase signal we used a heat denaturated c-terminal antibody in addition to the unconjugated IgG control. We could not detect any significant changes in luciferase activity for either control antibody opposing possible unspecific effects. Looking at our antibodies of interest we found that the c-terminal antibody showed a significant reduction of TDP-43 dimerization by approximately 18% compared to both the untreated and the denaturated c-terminal antibody controls (Fig. 7B). This reduction resulted in TDP-43 aggregation levels comparable to the unstressed control condition. Additionally, treatment with the n-terminal antibody, the pTDP-s409/410 antibody as well as the RRM2 antibody all decreased TDP-43 aggregation. However, this decrease did not reach a statistically significant level for any of the afore mentioned antibodies (n-terminal antibody: $p=0.09$; pTDP-s409/410 antibody: $p=0.24$; RRM2 antibody: $p=0.08$)

Again, we performed an LDH release assay to also analyse cellular toxicity. Consistent with our findings under unstressed conditions (see 3.3.1) cytotoxic effects could not be detected upon treatment with our antibodies (Fig. 7C). Interestingly, the positive effect of pTDP-s409/410 antibody treatment on cytotoxicity observed under unstressed conditions could not be reproduced under sorbitol-induced stress.

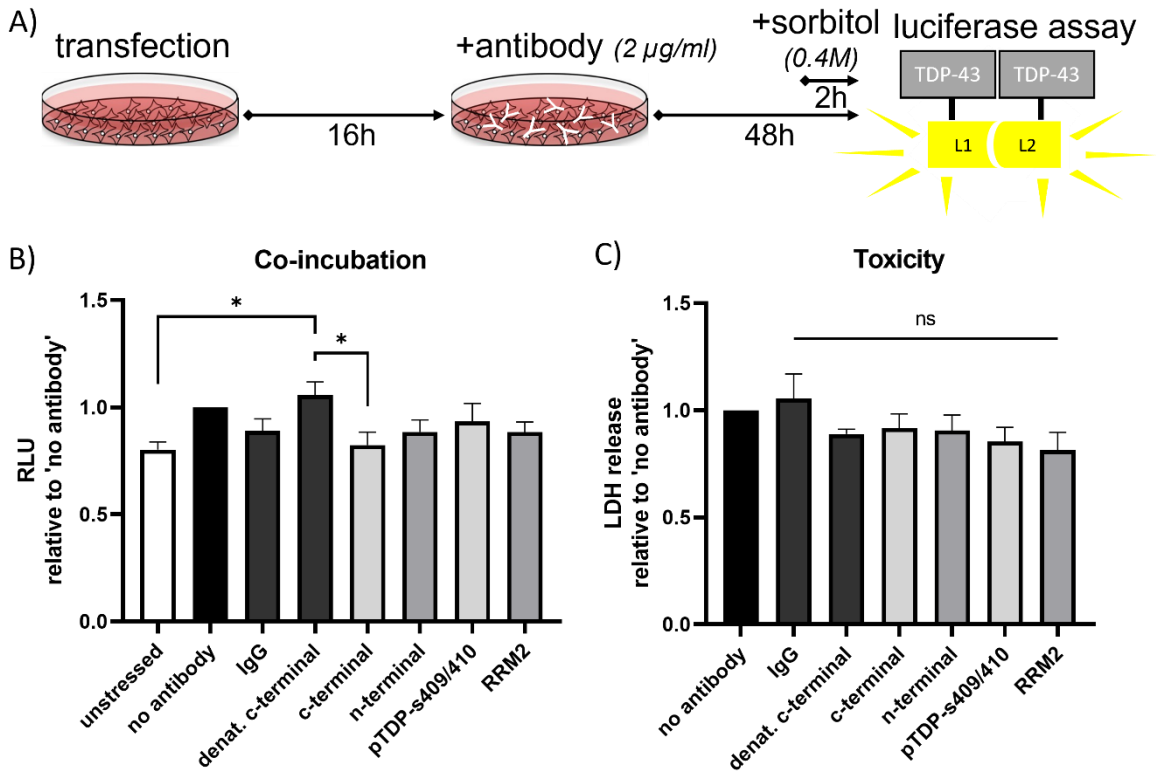

Figure 7: Effects of TDP-43 antibodies on TDP-43 aggregation and toxicity under conditions of oxidative stress

The effects of antibody treatment on TDP-43 dimerization were tested using a split-luciferase assay. To apply oxidative stress to transfected human embryonic kidney cells 293 (HEK-293 cells), 0.4M sorbitol was added 2 hours prior to measuring relative luminescence units (RLU). Media from these experiments was further used to address a possible effect of antibody treatment on cellular toxicity. A) Experimental design of the co-incubation experiments under oxidative stress. B) Luciferase activity normalised per n to the 'no antibody' group when co-incubating different antibodies. C) Growth media of the cells used in 'B' was used to perform a lactate dehydrogenase (LDH) release assay. LDH release was normalised to 'no antibody' for each replicate. Bars indicate mean ± SEM (n=7, unpaired t-test against 'no antibody': *p≤0.05; ns = not significant.)

3.3.3 Co-incubation of HEK-293 cells with TDP-43 antibodies increases TDP-43 solubility

To further investigate the effects of the indicated antibodies on TDP-43 aggregation and to validate our results with a secondary method, we looked at the solubility of TDP-43. Therefore, we compared the Urea and RIPA soluble TDP-43 fractions of HEK-293 cells transfected with myc-tagged wild type TDP-43 and incubated with TDP-43 antibodies. Additionally, cells were treated with 0.4M sorbitol for 2 hours to induce a shift in TDP-43 solubility[102, 132, 249]. Proteins were isolated, fractionated and the ratios of Urea and RIPA soluble TDP-43 were compared after densitometrical analysis of Western Blots (Fig. 8A).

In addition to the observed effect on TDP-43 aggregation, the c-terminal, the n-terminal and the pTDP-s409/410 antibody significantly increased TDP-43 solubility (Fig. 8B). Here, the pTDP-s409/410 and the n-terminal antibodies each showed a reduction of roughly 66%. Similarly, the c-terminal antibody reduced the amount of insoluble TDP-43 by approximately ~ 60%. Treatment with the RRM2 antibody on the other hand did not have an influence on TDP-43 solubility.

We could also see fragmented TDP-43 on our blots slightly above 35 kDa but, most likely due to sensitivity, not at 25 kDa (Fig. 8A). These ~ 35 kDa fragments have been described to have a high influence on TDP-43 aggregation and seeding ability[98, 161]. Densitometric analysis of these fragments showed an increase of solubility upon antibody treatment that was comparable to full length TDP-43 (Fig. 8C). Both, the n-terminal and the pTDP-s409/410 antibody showed a significant reduction of the insoluble fraction by more than 50% compared to the untreated control. Treatment with the c-terminal antibody showed a trend towards an increase of TDP-43 solubility as well ($p=0.06$).

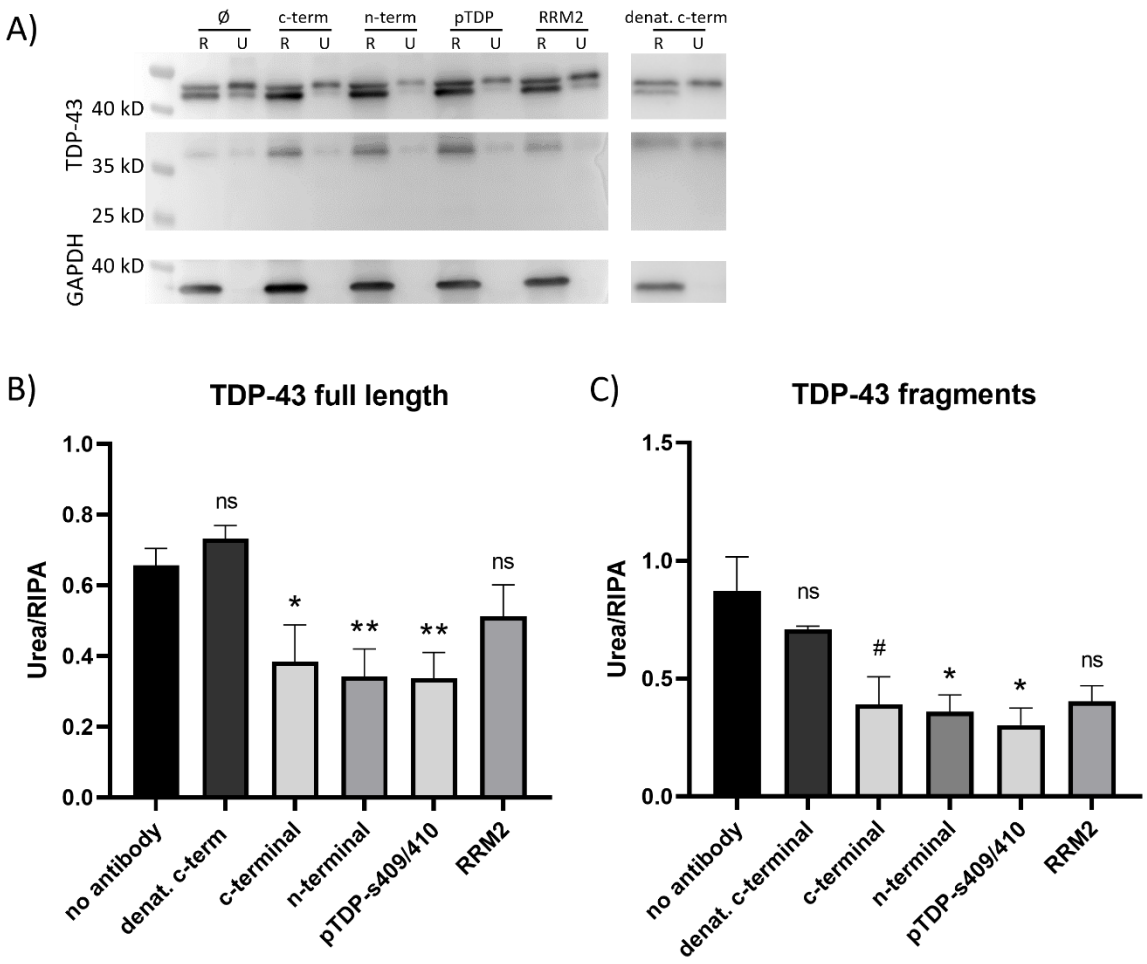

Figure 8: Effects of TDP-43 antibodies on TDP-43 solubility under conditions of oxidative stress

Human embryonic kidney cells 293 (HEK-293 cells) transiently overexpressing myc-TDP-43 were incubated with the respective antibodies and stressed for 2 hours with 0.4M sorbitol. After lysis, RIPA buffer (Radioimmunoprecipitation assay buffer) and urea soluble fractions were analysed by Western Blotting.

A) Representative Western Blots of RIPA (R) and urea (U) soluble fractions. B) Ratios of urea to RIPA soluble fractions after densitometrical analysis of exogenous and endogenous TDP-43. C) Ratios of urea to RIPA soluble fractions after densitometrical analysis of TDP-43 fragments detected at ~35 kDa.

Bars indicate mean ± SEM (n=3, unpaired t-test against 'no antibody': $^{\#}p \leq 0.1$; $*p \leq 0.05$; $**p \leq 0.01$; ns = not significant.)

3.4 The c-terminal TDP-43 antibody reduces TDP-43 propagation

It has been shown that TDP-43 is secreted in the growth media and can be taken up by cells. This uptaken TDP-43 may then act as a seed to induce further aggregation in recipient cells[102, 161] in a prion like spreading mechanism which is in line with the observed progression of TDP-43 pathology throughout the nervous system of ALS patients. Therefore, we hypothesized that TDP-43 uptake may be inhibited by the presence of antibodies targeting TDP-43 in the media. To further investigate this hypothesis, we adapted a propagation assay first established by *Feiler et al.* in 2015[75]. Thus, we generated conditioned media containing TDP-43 tagged with full length Gaussia luciferase and our antibodies of interest which was then added to naïve HEK-293 cells. After 60 hours, the recipient cells were washed and luciferase activity was measured (Fig. 9A).

Surprisingly, contrary to our initial hypothesis, our control group treated with denaturated antibody showed a significant increase in luciferase signal by ~30% compared to the untreated control, indicating an increase in TDP-43 uptake and most likely oligomerization in recipient cells. Similar results were observed for the pTDP-s409/410 antibody and to a lesser extent also the n-terminal antibody ($p=0.08$) compared to the untreated control. However, both antibodies did not show any difference when compared to our denaturated antibody control (Fig. 9B). Interestingly, the c-terminal antibody significantly reduced luciferase signal in the recipient cells compared to both, the antibody control and the untreated control group (reduction of ~14%; $p \leq 0.05$), indicating the antibody's ability to inhibit TDP-43 uptake and/or further oligomerization.

Again, we analysed cellular toxicity with and without antibody treatment by measuring LDH release into the media. Expectedly, the addition of conditioned media from cells overexpressing TDP-43 lead to a significantly higher toxicity compared to adding conditioned media generated from empty vector (pcDNA3) transfected cells (Fig. 9C). However, neither our antibody control nor any of the other antibodies were able to reduce cellular toxicity. On the contrary, all our antibodies, including the denaturated antibody control, increased toxicity to a similar level compared to the untreated control. This was also true for the c-terminal antibody, suggesting that the toxic effects of TDP-43 and antibody co-incubation interfere with any potential positive antibody effects.

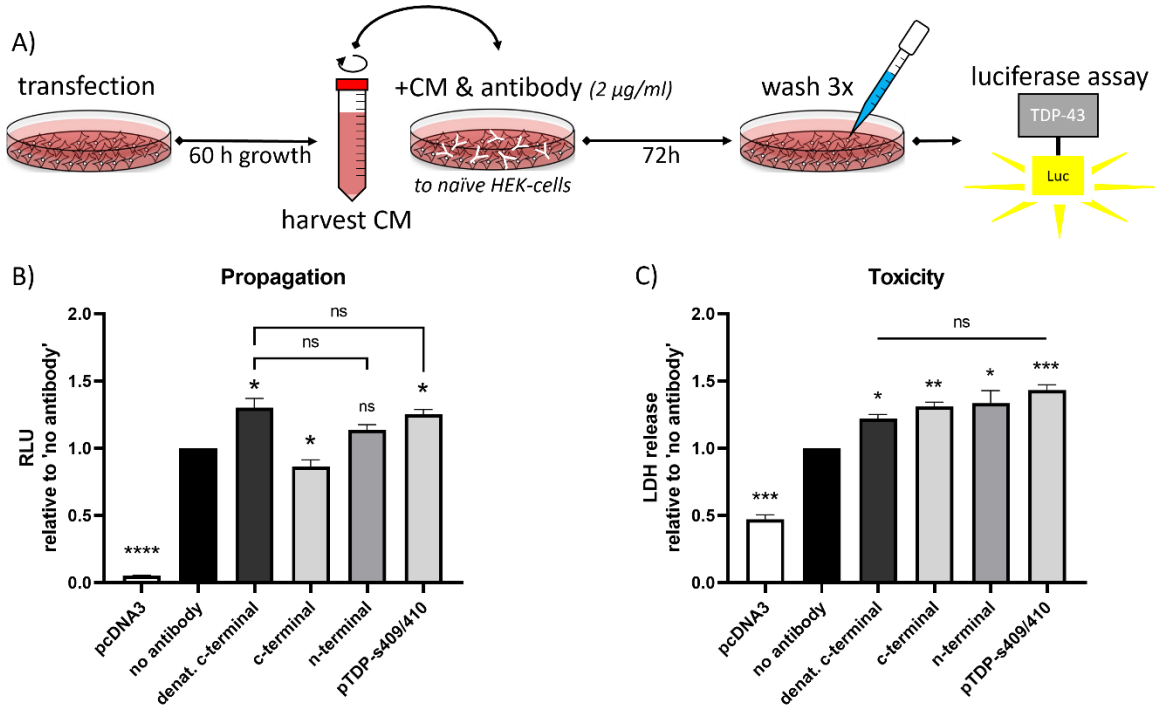

Figure 9: Effects of TDP-43 antibodies on TDP-43 propagation

Human embryonic kidney cells 293 (HEK-293) were transfected with full length Gaussia luciferase tagged TDP-43. Conditioned media (CM) was collected after 60 hours of growth and antibodies of interest and CM were added to naïve HEK-293 cells. After an additional 72 hours of co-culturing cells were washed, and relative luminescence units (RLU) were measured.

A) Experimental setup of propagation experiments. B) Luciferase count in target cells after washing with (phosphate-buffered saline) PBS. C) Lactate dehydrogenase (LDH) release was measured in the growth media of the recipient cells to analyse cellular toxicity.

Bars indicate mean ± SEM (n=3, unpaired t-test against 'no antibody' if not stated otherwise: $^{\#}p\leq0.1$; $*p\leq0.05$; $**p\leq0.01$; $***p\leq0.001$; $****p\leq0.0001$; ns = not significant.)

3.5 Side-by-side comparison of TDP-43 antibodies and their impacts on TDP-43 aggregation, solubility, propagation and toxicity

Taken together, TDP-43antibodies can influence aggregation, solubility and propagation of TDP-43 to various extents. However, their effects seem to be greatly impacted by additional variables like cellular stress. While it appears that their influence on solubility is the most prominent and their effects differ greatly from antibody to antibody, a distinct pattern can be observed over all these experiments performed so far (Table 7). The c-terminal antibody, which is a polyclonal antibody, has effects on aggregation, solubility and propagation in

every setup but has no impact on cytotoxicity. Similarly, the other polyclonal antibody, that targets the n-terminal part of TDP-43 has effects on aggregation and solubility but does not show any effect on propagation or cytotoxicity. The monoclonal pTDP-s409/410 antibody slightly reduces toxicity in unstressed cells and has the most pronounced effect on solubility, but has only minor effects, on TDP-43 aggregation as determined by luciferase assays. No positive effects were detected for the monoclonal RRM2 antibody, which did neither have a significant impact on toxicity, aggregation nor solubility and was therefore excluded from the propagation experiments.

Table 7: Comparison of antibodies used in this study

Side by side comparison of tested antibodies showing their respective effect in percent relative to the untreated control (unpaired t-test: $^{\#}p\leq0.1$; $^{*}p\leq0.05$; $^{**}p\leq0.01$; $^{***}p\leq0.001$)

	toxicity		aggregation		solubility		propagation	
	unstressed	stressed	unstressed	stressed	full length	Fragments	luciferase	toxicity
C-terminal	-	-	↓ 9.2% *	↓ 17.7% *	↓ 61.5% *	⇣ 61% #	↓ 13,4% *	↑30.9% **
N-terminal	-	-	↓ 9.3% *	-	↓ 65.9% **	↓ 64.2% *	-	↑33.5% **
pTDP-s409/410	↓ 10.2% **	-	⇣ 6.4% #	-	↓ 66.3% **	↓ 69.7% *	↑ 25.3% *	↑43.2% ***
RRM2	-	-	-	-	-	-		
Antibody control	↓ 8.2% *	-	-	-	-	-	↑ 30.1% *	↑21.9% *

4. Discussion

In multiple neurodegenerative diseases like Alzheimer's and Parkinson's antibody-based therapies have already emerged as a viable option to hinder protein aggregation and consequential neuronal toxicity. TDP-43 antibodies are suggested to act in a similar manner, but only little research has been conducted so far. In this study we used an *in vitro* model utilizing an established split protein assay to test commercially available antibodies against TDP-43 on their ability to influence aggregation, solubility and propagation of TDP-43. A positive effect of these antibodies on TDP-43 pathology can then further strengthen the idea that TDP-43 antibody-based therapies may represent a viable treatment option in ALS.

4.1 Antibody availability, toxicity and co-localisation with TDP-43

In 2013, *Congdon et al.* were able to show that antibodies are taken up into neurons, largely via Clathrin-dependent Fc-γ receptors[57]. Antibody uptake into neurons as well as microglia has also been reported in multiple neurodegenerative diseases[83, 116]. Using immunocytochemistry followed by fluorescence imaging via confocal microscopy, we were able to show that our antibodies readily enter the cytoplasm of target HEK-293 cells. Therefore, our findings suggest that HEK-293 cells can be used as a model to study antibody effects. Knowing that antibodies are able to cross the blood-brain barrier[13, 20, 158, 232, 256], a potential positive effect of antibodies in cell culture has reasonable promise to be effective as a therapeutic approach in ALS similar to what has been shown in other neurodegenerative diseases like AD and PD[103, 116, 176, 256].

ALS associated nuclear depletion of TDP-43 and an associated loss of function as well as overexpression of TDP-43 have been proposed to be toxic[12, 101, 134, 140, 203, 234]. We hypothesized that our antibodies could bind and interact with cytosolic TDP-43, which could likely lead to a loss of function of TDP-43. Consequently, we wanted to ensure that our unspecifically binding antibodies were not inherently toxic to HEK-293 cells. Indeed, we observed an increase of toxicity with rising antibody concentrations. In addition, unconjugated IgG, which we used as negative control, also showed a decrease in cell viability suggesting an unspecific toxic effect that is proportional to rising antibody concentrations.

Suggesting a potential interaction between TDP-43 and our co-cultured antibodies we further evaluated confocal images of HEK-293 cells overexpressing TDP-43 that had been co-incubated with anti TDP-43 antibodies. However, all tested antibodies did not co-localize with TDP-43. Co-incubated antibodies could have already interacted with TDP-43 inducing it's clearance as it has been reported for other antibodies against TDP-43 and other aggregation-prone proteins like tau or α-synuclein[57, 116, 227, 232]. This would have led to reduced TDP-43 levels in cells that had previously taken up our antibodies of interest leading to no significant co-localization during the imaging. However, it needs to be mentioned that these afore mentioned studies were performed in vivo which means aggregate clearance was observed in neurons with other non cell-autonomous clearance pathways available compared to our cultured HEK-293 cells.

4.2 Influence of TDP-43 antibodies on aggregation

After showing that antibodies reach their site of action, we were also able to show that co-culturing of certain TDP-43 specific antibodies reduces TDP-43 dimerization in HEK-293 cells.

Whilst antibody-based approaches targeting key proteins in other neurodegenerative diseases, like tau or α-synuclein, have been established in the past decade[116, 139, 161, 171, 197, 232, 255], so far only one antibody-based study targeting TDP-43 has been published[227]. Tamaki *et al.* used an intrabody that specifically binds to misfolded TDP-43 and was conjugated to a proteolytic signal to further enhance TDP-43 aggregate clearance. With this system, they were able to effectively reduce aggregate load and toxicity in HEK-293 cells and mice[227]. A common readout in studies targeting protein aggregation is the number of visible aggregates in tissue. Antibodies reducing the number of visible aggregates have yielded positive effects on survival and burden of disease in rodent models and in first clinical trials[116, 139, 158, 248, 255].

In this study, we showed that TDP-43 aggregation can also be reduced by antibodies targeting TDP-43, the most prominent effects being achieved with broadly binding polyclonal TDP-43 antibodies against the c- or n-terminus of the protein. Comparing our results to previous studies, one must consider the different readouts of aggregation. As stated, the most frequently used readout is the number of microscopically visible aggregates in tissue or cells. However, due to the nature of our split protein system, we are able to

additionally detect dimers and smaller oligomeric species[75]. These oligomers are often not detected when using microscopy as a readout for aggregates, but are suspected to play a crucial role in TDP-43 associated toxicity[147, 188, 205], as TDP-43 toxicity has been reported to occur before aggregates are microscopically visible in cells[11, 99]. Thus, changes detected with our protein complementation assay may be more precise compared to the counting of microscopically visible aggregates.

Indeed, we were able to show that TDP-43 antibodies, especially the broadly binding antibody against the c-terminus of TDP-43, were able to reduce aggregate formation in HEK-293 cells. However, the positive effects that were observed upon antibody treatment are mostly diminished after the addition of oxidative stress. This was surprising, since oxidative stress is known to increase the number and size of protein aggregates[33, 71, 75, 132], suggesting that a beneficial antibody effect may be even more pronounced. This might be in part due to working with a defined concentration of antibody under unstressed and stressed conditions as additional stress might have increased the amount of aggregates so drastically that a potential positive effect of our administered antibodies became negligible. Additionally, as mentioned above, we primarily measured the formation of dimers and potentially oligomers, whereas the afore mentioned studies on increased number of aggregates under cellular stress all refer to bigger, microscopically visible, aggregates[33, 71, 132]. Therefore, the antibodies used in this study could simply be unable to reduce the number of dimers under stress but might still prevent further aggregation.

While we were able to show that TDP-43 antibodies can interfere with TDP-43 aggregation, the exact mechanism of action needs further investigation. For tau, ß-amyloid and α-synuclein antibodies intracellular neutralization and increased clearance of target proteins (e.g. via TRIM21) have been reported[20, 57, 116, 158, 194] and can therefore also be a possibility for TDP-43 targeting antibodies. To address this, further studies are necessary, ideally in a setting that enables an evaluation of non-cell autonomous clearance as well[247, 248] as this would mirror the *in vivo* situation.

4.3 Influence of selected TDP-43 antibodies on protein solubility

During the last decade, liquid-liquid phase separation as well as the ensuing liquid-solid state separation and their association with stress granules have become a focal point of research regarding aggregate formation in different neurodegenerative diseases like ALS[39, 164,

188, 265]. As such, changes to the solubility of a protein are closely linked to aggregate formation and persistence.

In this study, we were able to show that different TDP-43 antibodies can reduce the amount of insoluble TDP-43 in HEK-293 cells that can be detected when overexpressing TDP-43[102, 249]. This was especially true for the phosphorylation specific TDP-43 antibody TDP-s409/410 which had the biggest impact on solubility out of all four antibodies tested.

This raises the question why the tested antibodies have an even greater effect on solubility than on overall aggregation. Phosphorylation of TDP-43 has been closely linked to solubility and protein degradation by multiple studies in the past years[85, 102, 260]. Similarly, our pTDP-s409/410 antibody has the greatest impact on solubility among the antibodies tested in our study. Comparing our results regarding solubility and aggregation it is possible that our antibodies are unable to interfere with the formation of smaller oligomers under stressed conditions but effectively prevent the formation of larger, in general more insoluble, aggregates which would be consistent with previously mentioned findings using antibody treatment in AD and PD[139, 158, 256].

Furthermore, changes in TDP-43 solubility may prove to be more important than the reduction of visible TDP-43 aggregates itself. For example, Yanamandra et al. were able to show that antibodies affecting tau solubility yielded positive results *in vivo*[255]. Biochemical fractionation has also been used as a readout for aggregation in many other studies working with protein aggregation and high loads of insoluble protein have been linked to increased cellular toxicity[102, 132, 147, 204, 254]. Beel et al. were able to reduce toxicity in a neuronal disease model for TDP-43 by reducing the amount of insoluble protein via overexpression of progranulin[23]. Lastly, increasing protein solubility does not only effect LLPS and further aggregate stabilization[85, 164, 188, 265] and formation[102, 129, 249] but is also closely linked to protein degradation and hence clearance of aggregates[148, 204, 260].

In addition to the changes observed for full length TDP-43, we found similar results to be true for TDP-43 fragments at a size of roughly 35 kDa in our western blots. According to the literature, these bands most likely resemble TDP-43 fragments which have been described as early as 2006 by Neumann et al[170, 173, 175, 246, 252]. While we did not further analyse these fragments and hence cannot distinctly label them as c- or n-terminal, both n- and c-terminal fragments are reported to be heavily involved in aggregate formation, seeding and increased cellular toxicity[49, 72, 98, 100, 114, 246, 252, 263]. This is especially

true for their insoluble forms, further supporting a possible positive effect our antibodies may have on TDP-43 solubility as a therapeutic option in TDP-43 proteinopathies.

4.4 Influences of selected TDP-43 antibodies on cellular toxicity

Since TDP-43 overexpression results in aggregate formation and is toxic in *in vitro* and *in vivo* models[12, 108, 112, 253], we wanted to see whether different TDP-43 antibodies could counteract cellular toxicity in our model as well.

However, only the phosphorylation specific TDP-s409/410 antibody was able to reduce cellular toxicity under unstressed conditions. Phosphorylation had previously been described to increase cellular toxicity in two studies from 2013[53, 147] with one of these studies also showing that solely reducing the amount of visible aggregates does not reduce TDP-43 associated toxicity[147].

TDP-43 levels in cells are tightly regulated since not only TDP-43 overexpression and aggregate formation but TDP-43 depletion and loss of TDP-43's physiological functions as well have been associated with cellular toxicity[101, 134, 140, 234]. By using broadly binding, not pathology specific TDP-43 antibodies, it is very likely that the antibodies we used not only interfere with pathological TDP-43 and TDP-43 aggregates but also affect physiological TDP-43 and interfere with its desired cellular functions. As a result, these antibodies should have an inherent toxicity that is aggravated upon stress to the cells. Hence, possible positive effects on toxicity by reducing aggregate formation as well as promoting changes in solubility would be reduced or even masked in our experimental setup. This could be a possible explanation why the c-terminal and n-terminal TDP-43 antibodies used in our experiments were able to increase solubility and reduce aggregate formation but did not affect cellular toxicity. In this regard, the pathology specific phospho-TDP-43 antibody showed a positive effect on toxicity in addition to increasing solubility.

Therefore, the use of pathology specific TDP-43 antibodies might be a valuable treatment option. On a related note, misfolding specific antibodies have been successfully used for SOD1 in ALS models and are a main focus of antibody mediated therapies in other neurodegenerative diseases like Parkinson's and Alzheimer's already[18, 139, 145, 171].

Interestingly, the unconjugated IgG control, also slightly reduced toxicity under unstressed conditions. However, we did not observe an effect under conditions of cellular stress. A

second antibody control, a heat denaturized c-terminal TDP-43 antibody, did not show a positive effect on toxicity. In other neurodegenerative disease models it has been shown that unspecific antibody effects can have an impact on antibody mediated enhancement of protein degradation[157, 158] which in turn has been shown to reduce toxicity in models of TDP-43 proteinopathies[21, 227]. However, these effects were mainly observed related to Fc-γ mediated phagocytosis in surrounding cells like microglia, rather than enhanced intracellular protein degradation that could occur in HEK-293 cells[20, 57, 248].

In conclusion, while able to observe small changes under unstressed conditions, all antibodies tested did not show an effect on toxicity after induction of oxidative stress. Combined with our results on TDP-43 aggregation it is likely that increasing toxicity by adding oxidative stress may outweigh potential positive effects by our antibodies at the concentrations used in this model. Further testing will be needed as non-cell autonomous clearing mechanisms and pathology specific antibodies are becoming focal points of antibody therapies in many other neurodegenerative disease[116, 139, 171, 227] and could circumvent some of discussed problems.

4.5 Alteration of TDP-43 uptake

Studies in different cellular models have shown that TDP-43 is released into the growth media and taken up by surrounding cells[75, 102]. Preventing the uptake of disease-relevant proteins such as tau[30, 83] or α-synuclein[103, 139, 232] is thought to be one of the driving factors in the effectiveness of antibody-based therapies in different neurodegenerative diseases[120, 135, 218]. Therefore, the idea to inhibit TDP-43 cell-to-cell transmission is thought to be a promising therapeutic strategy in ALS[18, 116, 161].

In our propagation experiments we found that the c-terminal antibody is capable of reducing TDP-43 uptake. On the contrary, all other antibodies that were tested increased TDP-43 uptake similarly to the control, a heat-denaturized TDP-43 antibody.

As mentioned, antibodies against α-synuclein and tau are able to interfere with the propagation of said proteins and hence reduce cellular toxicity. TDP-43 is directly released into the media similar to α-synuclein and tau suggesting that our c-terminal antibody similarly interferes and inhibits TDP-43 uptake. While direct interference with secreted proteins is one likely option, Feiler et al. were able to show that TDP-43 is transmitted more

effectively via vesicles rather than uncoated. These extracellular vesicles are suggested to be taken up by target cells via a multitude of different mechanisms, e.g. clathrin dependent and independent membrane fusion and pinocytosis (reviewed in [159, 167]). Antibodies binding to TDP-43 that can be present on the surface of these vesicles[75] may thus hamper protein internalization by inhibiting docking of the vesicle to cellular membranes, interfering with receptor mediated vesicle uptake, or by directing vesicles that have been taken up to degradation pathways rather than internalisation of the containing proteins.

In vivo a third pathway is microglia mediated uptake and degradation of extracellular protein[17, 116, 237, 247]. Supposedly antibody bound to target protein is cleared from the extracellular space and degraded by microglia via e.g. Fc-γ receptors[13, 232]. While this can only be speculated for TDP-43, as studies on the matter are all performed with antibodies targeting other key proteins in neurodegenerative disease like tau or α-synuclein, the underlying diseases and the role of key proteins in these diseases share many similarities with TDP-43 and ALS[152, 161, 188]. With activated microglia being a key feature in ALS as well[219, 226], it appears to be beneficial to further investigate this interaction and its potential use in possible antibody mediated therapies in ALS.

On the contrary, increased cellular stress due to the co-incubation of our antibodies could instead be the reason TDP-43 uptake is increased when using antibodies and our control. Similar results were found in a study by Flack et al as membrane permeability was increased for tau oligomers after applying stress. Additionally, the c-terminal region of TDP-43 has also been shown to impair membranes[80, 143].

The only antibody that had a positive effect on propagation was the c-terminally binding TDP-43 antibody, suggesting an important role of the c-terminal end of TDP-43 in uptake of the protein into adjacent cells. This might in part be due to a membrane de-stabilising effect that has been reported for the c-terminal region of TDP-43[80, 143]. This way the c-terminal antibody may not directly inhibit TDP-43 uptake, but instead stabilize cellular membranes thus reducing the amount of TDP-43 that is taken up. It is also interesting to note that the c-terminus and the n-terminus of TDP-43 are associated with the formation of different types of aggregates. While both ends of the protein may play a role in aggregation[46, 199], the c-terminus is suggested to be more important in the formation of pathologic, large TDP-43 aggregates[2, 133, 161, 174] whereas the n-terminus seems to be crucial in TDP-43 dimerization, a process that is suggested to be important to TDP-43's physiological functions[106, 209, 235, 259]. With the c-terminal antibody being the only

one with significant effects on TDP-43 propagation, one could suggest that c-terminally mediated larger TDP-43 aggregates are more important for cell-to-cell propagation.

While our findings suggest an important role of the c-terminal part of the protein in propagation, the importance of non-cell autonomous clearing mechanisms of extracellular aggregates must be highlighted. It is also important to note that we did not investigate further seeding or aggregate formation in the target cells, which could be another important step in which antibodies could impact TDP-43 propagation and its role in TDP-43 mediated diseases.

4.6 Side-by-side comparison of different TDP-43 antibodies

When comparing the antibodies used in each experimental setup side-by-side (see Table 7, chapter 3.5), the c-terminal antibody seems to outperform the other antibodies in question in most of our experimental setups. This becomes even more apparent when comparing it to the n-terminal antibody, which has its most positive effects in the same experimental setups as our c-terminal antibody does but was overall less effective than the c-terminal antibody. Across literature the c-terminal region is reported to have the biggest impact on aggregation[72, 98, 124, 132, 161, 175, 188]. Despite the fact that some n-terminal TDP-43 sequences have been discovered to play a role in aggregation, their impact seems to be less than that of c-terminal parts of the protein and more closely related to physiological properties of TDP-43[124, 199, 209, 235].

Interestingly, the biggest impact of antibody functionality seems to be achieved by higher binding affinity that comes with both broadly binding TDP-43 antibodies, leaving the n-terminal antibody still more effective than another c-terminal binding, yet pathology specific, antibody against phosphorylated Serins at position 409 and 410.

However, this antibody does have advantages compared to both broadly binding TDP-43 antibodies. It influences TDP-43 solubility which is closely linked to phosphorylation and cellular toxicity as we discussed previously (see 4.3 and [53, 164, 188]), and has a positive effect on overall toxicity in our experimental setting.

Whilst it has been reported that the RRM2 does also play a role in TDP-43 aggregation[229, 243], using an antibody targeted at the RRM2 did not show any meaningful impact on aggregation, toxicity or solubility in our study.

In summary, the greatest combined effects on solubility, aggregation and propagation could be observed for our broadly binding c-terminal antibody leaving it as the most effective antibody in testing. While the pTDP-s409/410 antibody was overall not as effective as the c-terminal antibody, its effects on solubility and improvements in cellular health during treatment make it worth noting as well.

4.7 Outlook

Using HEK-293 cells we were able to show that different antibodies against TDP-43 can alter TDP-43 solubility and aggregation. More precisely, co-incubating broadly binding antibodies against the c-terminus, n-terminus and phosphorylated TDP-43 can reduce the number of aggregates as well as the amount of insoluble TDP-43 in HEK-293 cells overexpressing TDP-43. Additionally, reduced toxicity and cell-to-cell propagation could be observed upon treatment with the pTDP-409/410 antibody and the c-terminal antibody respectively. However, the underlying mechanisms are still unknown.

To address this, studies performed for α-synuclein and tau might function as prime examples for future research. In this regard, misfolding specific antibodies have been used in experiments and first clinical trials for AD and PD to address antibody specificity. In ALS, one study utilized misfolding specific antibodies against SOD1[18, 139, 145, 171, 231].

Additional testing will also be mandatory regarding non-cell autonomous and neuron specific clearance mechanisms. These have been shown to be of great significance in other antibody mediated therapies, e.g. in AD or PD. Microglia and neuron co-cultures could provide a valuable tool to further evaluate TDP-43 propagation and aggregation.

Taken together, we were able to show that TDP-43 antibodies, especially a broadly binding c-terminal antibody in question, can in fact interfere with pathologic properties of TDP-43, as has been shown and translated into therapeutic options for the likes of α-synuclein in Parkinson's and tau in Alzheimer's diseases and has been suggested by multiple groups throughout the last couple of years[18, 59, 161].

5. Summary

Over the last years antibody-based therapies have become increasingly important as novel therapeutic options in many different neurodegenerative diseases like Alzheimer's and Parkinson's. With protein aggregation being a key pathophysiological feature of amyotrophic lateral sclerosis (ALS), we hypothesized that antibodies targeted against TDP-43 may also be beneficial in inhibiting protein aggregation, propagation and ultimately associated cellular toxicity.

Therefore, we chose commercially available antibodies against different parts of TDP-43 that had been reported to play a role in TDP-43 aggregation and solubility (a broadly binding c-terminal antibody, a broadly binding n-terminal antibody and an antibody against the RRM of TDP-43) as well as an antibody specifically binding to phosphorylated TDP-43 (phosphorylated Serin at 409 and 410). This modification is only found in TDP-43 clustered in pathological aggregates but not in the physiological form of the protein.

As a readout, we used a luciferase-based protein complementation assay for TDP-43 that had been previously established. Both broadly binding antibodies, the c-terminal one as well as the n-terminal antibody, did show a significant reduction of TDP-43 aggregation (~9%). When additionally adding oxidative stress to the cells, this positive effect was lost for the n-terminal antibody, while the c-terminal antibody did show an even greater reduction in aggregation of TDP-43 (~17%). To further investigate this aspect, we performed a Urea/RIPA solubility assay with the same antibodies und conditions of oxidative stress. Interestingly, not only the c-terminal, n-terminal but the pTDP-s409/410 antibody as well were all able to greatly reduce insoluble TDP-43 by up to 66% with the greatest effects detected for the pTDP-s409/410 antibody. An antibody that had only shown a trend towards reducing aggregation before (reduction of ~6%; p=0,08). However, while the RRM2 domain had previously been linked to aggregation, the tested RRM2 antibody was not able to reduce either aggregation nor did it promote changes in TDP-43 solubility and was hence ruled out for further testing.

Further, we wanted to test if our antibodies were also able to reduce cellular toxicity associated with TDP-43 overexpression and the addition of cellular stress. However, only the pathology specific pTDP-s409/410 antibody, was able to reduce toxicity, and only without the addition of oxidative stress to the cells.

Lastly, we tested the ability of our antibodies to inhibit cellular propagation of TDP-43, a mechanism that is hypothesized to play a major role in the spread of TDP-43 associated diseases. The c-terminal antibody was able to reduce the uptake of TDP-43 from the media into cells but did not reduce toxicity in this setting compared to our control. All other antibodies, including our antibody control, were even slightly increasing TDP-43 uptake compared to the untreated control.

With further research still being necessary to understand the treatment possibilities of antibodies in TDP-43-associated disease fully, we could show that a treatment of TDP-43 targeting antibodies can have an impact on TDP-43 aggregation, solubility propagation and even toxicity in cell culture. Therefore, the results gained in this study hint towards a possible benefit of antibody-based therapeutics in ALS and other TDP-43 proteinopathies, similar to Parkinson's and Alzheimer's disease, as has been suggested during the last couple of years.

6. References

1. Abu-Rumeileh Samir, Vacchiano Veria, Zenesini Corrado, Polischi Barbara, de Pasqua Silvia, Fileccia Enrico, Mammana Angela, Di Stasi Vitantonio, Capellari Sabina, Salvi Fabrizio, Liguori Rocco, Parchi Piero, Bartolomei Ilaria, Plasmati Rosaria, Pastorelli Francesca, Quarta Cecilia Celidea, Reale Vincenzo, Mariano Vincenza, Milletti David, Nasca Raffaella, Rizzi Francesca, Cherici Arianna, Rusolo Diletta, Valeriani Luca, Anzolin Francesca, Fantoni Elisabetta, Fiorito Alessia, Andrini Luciana, Avoni Patrizia, Donadio Vincenzo, Rizzo Giovanni, Morandi Luca, Marliani Federica, Albini-Riccioli Luca, Foschini Maria Pia, Pession Annalisa, Battaglia Stella, Poda Roberto, Valenti Danila, Asioli Sofia, Vignatelli Luca, Oppi Federico, Stanzani-Maserati Michelangelo, Bartoletti-Stella Anna, Colombo Carolina, Maselli Serena, Giannoccaro Maria Pia, Moresco Monica (2020) Diagnostic-prognostic value and electrophysiological correlates of CSF biomarkers of neurodegeneration and neuroinflammation in amyotrophic lateral sclerosis. J Neurol 267:1699–1708
2. Afroz Tariq, Hock Eva Maria, Ernst Patrick, Foglieni Chiara, Jambeau Melanie, Gilhespy Larissa AB, Laferriere Florent, Maniecka Zuzanna, Plückthun Andreas, Mittl Peer, Paganetti Paolo, Allain Frédéric HT, Polymenidou Magdalini (2017) Functional and dynamic polymerization of the ALS-linked protein TDP-43 antagonizes its pathologic aggregation. Nat Commun 8:45
3. Agrawal Sashank, Kuo Pan Hsien, Chu Lee Ya, Golzarroshan Bagher, Jain Monika, Yuan Hanna S (2019) RNA recognition motifs of disease-linked RNA-binding proteins contribute to amyloid formation. Sci Rep 9:1–12
4. Alami Nael H, Smith Rebecca B, Carrasco Monica A, Williams Luis A, Winborn Christina S, Han Steve SW, Kiskinis Evangelos, Winborn Brett, Freibaum Brian D, Kanagaraj Anderson, Clare Alison J, Badders Nisha M, Bilican Bilada, Chaum Edward, Chandran Siddharthan, Shaw Christopher E, Eggan Kevin C, Maniatis Tom, Taylor J Paul (2014) Axonal Transport of TDP-43 mRNA Granules Is Impaired by ALS-Causing Mutations. Neuron 81:536–543
5. Amlie-Wolf Alexandre, Ryvkin Paul, Tong Rui, Dragomir Isabelle, Suh Eunran, Xu Yan, Van Deerlin Vivianna M, Gregory Brian D, Kwong Linda K, Trojanowski John Q, Lee Virginia MY, Wang Li San, Lee Edward B (2015) Transcriptomic changes due to cytoplasmic TDP-43 expression reveal dysregulation of histone transcripts and nuclear chromatin. PLoS One 10:e0141836
6. Andersen Peter M, Abrahams Sharon, Borasio Gian D, de Carvalho Mamede, Chio Adriano, Van Damme Philip, Hardiman Orla, Kollewe Katja, Morrison Karen E, Petri Susanne, Pradat Pierre Francois, Silani Vincenzo, Tomik Barbara, Wasner Maria, Weber Markus (2012) EFNS guidelines on the Clinical Management of Amyotrophic Lateral Sclerosis (MALS) - revised report of an EFNS task force. Eur J Neurol 19:360–375
7. Anderson Paul, Kedersha Nancy (2008) Stress granules: the Tao of RNA triage. Trends Biochem Sci 33:141–150
8. Anderson Paul, Kedersha Nancy (2009) RNA granules: Post-transcriptional and epigenetic modulators of gene expression. Nat Rev Mol Cell Biol 10:430–436
9. Andrew Eisen, Heather Stewart, Michael Schulzer, Donald Cameron (1993) Anti-Glutamate Therapy in Amyotrophic Lateral Sclerosis: A Trial Using Lamotrigine. Can J Neurol Sci / J Can des Sci Neurol 20:297–301
10. Arai Tetsuaki, Hasegawa Masato, Akiyama Haruhiko, Ikeda Kenji, Nonaka Takashi, Mori Hiroshi, Mann David, Tsuchiya Kuniaki, Yoshida Mari, Hashizume Yoshio,

Oda Tatsuro (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 351:602–611
11. Arnold ES, Ling SC, Huelga SC, Lagier-Tourenne C, Polymenidou M, Ditsworth D, Kordasiewicz HB, McAlonis-Downes M, Platoshyn O, Parone PA, Da Cruz S, Clutario KM, Swing D, Tessarollo L, Marsala M, Shaw CE, Yeo GW, Cleveland DW (2013) ALS-linked TDP-43 mutations produce aberrant RNA splicing and adult-onset motor neuron disease without aggregation or loss of nuclear TDP-43. Proc Natl Acad Sci 110:E736–E745
12. Ash Peter EA, Zhang Yong-Jie, Roberts Christine M, Saldi Tassa, Hutter Harald, Buratti Emanuele, Petrucelli Leonard, Link Christopher D (2010) Neurotoxic effects of TDP-43 overexpression in C. elegans. Hum Mol Genet 19:3206–3218
13. Asuni Ayodeji A, Boutajangout Allal, Quartermain David, Sigurdsson Einar M (2007) Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. J Neurosci 27:9115–9129
14. Aulas Anaïs, Velde Christine Vande (2015) Alterations in stress granule dynamics driven by TDP-43 and FUS: A link to pathological inclusions in ALS? Front Cell Neurosci 9:423
15. Ayala Youhna M, De Conti Laura, Avendaño-Vázquez S Eréndira, Dhir Ashish, Romano Maurizio, D'Ambrogio Andrea, Tollervey James, Ule Jernej, Baralle Marco, Buratti Emanuele, Baralle Francisco E (2011) TDP-43 regulates its mRNA levels through a negative feedback loop. EMBO J 30:277–288
16. Ayala Youhna M, Zago Paola, D'Ambrogio Andrea, Xu Ya Fei, Petrucelli Leonard, Buratti Emanuele, Baralle Francisco E (2008) Structural determinants of the cellular localization and shuttling of TDP-43. J Cell Sci 121:3778–3785
17. Bae Eun-Jin, Lee He-Jin, Rockenstein Edward, Ho Dong-Hwan, Park Eun-Bi, Yang Na-Young, Desplats Paula, Masliah Eliezer, Lee Seung-Jae (2012) Antibody-aided clearance of extracellular α-synuclein prevents cell-to-cell aggregate transmission. J Neurosci 32:13454–13469
18. Baekelandt Veerle, Lobbestael Evy (2017) Disease-Modifying Targets in Neurodegenerative Disorders: Paving the Way for Disease-Modifying Therapies. Academic Press
19. Baloh Robert H (2011) TDP-43: the relationship between protein aggregation and neurodegeneration in amyotrophic lateral sclerosis and frontotemporal lobar degeneration. FEBS J 278:3539–3549
20. Bard Frédérique, Cannon Catherine, Barbour Robin, Burke Rae Lyn, Games Dora, Grajeda Henry, Guido Teresa, Hu Kang, Huang Jiping, Johnson-Wood Kelly, Khan Karen, Kholodenko Dora, Lee Mike, Lieberburg Ivan, Motter Ruth, Nguyen Minh, Soriano Ferdie, Vasquez Nicki, Weiss Kim, Welch Brent, Seubert Peter, Schenk Dale, Yednock Ted (2000) Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 6:916–919
21. Barmada Sami J, Serio Andrea, Arjun Arpana, Bilican Bilada, Daub Aaron, Ando D Michael, Tsvetkov Andrey, Pleiss Michael, Li Xingli, Peisach Daniel, Shaw Christopher, Chandran Siddharthan, Finkbeiner Steven (2014) Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. Nat Chem Biol 10:677–685
22. Barmada Sami J, Skibinski Gaia, Korb Erica, Rao Elizabeth J, Wu Jane Y, Finkbeiner Steven (2010) Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral

sclerosis. J Neurosci 30:639–649
23. Beel Sander, Herdewyn Sarah, Fazal Raheem, De Decker Mathias, Moisse Matthieu, Robberecht Wim, Van Den Bosch Ludo, Van Damme Philip (2018) Progranulin reduces insoluble TDP-43 levels, slows down axonal degeneration and prolongs survival in mutant TDP-43 mice. Mol Neurodegener 13:55
24. Belzil Veronique V, Katzman Rebecca B, Petrucelli Leonard (2016) ALS and FTD: an epigenetic perspective. Acta Neuropathol 132:487–502
25. Benkler Chen, O'Neil Alison L, Slepian Susannah, Qian Fang, Weinreb Paul H, Rubin Lee L (2018) Aggregated SOD1 causes selective death of cultured human motor neurons. Sci Rep 8:16393
26. Bensimon G, Lacomblez L, Meininger V (1994) A controlled trial of riluzole in amyotrophic lateral sclerosis. N Engl J Med 330:585–591
27. Bentmann Eva, Neumann Manuela, Tahirovic Sabina, Rodde Ramona, Dormann Dorothee, Haass Christian (2012) Requirements for stress granule recruitment of fused in sarcoma (FUS) and TAR DNA-binding protein of 43 kDa (TDP-43). J Biol Chem 287:23079–23094
28. Van Den Berg JP, Kalmijn S, Lindeman E, Veldink JH, De Visser M, Van Der Graaff MM, Wokke JHJ, Van Den Berg LH (2005) Multidisciplinary ALS care improves quality of life in patients with ALS. Neurology 65:1264–1267
29. Bhardwaj Amit, Myers Michael P, Buratti Emanuele, Baralle Francisco E (2013) Characterizing TDP-43 interaction with its RNA targets. Nucleic Acids Res 41:5062–5074
30. Bi Mian, Ittner Arne, Ke Yazi D, Götz Jürgen, Ittner Lars M (2011) Tau-targeted immunization impedes progression of neurofibrillary histopathology in aged P301L tau transgenic mice. PLoS One 6:e26860
31. Blokhuis Anna M, Groen Ewout JN, Koppers Max, Van Den Berg Leonard H, Pasterkamp R Jeroen (2013) Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol 125:777–794
32. Blokhuis Anna M, Koppers Max, Groen Ewout JN, van den Heuvel Dianne MA, Dini Modigliani Stefano, Anink Jasper J, Fumoto Katsumi, van Diggelen Femke, Snelting Anne, Sodaar Peter, Verheijen Bert M, Demmers Jeroen AA, Veldink Jan H, Aronica Eleonora, Bozzoni Irene, den Hertog Jeroen, van den Berg Leonard H, Pasterkamp R Jeroen (2016) Comparative interactomics analysis of different ALS-associated proteins identifies converging molecular pathways. Acta Neuropathol 132:175–196
33. Boyd Justin D, Lee-Armandt J Peter, Feiler Marisa S, Zaarur Nava, Liu Min, Kraemer Brian, Concannon John B, Ebata Atsushi, Wolozin Benjamin, Glicksman Marcie A (2014) A High-Content Screen Identifies Novel Compounds That Inhibit Stress-Induced TDP-43 Cellular Aggregation and Associated Cytotoxicity. J Biomol Screen 19:44–56
34. Bozzo F, Mirra A, Carrì MT (2017) Oxidative stress and mitochondrial damage in the pathogenesis of ALS: New perspectives. Neurosci Lett 636:3–8
35. Braak Heiko, Brettschneider Johannes, Ludolph Albert C, Lee Virginia M, Trojanowski John Q, Del Tredici Kelly (2013) Amyotrophic lateral sclerosis-a model of corticofugal axonal spread. Nat Rev Neurol 9:708–714
36. Brettschneider Johannes, Arai Kimihito, Del Tredici Kelly, Toledo Jon B, Robinson John L, Lee Edward B, Kuwabara Satoshi, Shibuya Kazumoto, Irwin David J, Fang Lubin, Brettschneider J, Arai K, Del Tredici contributed equally Braak KH, Trojanowski are Senior authors JQ (2014) TDP-43 pathology and neuronal loss in amyotrophic lateral sclerosis spinal cord HHS Public Access. Acta Neuropathol 128:423–437

37. Brettschneider Johannes, Del Tredici Kelly, Toledo Jon B, Robinson John L, Irwin David J, Grossman Murray, Suh EunRan, Van Deerlin Vivianna M, Wood Elisabeth M, Baek Young, Kwong Linda, Lee Edward B, Elman Lauren, McCluskey Leo, Fang Lubin, Feldengut Simone, Ludolph Albert C, M-Y Lee Virginia, Braak Heiko, Trojanowski John Q, Neurol Author manuscript Ann (2013) Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann Neurol 74:20–38
38. Vanden Broeck Lies, Callaerts Patrick, Dermaut Bart (2014) TDP-43-mediated neurodegeneration: Towards a loss-of-function hypothesis? Trends Mol Med 20:66–71
39. Brown Dean G, Shorter James, Wobst Heike J (2019) Emerging small-molecule therapeutic approaches for amyotrophic lateral sclerosis and frontotemporal dementia. Bioorg Med Chem Lett 30:126942
40. Budini Mauricio, Buratti Emanuele, Morselli Eugenia, Criollo Alfredo (2017) Autophagy and its impact on neurodegenerative diseases: New roles for TDP-43 and C9orf72. Front Mol Neurosci 10:170
41. Buratti Emanuele (2015) Functional Significance of TDP-43 Mutations in Disease. Adv Genet 91:1–53
42. Buratti Emanuele, Conti Laura De, Stuani Cristiana, Romano Maurizio, Baralle Marco, Baralle Francisco (2010) Nuclear factor TDP-43 can affect selected microRNA levels. FEBS J 277:2268–2281
43. Buratti Emanuele, Dörk Thilo, Zuccato Elisabetta, Pagani Franco, Romano Maurizio, Baralle Francisco E (2001) Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. EMBO J 20:1774–1784
44. De Carvalho Mamede, Swash Michael (2009) Awaji diagnostic algorithm increases sensitivity of El Escorial criteria for ALS diagnosis. Amyotroph Lateral Scler 10:53–57
45. Chang Chung Ke, Chiang Ming Hui, Toh Elsie Khai Woon, Chang Chi Fon, Huang Tai Huang (2013) Molecular mechanism of oxidation-induced TDP-43 RRM1 aggregation and loss of function. FEBS Lett 587:575–582
46. Chang Chung ke, Wu Tzong Huah, Wu Chu Ya, Chiang Ming hui, Toh Elsie Khai Woon, Hsu Yin Chih, Lin Ku Feng, Liao Yu heng, Huang Tai huang, Huang Joseph Jen Tse (2012) The N-terminus of TDP-43 promotes its oligomerization and enhances DNA binding affinity. Biochem Biophys Res Commun 425:219–224
47. Charcot JM (1874) Amyotrophies spinales deuteropathiques sclérose latérale amyotrophique & Sclérose latérale amyotrophique. Bur du Progrès Médical 2:234–266
48. Chen-Plotkin Alice S, Lee Virginia MY, Trojanowski John Q (2010) TAR DNA-binding protein 43 in neurodegenerative disease. Nat Rev Neurol 6:211–220
49. Chen Allan KH, Lin Ryan YY, Hsieh Eva ZJ, Tu Pang Hsien, Chen Rita PY, Liao Tai Yan, Chen Wenlung, Wang Chih Hsien, Huang Joseph JT (2010) Induction of amyloid fibrils by the C-terminal fragments of TDP-43 in amyotrophic lateral sclerosis. J Am Chem Soc 132:1186–1187
50. Chen Tong, Turner Bradley J, Beart Philip M, Sheehan-Hennessy Lucy, Elekwachi Chinasom, Muyderman Hakan (2018) Glutathione monoethyl ester prevents TDP-43 pathology in motor neuronal NSC-34 cells. Neurochem Int 112:278–287
51. Chiò Adriano, Battistini Stefania, Calvo Andrea, Caponnetto Claudia, Conforti Francesca L, Corbo Massimo, Giannini Fabio, Mandrioli Jessica, Mora Gabriele, Sabatelli Mario, Ajmone Clara, Mastro Enza, Pain Debora, Mandich Paola, Penco Silvana, Restagno Gabriella, Zollino Marcella, Surbone Antonella, Monsurrò Maria Rosaria, Tedeschi Gioacchino, Conte Amelia, Luigetti Marco, Lattante Serena, Marangi Giuseppe, Volanti Paolo, Marinou Kalliopi, Papetti Laura, Lunetta

Christian, Pintor Giuseppe Lauria, Salvi Fabrizio, Bartolomei Ilaria, Quattrone Aldo, Gambardella Antonio, Logroscino Giancarlo, Simone Isabella, Pisano Fabrizio, Spataro Rossella, La Bella Vincenzo, Colletti Tiziana, Mancardi Gianluigi, Origone Paola, Sola Patrizia, Borghero Giuseppe, Marrosu Francesco, Marrosu Maria Giovanna, Murru Maria Rita, Floris Gianluca, Cannas Antonino, Piras Valeria, Costantino Emanuela, Pani Carla, Sotgiu Maria Alessandra, Pugliatti Maura, Parish Leslie D, Cossu Paola, Ticca Anna, Rodolico Carmelo, Portaro Simona, Ricci Claudia, Moglia Cristina, Ossola Irene, Brunetti Maura, Barberis Marco, Canosa Antonio, Cammarosano Stefania, Bertuzzo Davide, Fuda Giuseppe, Ilardi Antonio, Manera Umberto, Pastore Ilaria, Sproviero William, Logullo Francesco, Tanel Raffaella (2014) Genetic counselling in ALS: Facts, uncertainties and clinical suggestions. J Neurol Neurosurg Psychiatry 85:478–485

52. Chio Adriano, Calvo Andrea, Mazzini Letizia, Cantello Roberto, Mora Gabriele, Moglia Cristina, Corrado Lucia, D'Alfonso Sandra, Majounie Elisa, Renton Alan, Pisano Fabrizio, Ossola Irene, Brunetti Maura, Traynor Bryan J, Restagno Gabriella (2012) Extensive genetics of ALS : A population-based study in Italy. Neurology 79:1983–1989
53. Choksi Darshana K, Roy Bidisha, Chatterjee Shreyasi, Yusuff Tanzeen, Bakhoum Mathieu F, Sengupta Urmi, Ambegaokar Suren, Kayed Rakez, Jackson George R (2013) TDP-43 Phosphorylation by casein kinase Iε promotes oligomerization and enhances toxicity in vivo. Hum Mol Genet 23:1025–1035
54. Cistaro Angelina, Pagani Marco, Montuschi Anna, Calvo Andrea, Moglia Cristina, Canosa Antonio, Restagno Gabriella, Brunetti Maura, Traynor Bryan J, Nobili Flavio, Carrara Giovanna, Fania Piercarlo, Lopiano Leonardo, Valentini M Consuelo, Chiò Adriano (2014) The metabolic signature of C9ORF72-related ALS: FDG PET comparison with nonmutated patients. Eur J Nucl Med Mol Imaging 41:844–852
55. Collins Mahlon, Riascos David, Kovalik Tina, An Jiyan, Krupa Kelly, Krupa Kristin, Hood Brian L, Conrads Thomas P, Renton Alan E, Traynor Bryan J, Bowser Robert (2012) The RNA-binding motif 45 (RBM45) protein accumulates in inclusion bodies in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) patients. Acta Neuropathol 124:717–732
56. Colombrita Claudia, Zennaro Eleonora, Fallini Claudia, Weber Markus, Sommacal Andreas, Buratti Emanuele, Silani Vincenzo, Ratti Antonia (2009) TDP-43 is recruited to stress granules in conditions of oxidative insult. J Neurochem 111:1051–1061
57. Congdon Erin E, Gu Jiaping, Sait Hameetha BR, Sigurdsson Einar M (2013) Antibody Uptake into Neurons Occurs Primarily via Clathrin-dependent Fcγ Receptor Endocytosis and Is a Prerequisite for Acute Tau Protein Clearance. J Biol Chem 288:35452–35465
58. Conicella Alexander E, Zerze Gül H, Mittal Jeetain, Fawzi Nicolas L (2016) ALS Mutations Disrupt Phase Separation Mediated by α-Helical Structure in the TDP-43 Low-Complexity C-Terminal Domain. Structure 24:1537–1549
59. De Conti Laura, Borroni Barbara, Baralle Marco (2017) New routes in frontotemporal dementia drug discovery. Expert Opin Drug Discov 12:659–671
60. Cooper-Knock Johnathan, Higginbottom Adrian, Connor-Robson Natalie, Bayatti Nadhim, Bury Joanna J, Kirby Janine, Ninkina Natalia, Buchman Vladimir L, Shaw Pamela J (2013) Clinical/Scientific Notes C9ORF72 transcription in a frontotemporal dementia case with two expanded alleles. Neurology 81:1719–1721
61. Correia Ana Sofia, Patel Priyanka, Dutta Kallol, Julien Jean Pierre (2015)

Inflammation induces TDP-43 mislocalization and aggregation. PLoS One 10:e0140248
62. Costa Joã, Swash Michael, de Carvalho Mamede (2012) Awaji Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis A Systematic Review. Arch Neurol 69:1410–1416
63. Costa Julia, Gomes Catarina, de Carvalho Mamede (2012) Diagnosis, Pathogenesis and Therapeutic Targets in Amyotrophic Lateral Sclerosis. CNS Neurol Disord - Drug Targets 9:764–778
64. Coyne Alyssa N, Zaepfel Benjamin L, Zarnescu Daniela C (2017) Failure to deliver and translate—new insights into RNA dysregulation in ALS. Front Cell Neurosci 11:243
65. Cozzolino Mauro, Rossi Simona, Mirra Alessia, Carrì Maria Teresa (2015) Mitochondrial dynamism and the pathogenesis of amyotrophic lateral sclerosis. Front Cell Neurosci 9:31
66. D'Amico Emanuele, Factor-Litvak Pam, Santella Regina M, Mitsumoto Hiroshi (2013) Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. Free Radic Biol Med 65:509–527
67. Davis Stephani A, Itaman Sheed, Khalid-Janney Christopher M, Sherard Justin A, Dowell James A, Cairns Nigel J, Gitcho Michael A (2018) TDP-43 interacts with mitochondrial proteins critical for mitophagy and mitochondrial dynamics. Neurosci Lett 678:8–15
68. Decker Thomas, Lohmann-Matthes Marie Luise (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. J Immunol Methods 115:61–69
69. DeJesus-Hernandez Mariely, Mackenzie Ian R, Boeve Bradley F, Boxer Adam L, Baker Matt, Rutherford Nicola J, Nicholson Alexandra M, Finch Ni Cole A, Flynn Heather, Adamson Jennifer, Kouri Naomi, Wojtas Aleksandra, Sengdy Pheth, Hsiung Ging Yuek R, Karydas Anna, Seeley William W, Josephs Keith A, Coppola Giovanni, Geschwind Daniel H, Wszolek Zbigniew K, Feldman Howard, Knopman David S, Petersen Ronald C, Miller Bruce L, Dickson Dennis W, Boylan Kevin B, Graff-Radford Neill R, Rademakers Rosa (2011) Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. Neuron 72:245–256
70. Deng Han Xiang, Chen Wenjie, Hong Seong Tshool, Boycott Kym M, Gorrie George H, Siddique Nailah, Yang Yi, Fecto Faisal, Shi Yong, Zhai Hong, Jiang Hujun, Hirano Makito, Rampersaud Evadnie, Jansen Gerard H, Donkervoort Sandra, Bigio Eileen H, Brooks Benjamin R, Ajroud Kaouther, Sufit Robert L, Haines Jonathan L, Mugnaini Enrico, Pericak-Vance Margaret A, Siddique Teepu (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477:211–215
71. Dewey CM, Cenik B, Sephton CF, Dries DR, Mayer P, Good SK, Johnson BA, Herz J, Yu G (2011) TDP-43 Is Directed to Stress Granules by Sorbitol, a Novel Physiological Osmotic and Oxidative Stressor. Mol Cell Biol 31:1098–1108
72. Ding Xuebing, Ma Mingming, Teng Junfang, Teng Robert KF, Zhou Shuang, Yin Jingzheng, Fonkem Ekokobe, Huang Jason H, Wu Erxi, Wang Xuejing (2015) Exposure to ALS-FTD-CSF generates TDP-43 aggregates in glioblastoma cells through exosomes and TNTs-like structure. Oncotarget 6:24178–24191
73. Fang Yu Sheng, Tsai Kuen Jer, Chang Yu Jen, Kao Patricia, Woods Rima, Kuo Pan Hsien, Wu Cheng Chun, Liao Jhih Ying, Chou Shih Chieh, Lin Vinson, Jin Lee Way, Yuan Hanna S, Cheng Irene H, Tu Pang Hsien, Chen Yun Ru (2014) Full-

length TDP-43 forms toxic amyloid oligomers that are present in frontotemporal lobar dementia-TDP patients. Nat Commun 5:4824
74. Fecto Faisal, Yan Jianhua, Vemula S Pavan, Liu Erdong, Yang Yi, Chen Wenjie, Zheng Jian Guo, Shi Yong, Siddique Nailah, Arrat Hasan, Donkervoort Sandra, Ajroud-Driss Senda, Sufit Robert L, Heller Scott L, Deng Han Xiang, Siddique Teepu (2011) SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch Neurol 68:1440–1446
75. Feiler Marisa S, Strobel Benjamin, Freischmidt Axel, Helferich Anika M, Kappel Julia, Brewer Bryson M, Li Deyu, Thal Dietmar R, Walther Paul, Ludolph Albert C, Danzer Karin M, Weishaupt Jochen H (2015) TDP-43 is intercellularly transmitted across axon terminals. J Cell Biol 211:897–911
76. Feneberg Emily, Gray Elizabeth, Ansorge Olaf, Talbot Kevin, Turner Martin R (2018) Towards a TDP-43-Based Biomarker for ALS and FTLD. Mol Neurobiol 55:1–13
77. Ferguson Toby A, Elman Lauren B (2007) Clinical presentation and diagnosis of Amyotrophic Lateral Sclerosis. NeuroRehabilitation 22:409–416
78. Filimonenko Maria, Stuffers Susanne, Raiborg Camilla, Yamamoto Ai, Malerød Lene, Fisher Elizabeth MC, Isaacs Adrian, Brech Andreas, Stenmark Harald, Simonsen Anne (2007) Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. J Cell Biol 179:485–500
79. Fitzmaurice PS, Shaw IC, Kleiner HE, Miller RT, Monks TJ, Lau SS, Mitchell JD, Lynch PG (1996) Evidence for DNA damage in amyotrophic lateral sclerosis. Muscle Nerve 19:797–798
80. Flach Katharina, Hilbrich Isabel, Schiffmann Andrea, Gärtner Ulrich, Krüger Martin, Leonhardt Marion, Waschipky Hanka, Wick Lukas, Arendt Thomas, Holzer Max (2012) Tau oligomers impair artificial membrane integrity and cellular viability. J Biol Chem 287:43223–43233
81. Forsberg Karin, Andersen Peter M, Marklund Stefan L, Brännström Thomas (2011) Glial nuclear aggregates of superoxide dismutase-1 are regularly present in patients with amyotrophic lateral sclerosis. Acta Neuropathol 121:623–634
82. Freibaum Brian D, Chitta Raghu K, High Anthony A, Taylor J Paul (2010) Global analysis of TDP-43 interacting proteins reveals strong association with RNA splicing and translation machinery. J Proteome Res 9:1104–1120
83. Funk Kristen E, Mirbaha Hilda, Jiang Hong, Holtzman David M, Diamond Marc I (2015) Distinct Therapeutic Mechanisms of Tau Antibodies: Promoting Microglial Clearance Versus Blocking Neuronal Uptake. J Biol Chem 290:21652–21662
84. Furukawa Yoshiaki, Kaneko Kumi, Watanabe Shoji, Yamanaka Koji, Nukina Nobuyuki (2011) A seeding reaction recapitulates intracellular formation of sarkosyl-insoluble transactivation response element (TAR) DNA-binding protein-43 inclusions. J Biol Chem 286:18664–18672
85. Gasset-Rosa Fatima, Lu Shan, Yu Haiyang, Chen Cong, Melamed Ze'ev, Guo Lin, Shorter James, Da Cruz Sandrine, Cleveland Don W (2019) Cytoplasmic TDP-43 De-mixing Independent of Stress Granules Drives Inhibition of Nuclear Import, Loss of Nuclear TDP-43, and Cell Death. Neuron 102:339-357.e7
86. Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Boada Rovira M, Forette F, Orgogozo JM (2005) Clinical effects of Aβ immunization (AN1792) in patients with AD in an interrupted trial. Neurology 64:1553–1562
87. Goossens Joery, Vanmechelen Eugeen, Trojanowski John Q, Lee Virginia MY, Van Broeckhoven Christine, van der Zee Julie, Engelborghs Sebastiaan (2015) TDP-43

as a possible biomarker for frontotemporal lobar degeneration: a systematic review of existing antibodies. Acta Neuropathol Commun 3:15
88. Gornall AG, Bardawill CJ, David MM (1949) Determination of serum proteins by means of the biuret reaction. J Biol Chem 177:751–766
89. Guenther Elizabeth L, Cao Qin, Trinh Hamilton, Lu Jiahui, Sawaya Michael R, Cascio Duilio, Boyer David R, Rodriguez Jose A, Hughes Michael P, Eisenberg David S (2018) Atomic structures of TDP-43 LCD segments and insights into reversible or pathogenic aggregation. Nat Struct Mol Biol 25:463–471
90. Guo Weirui, Chen Yanbo, Zhou Xiaohong, Kar Amar, Ray Payal, Chen Xiaoping, Rao Elizabeth J, Yang Mengxue, Ye Haihong, Zhu Li, Liu Jianghong, Xu Meng, Yang Yanlian, Wang Chen, Zhang David, Bigio Eileen H, Mesulam Marsel, Shen Yan, Xu Qi, Fushimi Kazuo, Wu Jane Y (2011) An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity. Nat Struct Mol Biol 18:822–831
91. Hans Friederike, Fiesel Fabienne C, Strong Jennifer C, Jackel Sändra, Rasse Tobias M, Geisler Sven, Springer Wolfdieter, Schulz Jörg B, Voigt Aaron, Kahle Philipp J (2014) UBE2E ubiquitin-conjugating enzymes and ubiquitin isopeptidase y regulate TDP-43 protein ubiquitination. J Biol Chem 289:19164–19179
92. Hardiman Orla, Al-Chalabi Ammar, Chio Adriano, Corr Emma M, Logroscino Giancarlo, Robberecht Wim, Shaw Pamela J, Simmons Zachary, Van Den Berg Leonard H (2017) Amyotrophic lateral sclerosis. Nat Rev Dis Prim 3:17071
93. Hardiman Orla, van den Berg Leonard H (2017) Edaravone: a new treatment for ALS on the horizon? Lancet Neurol 16:490–491
94. Harrison Alice Ford, Shorter James (2017) RNA-binding proteins with prion-like domains in health and disease. Biochem J 474:1417–1438
95. Hebron Michaeline L, Lonskaya Irina, Sharpe Kaydee, Weerasinghe Puwakdandawe PK, Algarzae Norah K, Shekoyan Ashot R, Moussa Charbel EH (2013) Parkin ubiquitinates tar-DNA binding protein-43 (TDP-43) and promotes its cytosolic accumulation via interaction with histone deacetylase 6 (HDAC6). J Biol Chem 288:4103–4115
96. Hock Eva Maria, Polymenidou Magdalini (2016) Prion-like propagation as a pathogenic principle in frontotemporal dementia. J Neurochem 138:163–183
97. Hong Kun, Li Yi, Duan Weisong, Guo Yansu, Jiang Hong, Li Wenju, Li Chunyan (2012) Full-length TDP-43 and its C-terminal fragments activate mitophagy in NSC34 cell line. Neurosci Lett 530:144–149
98. Igaz Lionel M, Kwong Linda K, Chen-Plotkin Alice, Winton Matthew J, Unger Travis L, Xu Yan, Neumann Manuela, Trojanowski John Q, Lee Virginia MY (2009) Expression of TDP-43 C-terminal fragments in vitro recapitulates pathological features of TDP-43 proteinopathies. J Biol Chem 284:8516–8524
99. Igaz Lionel M, Kwong Linda K, Lee Edward B, Chen-Plotkin Alice, Swanson Eric, Unger Travis, Malunda Joe, Xu Yan, Winton Matthew J, Trojanowski John Q, Lee Virginia MY (2011) Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. J Clin Invest 121:726–738
100. Igaz Lionel M, Kwong Linda K, Xu Yan, Truax Adam C, Uryu Kunihiro, Neumann Manuela, Clark Christopher M, Elman Lauren B, Miller Bruce L, Grossman Murray, McCluskey Leo F, Trojanowski John Q, Lee Virginia MY (2008) Enrichment of C-terminal fragments in TAR DNA-binding protein-43 cytoplasmic inclusions in brain but not in spinal cord of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Am J Pathol 173:182–194
101. Iguchi Yohei, Katsuno Masahisa, Niwa Jun-ichi, Takagi Shinnosuke, Ishigaki Shinsuke, Ikenaka Kensuke, Kawai Kaori, Watanabe Hirohisa, Yamanaka Koji,

Takahashi Ryosuke (2013) Loss of TDP-43 causes age-dependent progressive motor neuron degeneration. Brain 136:1371–1382
102. Ishii Tomohiro, Kawakami Emiko, Endo Kentaro, Misawa Hidemi, Watabe Kazuhiko (2017) Formation and spreading of TDP-43 aggregates in cultured neuronal and glial cells demonstrated by time-lapse imaging. PLoS One 12:e0179375
103. Jankovic J, Goodman I, Safirstein B, Schenk DB, Kinney GG, Koller M, Ness DK, Griffith SG, Grundman M, Soto J, Ostrowitzki S, Boess FG, Martin-Facklam M, Quinn JF, Issacson SH, Jennings D, Omidvar O, Ellenbogen A (2018) Results from a phase 1b multiple ascending-dose study of PRX002/RG7935, an Anti-alpha-synuclein monoclonal antibody, in patients with Parkinson's disease. Parkinsonism Relat Disord 46:e25
104. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, St George-Hyslop P, Westaway D (2000) A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 408:979–982
105. Jiang Lei-Lei Lei, Che Mei-Xia Xia, Zhao Jian, Zhou Chen-Jie Jie, Xie Mu-Yun Yun, Li Hai-Yin Yin, He Jian-Hua Hua, Hu Hong-Yu Yu (2013) Structural transformation of the amyloidogenic core region of TDP-43 protein initiates its aggregation and cytoplasmic inclusion. J Biol Chem 288:19614–19624
106. Jiang Lei Lei, Xue Wei, Hong Jun Ye, Zhang Jun Ting, Li Min Jun, Yu Shao Ning, He Jian Hua, Hu Hong Yu (2017) The N-terminal dimerization is required for TDP-43 splicing activity. Sci Rep 7:6196
107. Jiang Lei Lei, Zhao Jian, Yin Xiao Fang, He Wen Tian, Yang Hui, Che Mei Xia, Hu Hong Yu (2016) Two mutations G335D and Q343R within the amyloidogenic core region of TDP-43 influence its aggregation and inclusion formation. Sci Rep 6:23928
108. Johnson Brian S, McCaffery J Michael, Lindquist Susan, Gitler Aaron D (2008) A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. Proc Natl Acad Sci U S A 105:6439–6444
109. Johnson Brian S, Snead David, Lee Jonathan J, McCaffery J Michael, Shorter James, Gitler Aaron D (2009) TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. J Biol Chem 284:20329–20339
110. Jordan M, Wurm F (2004) Transfection of adherent and suspended cells by calcium phosphate. Methods 33:136–143
111. Juneja T, Pericak-Vance MA, Laing NG, Dave S, Siddique T (1997) Prognosis in familial amyotrophic lateral sclerosis: progression and survival in patients with glu100gly and ala4val mutations in Cu,Zn superoxide dismutase. Neurology 48:55–57
112. Kabashi Edor, Lin Li, Tradewell Miranda L, Dion Patrick A, Bercier Valérie, Bourgouin Patrick, Rochefort Daniel, Bel Hadj Samar, Durham Heather D, Velde Christine Vande, Rouleau Guy A, Drapeau Pierre (2009) Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits in vivo. Hum Mol Genet 19:671–683
113. Kabashi Edor, Valdmanis Paul N, Dion Patrick, Spiegelman Dan, McConkey Brendan J, Velde Christine Vande, Bouchard Jean Pierre, Lacomblez Lucette, Pochigaeva Ksenia, Salachas Francois, Pradat Pierre Francois, Camu William, Meininger Vincent, Dupre Nicolas, Rouleau Guy A (2008) TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat Genet

40:572–574
114. Kasu Yasar Arfat T, Alemu Samrawit, Lamari Angela, Loew Nicole, Brower Christopher S (2018) The N Termini of TAR DNA-Binding Protein 43 (TDP43) C-Terminal Fragments Influence Degradation, Aggregation Propensity, and Morphology. Mol Cell Biol 38:e00243-18
115. Kato Shinsuke (2008) Amyotrophic lateral sclerosis models and human neuropathology: Similarities and differences. Acta Neuropathol 115:97–114
116. Katsinelos Taxiarchis, Tuck Benjamin J, Mukadam Aamir S, McEwan William A (2019) The role of antibodies and their receptors in protection against ordered protein assembly in neurodegeneration. Front Immunol 10:1139
117. Kawahara Yukio, Mieda-Sato Ai (2012) TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. Proc Natl Acad Sci U S A 109:3347–3352
118. Kedersha Nancy L, Gupta Mita, Li Wei, Miller Ira, Anderson Paul (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2α to the assembly of mammalian stress granules. J Cell Biol 147:1431–1441
119. Kerman Aaron, Liu Hsueh Ning, Croul Sidney, Bilbao Juan, Rogaeva Ekaterina, Zinman Lorne, Robertson Janice, Chakrabartty Avijit (2010) Amyotrophic lateral sclerosis is a non-amyloid disease in which extensive misfolding of SOD1 is unique to the familial form. Acta Neuropathol 119:335–344
120. Kfoury Najla, Holmes Brandon B, Jiang Hong, Holtzman David M, Diamond Marc I (2012) Trans-cellular Propagation of Tau Aggregation by Fibrillar Species. J Biol Chem 287:19440–19451
121. Kiernan Matthew C, Vucic Steve, Cheah Benjamin C, Turner Martin R, Eisen Andrew, Hardiman Orla, Burrell James R, Zoing Margaret C (2011) Amyotrophic lateral sclerosis. In: Lancet. S 942–955
122. Kim Hong Joo, Kim Nam Chul, Wang Yong Dong, Scarborough Emily A, Moore Jennifer, Diaz Zamia, MacLea Kyle S, Freibaum Brian, Li Songqing, Molliex Amandine, Kanagaraj Anderson P, Carter Robert, Boylan Kevin B, Wojtas Aleksandra M, Rademakers Rosa, Pinkus Jack L, Greenberg Steven A, Trojanowski John Q, Traynor Bryan J, Smith Bradley N, Topp Simon, Gkazi Athina Soragia, Miller Jack, Shaw Christopher E, Kottlors Michael, Kirschner Janbernd, Pestronk Alan, Li Yun R, Ford Alice Flynn, Gitler Aaron D, Benatar Michael, King Oliver D, Kimonis Virginia E, Ross Eric D, Weihl Conrad C, Shorter James, Taylor J Paul (2013) Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. Nature 495:467–473
123. Krug Lisa, Chatterjee Nabanita, Borges-Monroy Rebeca, Hearn Stephen, Liao Wen Wei, Morrill Kathleen, Prazak Lisa, Rozhkov Nikolay, Theodorou Delphine, Hammell Molly, Dubnau Josh (2017) Retrotransposon activation contributes to neurodegeneration in a Drosophila TDP-43 model of ALS. PLoS Genet 13:e1006635
124. Kumar Vijay, Wahiduzzaman, Prakash Amresh, Tomar Anil Kumar, Srivastava Ankit, Kundu Bishwajit, Lynn Andrew M, Imtaiyaz Hassan Md (2019) Exploring the aggregation-prone regions from structural domains of human TDP-43. Biochim Biophys Acta - Proteins Proteomics 1867:286–296
125. Kuo Pan-Hsien, Doudeva Lyudmila G, Wang Yi-Ting, James Shen Che-Kun, Yuan Hanna S (2009) Structural insights into TDP-43 in nucleic-acid binding and domain interactions. Nucleic Acids Res 37:1799–1808
126. Kurian KM, Forbes RB, Colville S, Swingler RJ (2009) Cause of death and clinical grading criteria in a cohort of amyotrophic lateral sclerosis cases undergoing autopsy from the Scottish Motor Neurone Disease Register. J Neurol Neurosurg

Psychiatry 80:84–87
127. Kwiatkowski TJ, Bosco DA, LeClerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, Valdmanis P, Rouleau GA, Hosler BA, Cortelli P, De Jong PJ, Yoshinaga Y, Haines JL, Pericak-Vance MA, Yan J, Ticozzi N, Siddique T, McKenna-Yasek D, Sapp PC, Horvitz HR, Landers JE, Brown RH (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science (80-) 323:1205–1208
128. Lacomblez Lucette, Bensimon Gilbert, Leigh P Nigel, Guillet Philippe, Meininger Vincent (1996) Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Lancet 347:1425–1431
129. Laferrière Florent, Maniecka Zuzanna, Pérez-Berlanga Manuela, Hruska-Plochan Marian, Gilhespy Larissa, Hock Eva-Maria, Wagner Ulrich, Afroz Tariq, Boersema Paul J, Barmettler Gery, Foti Sandrine C, Asi Yasmine T, Isaacs Adrian M, Al-Amoudi Ashraf, Lewis Amanda, Stahlberg Henning, Ravits John, De Giorgi Francesca, Ichas François, Bezard Erwan, Picotti Paola, Lashley Tammaryn, Polymenidou Magdalini (2019) TDP-43 extracted from frontotemporal lobar degeneration subject brains displays distinct aggregate assemblies and neurotoxic effects reflecting disease progression rates. Nat Neurosci 22:65–77
130. Laferriere Florent, Polymenidou Magdalini (2015) Advances and challenges in understanding the multifaceted pathogenesis of amyotrophic lateral sclerosis. Swiss Med Wkly 145:w14054
131. Lanka Veena, Cudkowicz Merit (2008) Therapy development for ALS: Lessons learned and path forward. Amyotroph Lateral Scler 9:131–140
132. Lanznaster, Bourgeais, Bruno, Hergesheimer, Thepault, Vourc'h, Corcia, Andres, Herault, Blasco (2019) TDP-43-Mediated Toxicity in HEK293T Cells: A Fast and Reproducible Protocol To Be Employed in the Search of New Therapeutic Options against Amyotrophic Lateral Sclerosis. Cells 9:68
133. Laos Veronica, Bishop Dezmond, Lang Christian A, Marsh Nicole M, Cantrell Kristi Lazar, Buratto Steven K, Singh Ambuj K, Bowers Michael T (2020) Modulating ALS-Related Amyloidogenic TDP-43307-319 Oligomeric Aggregates with Computationally Derived Therapeutic Molecules. Biochemistry 59:499–508
134. Lee Edward B, Lee Virginia MY, Trojanowski John Q (2012) Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. Nat Rev Neurosci 13:38–50
135. Lee Seung-Jae, Desplats Paula, Sigurdson Christina, Tsigelny Igor, Masliah Eliezer (2010) Cell-to-cell transmission of non-prion protein aggregates. Nat Rev Neurol 6:702–706
136. Lee Youn Bok, Chen Han Jou, Peres João N, Gomez-Deza Jorge, Attig Jan, Štalekar Maja, Troakes Claire, Nishimura Agnes L, Scotter Emma L, Vance Caroline, Adachi Yoshitsugu, Sardone Valentina, Miller Jack W, Smith Bradley N, Gallo Jean Marc, Ule Jernej, Hirth Frank, Rogelj Boris, Houart Corinne, Shaw Christopher E (2013) Hexanucleotide repeats in ALS/FTD form length-dependent RNA Foci, sequester RNA binding proteins, and are neurotoxic. Cell Rep 5:1178–1186
137. Lei Yang, Zhang Zhi Feng, Lei Rui Xue, Wang Shu, Zhuang Yang, Liu An Chun, Wu Yan, Chen Juan, Tang Jun Chun, Pan Meng Xian, Liu Rui, Liao Wei Jing, Feng Yu Gong, Wan Qi, Zheng Mei (2018) DJ-1 Suppresses Cytoplasmic TDP-43 Aggregation in Oxidative Stress-Induced Cell Injury. J Alzheimer's Dis 66:1001–1014
138. Lenzken Silvia C, Romeo Valentina, Zolezzi Francesca, Cordero Francesca, Lamorte Giuseppe, Bonanno Davide, Biancolini Donatella, Cozzolino Mauro, Pesaresi Maria Grazia, Maracchioni Alessia, Sanges Remo, Achsel Tilmann, Carrì

Maria Teresa, Calogero Raffaele A, Barabino Silvia ML (2011) Mutant SOD1 and mitochondrial damage alter expression and splicing of genes controlling neuritogenesis in models of neurodegeneration. Hum Mutat 32:168–182
139. Li Xinyi, Koudstaal Wouter, Fletcher Lauren, Costa Martha, van Winsen Margot, Siregar Berdien, Inganäs Hanna, Kim Julie, Keogh Elissa, Macedo Jeremy, Holland Trevin, Perry Stuart, Bard Frederique, Hoozemans Jeroen J, Goudsmit Jaap, Apetri Adrian, Pascual Gabriel (2019) Naturally occurring antibodies isolated from PD patients inhibit synuclein seeding in vitro and recognize Lewy pathology. Acta Neuropathol 137:825–836
140. Li Yan, Ray Payal, Rao Elizabeth J, Shi Chen, Guo Weirui, Chen Xiaoping, Woodruff Elvin A, Fushimi Kazuo, Wu Jane Y, Wu Jane Y (2010) A Drosophila model for TDP-43 proteinopathy. Proc Natl Acad Sci U S A 107:3169–3174
141. Ling Shuo-Chien, Polymenidou Magdalini, Cleveland Don W (2013) Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron 79:416–438
142. Ling Shuo Chien, Albuquerque Claudio P, Han Joo Seok, Lagier-Tourenne Clotilde, Tokunaga Seiya, Zhou Huilin, Cleveland Don W (2010) ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. Proc Natl Acad Sci U S A 107:13318–13323
143. Liu Gerard Chun Hao, Chen Bryan Po Wen, Ye Nancy Ting Juan, Wang Chih Hsien, Chen Wenlung, Lee Hsien Ming, Chan Sunney I, Huang Joseph Jen Tse (2013) Delineating the membrane-disrupting and seeding properties of the TDP-43 amyloidogenic core. Chem Commun 49:11212–11214
144. Liu Guangbo, Coyne Alyssa N, Pei Fen, Vaughan Spencer, Chaung Matthew, Zarnescu Daniela C, Buchan J Ross (2017) Endocytosis regulates TDP-43 toxicity and turnover. Nat Commun 8:2092
145. Liu Hsueh-Ning, Tjostheim Sonja, Dasilva Kevin, Taylor David, Zhao Beibei, Rakhit Rishi, Brown Mary, Chakrabartty Avijit, McLaurin Joanne, Robertson Janice (2012) Targeting of monomer/misfolded SOD1 as a therapeutic strategy for amyotrophic lateral sclerosis. J Neurosci 32:8791–8799
146. Liu Ming-Sheng, Niu Jing-Wen, Li Yi, Guan Yu-Zhou, Cui Li-Ying (2016) Quantitating Changes in Jitter and Spike Number Using Concentric Needle Electrodes in Amyotrophic Lateral Sclerosis Patients. Chin Med J (Engl) 129:1036–1040
147. Liu Rui, Yang Guang, Nonaka Takashi, Arai Tetsuaki, Jia William, Cynader Max S (2013) Reducing TDP-43 aggregation does not prevent its cytotoxicity. Acta Neuropathol Commun 1:49
148. Loganathan Suvithanandhini, Lehmkuhl Erik M, Eck Randall J, Zarnescu Daniela C (2020) To Be or Not To Be…Toxic—Is RNA Association With TDP-43 Complexes Deleterious or Protective in Neurodegeneration? Front Mol Biosci 6:154
149. Longhena Francesca, Spano Pier Franco, Bellucci Arianna (2018) Targeting of disordered proteins by small molecules in neurodegenerative diseases. Handb. Exp. Pharmacol. S 85–110
150. Mackenzie Ian RA, Ansorge Olaf, Strong Michael, Bilbao Juan, Zinman Lorne, Ang Lee Cyn, Baker Matt, Stewart Heather, Eisen Andrew, Rademakers Rosa, Neumann Manuela (2011) Pathological heterogeneity in amyotrophic lateral sclerosis with FUS mutations: Two distinct patterns correlating with disease severity and mutation. Acta Neuropathol 122:87–98
151. Maharana Shovamayee, Wang Jie, Papadopoulos Dimitrios K, Richter Doris, Pozniakovsky Andrey, Poser Ina, Bickle Marc, Rizk Sandra, Guillén-Boixet Jordina, Franzmann Titus M, Jahnel Marcus, Marrone Lara, Chang Young Tae, Sterneckert

Jared, Tomancak Pavel, Hyman Anthony A, Alberti Simon (2018) RNA buffers the phase separation behavior of prion-like RNA binding proteins. Science 360:918–921
152. Maniecka Zuzanna, Polymenidou Magdalini (2015) From nucleation to widespread propagation: A prion-like concept for ALS. Virus Res 207:94–105
153. Maracchioni Alessia, Totaro Antonio, Angelini Daniela F, Di Penta Alessandra, Bernardi Giorgio, Carrì Maria Teresa, Achsel Tilmann (2007) Mitochondrial damage modulates alternative splicing in neuronal cells: Implications for neurodegeneration. J Neurochem 100:142–153
154. Marin B, Beghi E, Vial C, Bernard E, Lautrette G, Clavelou P, Guy N, Lemasson G, Debruxelles S, Cintas P, Antoine JC, Camdessanche JP, Logroscino G, Preux PM, Couratier P (2016) Evaluation of the application of the European guidelines for the diagnosis and clinical care of amyotrophic lateral sclerosis (ALS) patients in six French ALS centres. Eur J Neurol 23:787–795
155. Marin Benoît, Boumédiene Farid, Logroscino Giancarlo, Couratier Philippe, Babron Marie-Claude, Leutenegger Anne Louise, Copetti Massimilano, Preux Pierre-Marie, Beghi Ettore (2017) Variation in worldwide incidence of amyotrophic lateral sclerosis: a meta-analysis. Int J Epidemiol 46:57–74
156. Maruyama Hirofumi, Morino Hiroyuki, Ito Hidefumi, Izumi Yuishin, Kato Hidemasa, Watanabe Yasuhito, Kinoshita Yoshimi, Kamada Masaki, Nodera Hiroyuki, Suzuki Hidenori, Komure Osamu, Matsuura Shinya, Kobatake Keitaro, Morimoto Nobutoshi, Abe Koji, Suzuki Naoki, Aoki Masashi, Kawata Akihiro, Hirai Takeshi, Kato Takeo, Ogasawara Kazumasa, Hirano Asao, Takumi Toru, Kusaka Hirofumi, Hagiwara Koichi, Kaji Ryuji, Kawakami Hideshi (2010) Mutations of optineurin in amyotrophic lateral sclerosis. Nature 465:223–226
157. Masliah Eliezer, Rockenstein Edward, Adame Anthony, Alford Michael, Crews Leslie, Hashimoto Makoto, Seubert Peter, Lee Michael, Goldstein Jason, Chilcote Tamie, Games Dora, Schenk Dale (2005) Effects of α-synuclein immunization in a mouse model of Parkinson's disease. Neuron 46:857–868
158. Masliah Eliezer, Rockenstein Edward, Mante Michael, Crews Leslie, Spencer Brian, Adame Anthony, Patrick Christina, Trejo Margarita, Ubhi Kiren, Rohn Troy T, Mueller-Steiner Sarah, Seubert Peter, Barbour Robin, McConlogue Lisa, Buttini Manuel, Games Dora, Schenk Dale (2011) Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of lewy body disease. PLoS One 6:e19338
159. Mathieu Mathilde, Martin-Jaular Lorena, Lavieu Grégory, Théry Clotilde (2019) Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol 21:9–17
160. May Stephanie, Hornburg Daniel, Schludi Martin H, Arzberger Thomas, Rentzsch Kristin, Schwenk Benjamin M, Grässer Friedrich A, Mori Kohji, Kremmer Elisabeth, Banzhaf-Strathmann Julia, Mann Matthias, Meissner Felix, Edbauer Dieter (2014) C9orf72 FTLD/ALS-associated Gly-Ala dipeptide repeat proteins cause neuronal toxicity and Unc119 sequestration. Acta Neuropathol 128:485–503
161. McAlary Luke, Plotkin Steven S, Yerbury Justin J, Cashman Neil R (2019) Prion-Like Propagation of Protein Misfolding and Aggregation in Amyotrophic Lateral Sclerosis. Front Mol Neurosci 12:262
162. Mejzini Rita, Flynn Loren L, Pitout Ianthe L, Fletcher Sue, Wilton Steve D, Akkari P Anthony (2019) ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? Front Neurosci 13:1310
163. Mitra Joy, Guerrero Erika N, Hegde Pavana M, Liachko Nicole F, Wang Haibo, Vasquez Velmarini, Gao Junling, Pandey Arvind, Taylor J Paul, Kraemer Brian C, Wu Ping, Boldogh Istvan, Garruto Ralph M, Mitra Sankar, Rao KS, Hegde

Muralidhar L (2019) Motor neuron disease-associated loss of nuclear TDP-43 is linked to DNA double-strand break repair defects. Proc Natl Acad Sci 116:4696–4705
164. Molliex Amandine, Temirov Jamshid, Lee Jihun, Coughlin Maura, Kanagaraj Anderson P, Kim Hong Joo, Mittag Tanja, Taylor J Paul (2015) Phase Separation by Low Complexity Domains Promotes Stress Granule Assembly and Drives Pathological Fibrillization. Cell 163:123–133
165. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, Arendash GW (2000) A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 408:982–985
166. Mosmann Tim (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
167. Mulcahy Laura Ann, Pink Ryan Charles, Carter David Raul Francisco (2014) Routes and mechanisms of extracellular vesicle uptake. J Extracell Vesicles 3:24641
168. Münch Christoph, Sedlmeier R, Meyer T, Homberg V, Sperfeld AD, Kurt A, Prudlo J, Peraus G, Hanemann CO, Stumm G, Ludolph AC (2004) Point mutations of the p150 subunit of dynactin (DCTN1) gene ALS. Neurology 63:724–726
169. Neumann Manuela, Kwong Linda K, Lee Edward B, Kremmer Elisabeth, Flatley Andrew, Xu Yan, Forman Mark S, Troost Dirk, Kretzschmar Hans A, Trojanowski John Q, Lee Virginia MY (2009) Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. Acta Neuropathol 117:137–149
170. Neumann Manuela, Sampathu Deepak M, Kwong Linda K, Truax Adam C, Micsenyi Matthew C, Chou Thomas T, Bruce Jennifer, Schuck Theresa, Grossman Murray, Clark Christopher M, McCluskey Leo F, Miller Bruce L, Masliah Eliezer, Mackenzie Ian R, Feldman Howard, Feiden Wolfgang, Kretzschmar Hans A, Trojanowski John Q, Lee Virginia MY (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314:130–133
171. Nicholls Samantha B, DeVos Sarah L, Commins Caitlin, Nobuhara Chloe, Bennett Rachel E, Corjuc Diana L, Maury Eduardo, Eftekharzadeh Bahareh, Akingbade Ololade, Fan Zhanyun, Roe Allyson D, Takeda Shuko, Wegmann Susanne, Hyman Bradley T (2017) Characterization of TauC3 antibody and demonstration of its potential to block tau propagation. PLoS One 12:e0177914
172. Nicoll James AR, Wilkinson David, Holmes Clive, Steart Phil, Markham Hannah, Weller Roy O (2003) Neuropathology of human Alzheimer disease after immunization with amyloid-β peptide: a case report. Nat Med 9:448–452
173. Nishimoto Yoshinori, Ito Daisuke, Yagi Takuya, Nihei Yoshihiro, Tsunodo Yoshiko, Suzuki Norihiro (2010) Characterization of alternative isoforms and inclusion body of the TAR DNA-binding protein-43. J Biol Chem 285:608–619
174. Nonaka Takashi, Kametani Fuyuki, Arai Tetsuaki, Akiyama Haruhiko, Hasegawa Masato (2009) Truncation and pathogenic mutations facilitate the formation of intracellular aggregates of TDP-43. Hum Mol Genet 18:3353–3364
175. Nonaka Takashi, Masuda-Suzukake Masami, Arai Tetsuaki, Hasegawa Yoko, Akatsu Hiroyasu, Obi Tomokazu, Yoshida Mari, Murayama Shigeo, Mann David MA, Akiyama Haruhiko, Hasegawa Masato (2013) Prion-like properties of pathological TDP-43 aggregates from diseased brains. Cell Rep 4:124–134
176. Ondrus Matej, Novak Petr (2016) Design of the phase II clinical study of the tau vaccine AADvac1 in patients with mild Alzheimer's disease. Neurobiol Aging 39:26

177. Osaka Mayuko, Ito Daisuke, Suzuki Norihiro (2016) Disturbance of proteasomal and autophagic protein degradation pathways by amyotrophic lateral sclerosis-linked mutations in ubiquilin 2. Biochem Biophys Res Commun 472:324–331
178. Ou SH, Wu F, Harrich D, García-Martínez LF, Gaynor RB (1995) Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. J Virol 69:3584–3596
179. Park Sei Kyoung, Hong Joo Y, Arslan Fatih, Kanneganti Vydehi, Patel Basant, Tietsort Alex, Tank Elizabeth MH, Li Xingli, Barmada Sami J, Liebman Susan W (2017) Overexpression of the essential Sis1 chaperone reduces TDP-43 effects on toxicity and proteolysis. PLoS Genet 13:e1006805
180. Patel Avinash, Lee Hyun O, Jawerth Louise, Maharana Shovamayee, Jahnel Marcus, Hein Marco Y, Stoynov Stoyno, Mahamid Julia, Saha Shambaditya, Franzmann Titus M, Pozniakovski Andrej, Poser Ina, Maghelli Nicola, Royer Loic A, Weigert Martin, Myers Eugene W, Grill Stephan, Drechsel David, Hyman Anthony A, Alberti Simon (2015) A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell 162:1066–1077
181. Peters Owen M, Ghasemi Mehdi, Brown Robert H (2015) Emerging mechanisms of molecular pathology in ALS. J Clin Invest 125:1767–1779
182. Petrou Panayiota, Gothelf Yael, Argov Zohar, Gotkine Marc, Levy Yossef S, Kassis Ibrahim, Vaknin-Dembinsky Adi, Ben-Hur Tamir, Offen Daniel, Abramsky Oded, Melamed Eldad, Karussis Dimitrios (2016) Safety and clinical effects of mesenchymal stem cells secreting neurotrophic factor transplantation in patients with amyotrophic lateral sclerosis. JAMA Neurol 73:337–344
183. Phukan Julie, Elamin Marwa, Bede Peter, Jordan Norah, Gallagher Laura, Byrne Susan, Lynch Catherine, Pender Niall, Hardiman Orla (2012) The syndrome of cognitive impairment in amyotrophic lateral sclerosis: A population-based study. J Neurol Neurosurg Psychiatry 83:102–108
184. Pinarbasi Emile S, Cağatay Tolga, Fung Ho Yee Joyce, Li Ying C, Chook Yuh Min, Thomas Philip J (2018) Active nuclear import and passive nuclear export are the primary determinants of TDP-43 localization. Sci Rep 8:1–16
185. Pokrishevsky Edward, Grad Leslie I, Cashman Neil R (2016) TDP-43 or FUS-induced misfolded human wild-type SOD1 can propagate intercellularly in a prion-like fashion. Sci Rep 6:22155
186. Polymenidou Magdalini, Lagier-Tourenne Clotilde, Hutt Kasey R, Huelga Stephanie C, Moran Jacqueline, Liang Tiffany Y, Ling Shuo Chien, Sun Eveline, Wancewicz Edward, Mazur Curt, Kordasiewicz Holly, Sedaghat Yalda, Donohue John Paul, Shiue Lily, Bennett C Frank, Yeo Gene W, Cleveland Don W (2011) Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. Nat Neurosci 14:459–468
187. Porta Sílvia, Xu Yan, Restrepo Clark R, Kwong Linda K, Zhang Bin, Brown Hannah J, Lee Edward B, Trojanowski John Q, Lee Virginia MY (2018) Patient-derived frontotemporal lobar degeneration brain extracts induce formation and spreading of TDP-43 pathology in vivo. Nat Commun 9:4220
188. Prasad Archana, Bharathi Vidhya, Sivalingam Vishwanath, Girdhar Amandeep, Patel Basant K (2019) Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis. Front Mol Neurosci 12:25
189. Ramesh Nandini, Pandey Udai Bhan (2017) Autophagy dysregulation in ALS: When protein aggregates get out of hand. Front Mol Neurosci 10:263
190. Ravits John M, La Spada Albert R (2009) Als motor phenotype heterogeneity, focality, and spread: Deconstructing motor neuron degenerationsymbol. Neurology 73:805

191. Renton Alan E, Chiò Adriano, Traynor Bryan J (2014) State of play in amyotrophic lateral sclerosis genetics. Nat Neurosci 17:17–23
192. Riback Joshua A, Katanski Christopher D, Kear-Scott Jamie L, Pilipenko Evgeny V., Rojek Alexandra E, Sosnick Tobin R, Drummond D Allan (2017) Stress-Triggered Phase Separation Is an Adaptive, Evolutionarily Tuned Response. Cell 168:1028-1040.e19
193. Roggenbuck Jennifer, Quick Adam, Kolb Stephen J (2017) Genetic testing and genetic counseling for amyotrophic lateral sclerosis: An update for clinicians. Genet Med 19:267–274
194. Romero-Ramos Marina, Chelpin Marianne Von Euler, Sanchez-Guajardo Vanesa (2014) Vaccination strategies for Parkinson disease: Induction of a swift attack or raising tolerance? Hum Vaccines Immunother 10:852–867
195. Rooney James, Byrne Susan, Heverin Mark, Tobin Katy, Dick Alison, Donaghy Colette, Hardiman Orla (2015) A multidisciplinary clinic approach improves survival in ALS: a comparative study of ALS in Ireland and Northern Ireland. J Neurol Neurosurg Psychiatry 86:496–501
196. Rosen Daniel R, Siddique Teepu, Patterson David, Figlewicz Denise A, Sapp Peter, Hentati Afif, Donaldson Deirdre, Goto Jun, O'Regan Jeremiah P, Deng Han Xiang, Rahmani Zohra, Krizus Aldis, McKenna-Yasek Diane, Cayabyab Annarueber, Gaston Sandra M, Berger Ralph, Tanzi Rudolph E, Halperin John J, Herzfeldt Brian, Den Bergh Raymond Van, Hung Wu Yen, Bird Thomas, Deng Gang, Mulder Donald W, Smyth Celestine, Laing Nigel G, Soriano Edwin, Pericak-Vance Margaret A, Haines Jonathan, Rouleau Guy A, Gusella James S, Horvitz H Robert, Brown Robert H (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62
197. Sanchez-Guajardo Vanesa, Annibali Ambra, Jensen Poul Henning, Romero-Ramos Marina (2013) α-synuclein vaccination prevents the accumulation of parkinson disease-like pathologic inclusions in striatum in association with regulatory T cell recruitment in a rat model. J Neuropathol Exp Neurol 72:624–645
198. Santamaria Nikolas, Alhothali Marwa, Alfonso Maria Harreguy, Breydo Leonid, Uversky Vladimir N (2017) Intrinsic disorder in proteins involved in amyotrophic lateral sclerosis. Cell Mol Life Sci 74:1297–1318
199. Sasaguri Hiroki, Chew Jeannie, Xu Ya-Fei, Gendron Tania F, Garrett Aliesha, Lee Chris W, Jansen-West Karen, Bauer Peter O, Perkerson Emilie A, Tong Jimei, Stetler Caroline, Zhang Yong-Jie (2016) The extreme N-terminus of TDP-43 mediates the cytoplasmic aggregation of TDP-43 and associated toxicity in vivo. Brain Res 1647:57–64
200. Saxena Smita, Caroni Pico (2011) Selective Neuronal Vulnerability in Neurodegenerative Diseases: From Stressor Thresholds to Degeneration. Neuron 71:35–48
201. Schenk Dale, Barbour Robin, Dunn Whitney, Gordon Grace, Grajeda Henry, Guido Teresa, Hu Kang, Huang Jiping, Johnson-Wood Kelly, Khan Karen, Kholodenko Dora, Lee Mike, Liao Zhenmei, Lieberburg Ivan, Motter Ruth, Mutter Linda, Soriano Ferdie, Shopp George, Vasquez Nicki, Vandevert Christopher, Walker Shannan, Wogulis Mark, Yednock Ted, Games Dora, Seubert Peter (1999) Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400:173–177
202. Schrooten Maarten, Smetcoren Charlotte, Robberecht Wim, Van Damme Philip (2011) Benefit of the Awaji diagnostic algorithm for amyotrophic lateral sclerosis: A prospective study. Ann Neurol 70:79–83
203. Schwenk Benjamin M, Hartmann Hannelore, Serdaroglu Alperen, Schludi Martin

H, Hornburg Daniel, Meissner Felix, Orozco Denise, Colombo Alessio, Tahirovic Sabina, Michaelsen Meike, Schreiber Franziska, Haupt Simone, Peitz Michael, Brüstle Oliver, Küpper Clemens, Klopstock Thomas, Otto Markus, Ludolph Albert C, Arzberger Thomas, Kuhn Peer-Hendrik, Edbauer Dieter (2016) TDP-43 loss of function inhibits endosomal trafficking and alters trophic signaling in neurons. EMBO J 35:2350–2370

204. Scotter EL, Vance C, Nishimura AL, Lee YB, Chen HJ, Urwin H, Sardone V, Mitchell JC, Rogelj B, Rubinsztein DC, Shaw CE (2014) Differential roles of the ubiquitin proteasome system and autophagy in the clearance of soluble and aggregated TDP-43 species. J Cell Sci 127:1263–1278
205. Scotter Emma L, Chen Han Jou, Shaw Christopher E (2015) TDP-43 Proteinopathy and ALS: Insights into Disease Mechanisms and Therapeutic Targets. Neurotherapeutics 12:352–363
206. Sephton Chantelle F, Cenik Can, Kucukural Alper, Dammer Eric B, Cenik Basar, Han Yu Hong, Dewey Colleen M, Roth Frederick P, Herz Joachim, Peng Junmin, Moore Melissa J, Yu Gang (2011) Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. J Biol Chem 286:1204–1215
207. Seyfried Nicholas T, Gozal Yair M, Dammer Eric B, Xia Qiangwei, Duong Duc M, Cheng Dongmei, Lah James J, Levey Allan I, Peng Junmin (2010) Multiplex SILAC analysis of a cellular TDP-43 proteinopathy model reveals protein inclusions associated with SUMOylation and diverse polyubiquitin chains. Mol Cell Proteomics 9:705–718
208. Shaw Pamela J, Ince Paul G, Falkous Gavin, Mantle David (1995) Oxidative damage to protein in sporadic motor neuron disease spinal cord. Ann Neurol 38:691–695
209. Shiina Yuki, Arima Kunimasa, Tabunoki Hiroko, Satoh Jun Ichi (2010) TDP-43 dimerizes in human cells in culture. Cell Mol Neurobiol 30:641–652
210. Shimonaka Shotaro, Nonaka Takashi, Suzuki Genjiro, Hisanaga Shin Ichi, Hasegawa Masato (2016) Templated aggregation of TAR DNA-binding protein of 43 kDa (TDP-43) by seeding with TDP-43 peptide fibrils. J Biol Chem 291:8896–8907
211. Shin Yongdae, Brangwynne Clifford P (2017) Liquid phase condensation in cell physiology and disease. Science 357:eaaf4382
212. Sigurdsson Einar M, Brown David R, Daniels Maki, Kascsak Richard J, Kascsak Regina, Carp Richard, Meeker Harry C, Frangione Blas, Wisniewski Thomas (2002) Immunization Delays the Onset of Prion Disease in Mice. Am J Pathol 161:13–17
213. Smethurst Phillip, Newcombe Jia, Troakes Claire, Simone Roberto, Chen Yun Ru, Patani Rickie, Sidle Katie (2016) In vitro prion-like behaviour of TDP-43 in ALS. Neurobiol Dis 96:236–247
214. Smethurst Phillip, Sidle Katie Claire Louise, Hardy John (2015) Prion-like mechanisms of transactive response DNA binding protein of 43 kDa (TDP-43) in amyotrophic lateral sclerosis (ALS). Neuropathol Appl Neurobiol 41:578–597
215. Smith Bradley N, Ticozzi Nicola, Fallini Claudia, Gkazi Athina Soragia, Topp Simon, Kenna Kevin P, Scotter Emma L, Kost Jason, Keagle Pamela, Miller Jack W, Calini Daniela, Vance Caroline, Danielson Eric W, Troakes Claire, Tiloca Cinzia, Al-Sarraj Safa, Lewis Elizabeth A, King Andrew, Colombrita Claudia, Pensato Viviana, Castellotti Barbara, de Belleroche Jacqueline, Baas Frank, ten Asbroek Anneloor LMA, Sapp Peter C, McKenna-Yasek Diane, McLaughlin Russell L, Polak Meraida, Asress Seneshaw, Esteban-Pérez Jesús, Muñoz-Blanco JoséLuis L, Simpson Michael, van Rheenen Wouter, Diekstra Frank P, Lauria Giuseppe, Duga Stefano, Corti Stefania, Cereda Cristina, Corrado Lucia, Sorarù

Gianni, Morrison Karen E, Williams Kelly L, Nicholson Garth A, Blair Ian P, Dion Patrick A, Leblond Claire S, Rouleau Guy A, Hardiman Orla, Veldink Jan H, Van Den Berg Leonard H, Al-Chalabi Ammar, Pall Hardev, Shaw Pamela J, Turner Martin R, Talbot Kevin, Taroni Franco, García-Redondo Alberto, Wu Zheyang, Glass Jonathan D, Gellera Cinzia, Ratti Antonia, Brown Robert H, Silani Vincenzo, Shaw Christopher E, Landers John E, D'Alfonso Sandra, Mazzini Letizia, Comi Giacomo P, Del Bo Roberto, Ceroni Mauro, Gagliardi Stella, Querin Giorgia, Bertolin Cinzia (2014) Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. Neuron 84:324–331

216. Smith Emma F, Shaw Pamela J, De Vos Kurt J (2019) The role of mitochondria in amyotrophic lateral sclerosis. Neurosci Lett 710:132933
217. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85
218. Soto Claudio, Pritzkow Sandra (2018) Protein misfolding, aggregation, and conformational strains in neurodegenerative diseases. Nat Neurosci 21:1332–1340
219. Spiller Krista J, Restrepo Clark R, Khan Tahiyana, Dominique Myrna A, Fang Terry C, Canter Rebecca G, Roberts Christopher J, Miller Kelly R, Ransohoff Richard M, Trojanowski John Q, Lee Virginia MY (2018) Microglia-mediated recovery from ALS-relevant motor neuron degeneration in a mouse model of TDP-43 proteinopathy. Nat Neurosci 21:329–340
220. Sreedharan Jemeen, Blair Ian P, Tripathi Vineeta B, Hu Xun, Vance Caroline, Rogelj Boris, Ackerley Steven, Durnall Jennifer C, Williams Kelly L, Buratti Emanuele, Baralle Francisco, De Belleroche Jacqueline, Mitchell J Douglas, Leigh P Nigel, Al-Chalabi Ammar, Miller Christopher C, Nicholson Garth, Shaw Christopher E (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science (80-) 319:1668–1672
221. Steinacker Petra, Barschke Peggy, Otto Markus (2019) Biomarkers for diseases with TDP-43 pathology. Mol Cell Neurosci 97:43–59
222. Steinacker Petra, Feneberg Emily, Weishaupt Jochen, Brettschneider Johannes, Tumani Hayrettin, Andersen Peter M, Arnim Christine AF Vo, Böhm Sarah, Kassubek Jan, Kubisch Christian, Lulé Dorothée, Müller Hans Peter, Muche Rainer, Pinkhardt Elmar, Oeckl Patrick, Rosenbohm Angela, Anderl-Straub Sarah, Volk Alexander E, Weydt Patrick, Ludolph Albert C, Otto Markus (2016) Neurofilaments in the diagnosis of motoneuron diseases: A prospective study on 455 patients. J Neurol Neurosurg Psychiatry 87:12–20
223. Stoica Radu, De Vos Kurt J, Paillusson Sébastien, Mueller Sarah, Sancho Rosa M, Lau Kwok Fai, Vizcay-Barrena Gema, Lin Wen Lang, Xu Ya Fei, Lewis Jada, Dickson Dennis W, Petrucelli Leonard, Mitchell Jacqueline C, Shaw Christopher E, Miller Christopher CJ (2014) ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. Nat Commun 5:3996
224. Stribl Carola, Samara Aladin, Trümbach Dietrich, Peis Regina, Neumann Manuela, Fuchs Helmut, Gailus-Durner Valerie, De Angelis Martin Hrabě, Rathkolb Birgit, Wolf Eckhard, Beckers Johannes, Horsch Marion, Neff Frauke, Kremmer Elisabeth, Koob Sebastian, Reichert Andreas S, Hans Wolfgang, Rozman Jan, Klingenspor Martin, Aichler Michaela, Karl Walch Axel, Becker Lore, Klopstock Thomas, Glasl Lisa, Hölter Sabine M, Wurst Wolfgang, Floss Thomas (2014) Mitochondrial dysfunction and decrease in body weight of a transgenic knock-in mouse model for TDP-43. J Biol Chem 289:10769–10784
225. Talbott EO, Malek AM, Lacomis D (2016) The epidemiology of amyotrophic lateral

sclerosis. Handb Clin Neurol 138:225–238
226. Tam Oliver H, Rozhkov Nikolay V., Shaw Regina, Kim Duyang, Hubbard Isabel, Fennessey Samantha, Propp Nadia, Phatnani Hemali, Kwan Justin, Sareen Dhruv, Broach James R, Simmons Zachary, Arcila-Londono Ximena, Lee Edward B, Van Deerlin Vivianna M, Shneider Neil A, Fraenkel Ernest, Ostrow Lyle W, Baas Frank, Zaitlen Noah, Berry James D, Malaspina Andrea, Fratta Pietro, Cox Gregory A, Thompson Leslie M, Finkbeiner Steve, Dardiotis Efthimios, Miller Timothy M, Chandran Siddharthan, Pal Suvankar, Hornstein Eran, MacGowan Daniel J, Heiman-Patterson Terry, Hammell Molly G, Patsopoulos Nikolaos A, Butovsky Oleg, Dubnau Joshua, Nath Avindra, Bowser Robert, Harms Matt, Aronica Eleonora, Poss Mary, Phillips-Cremins Jennifer, Crary John, Atassi Nazem, Lange Dale J, Adams Darius J, Stefanis Leonidas, Gotkine Marc, Baloh Robert, Babu Suma, Raj Towfique, Paganoni Sabrina, Shalem Ophir, Smith Colin, Zhang Bin, Harris Brent T, Fagegaltier Delphine, Ravits John, Dubnau Joshua, Gale Hammell Molly (2019) Postmortem Cortex Samples Identify Distinct Molecular Subtypes of ALS: Retrotransposon Activation, Oxidative Stress, and Activated Glia. Cell Rep 29:1164-1177.e5
227. Tamaki Yoshitaka, Shodai Akemi, Morimura Toshifumi, Hikiami Ryota, Minamiyama Sumio, Ayaki Takashi, Tooyama Ikuo, Furukawa Yoshiaki, Takahashi Ryosuke, Urushitani Makoto (2018) Elimination of TDP-43 inclusions linked to amyotrophic lateral sclerosis by a misfolding-specific intrabody with dual proteolytic signals. Sci Rep 8:6030
228. Tao Qing Qing, Wu Zhi Ying (2017) Amyotrophic lateral sclerosis: Precise diagnosis and individualized treatment. Chin Med J (Engl) 130:2269–2272
229. Tavella Davide, Zitzewitz Jill A, Massi Francesca (2018) Characterization of TDP-43 RRM2 Partially Folded States and Their Significance to ALS Pathogenesis. Biophys J 115:1673–1680
230. Tollervey James R, Curk Tomaž, Rogelj Boris, Briese Michael, Cereda Matteo, Kayikci Melis, König Julian, Hortobágyi Tibor, Nishimura Agnes L, Zupunski Vera, Patani Rickie, Chandran Siddharthan, Rot Gregor, Zupan Blaž, Shaw Christopher E, Ule Jernej (2011) Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat Neurosci 14:452–458
231. Tóth Gergely, Gardai Shyra J, Zago Wagner, Bertoncini Carlos W, Cremades Nunilo, Roy Susan L, Tambe Mitali A, Rochet Jean-Christophe, Galvagnion Celine, Skibinski Gaia, Finkbeiner Steven, Bova Michael, Regnstrom Karin, Chiou San-San, Johnston Jennifer, Callaway Kari, Anderson John P, Jobling Michael F, Buell Alexander K, Yednock Ted A, Knowles Tuomas PJ, Vendruscolo Michele, Christodoulou John, Dobson Christopher M, Schenk Dale, McConlogue Lisa (2014) Targeting the Intrinsically Disordered Structural Ensemble of α-Synuclein by Small Molecules as a Potential Therapeutic Strategy for Parkinson's Disease. PLoS One 9:e87133
232. Tran Hien T, Chung Charlotte Hiu Yan, Iba Michiyo, Zhang Bin, Trojanowski John Q, Luk Kelvin C, Lee Virginia MY (2014) α-Synuclein Immunotherapy Blocks Uptake and Templated Propagation of Misfolded α-Synuclein and Neurodegeneration. Cell Rep 7:2054–2065
233. Traynor BJ, Alexander M, Corr B, Frost E, Hardiman O (2003) Effect of a multidisciplinary amyotrophic lateral sclerosis (ALS) clinic on ALS survival: A population based study, 1996-2000. J Neurol Neurosurg Psychiatry 74:1258–1261
234. Tsao William, Jeong Yun Ha, Lin Sophie, Ling Jonathan, Price Donald L, Chiang Po Min, Wong Philip C (2012) Rodent models of TDP-43: Recent advances. Brain Res 1462:26–39

235. Tsoi Phoebe S, Choi Kyoung Jae, Leonard Paul G, Sizovs Antons, Moosa Mahdi Muhammad, MacKenzie Kevin R, Ferreon Josephine C, Ferreon Allan Chris M (2017) The N-Terminal Domain of ALS-Linked TDP-43 Assembles without Misfolding. Angew Chemie - Int Ed 56:12590–12593
236. Udan-Johns Maria, Bengoechea Rocio, Bell Shaughn, Shao Jieya, Diamond Marc I, True Heather L, Weihl Conrad C, Baloh Robert H (2014) Prion-like nuclear aggregation of TDP-43 during heat shock is regulated by HSP40/70 chaperones. Hum Mol Genet 23:157–170
237. Urushitani Makoto, Ezzi Samer Abou, Julien JP Jean-Pierre (2007) Therapeutic effects of immunization with mutant superoxide dismutase in mice models of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 104:2495–2500
238. Vance Caroline, Rogelj Boris, Hortobágyi Tibor, De Vos Kurt J, Nishimura Agnes Lumi, Sreedharan Jemeen, Hu Xun, Smith Bradley, Ruddy Deborah, Wright Paul, Ganesalingam Jeban, Williams Kelly L, Tripathi Vineeta, Al-Saraj Safa, Al-Chalabi Ammar, Leigh P Nigel, Blair Ian P, Nicholson Garth, De Belleroche Jackie, Gallo Jean Marc, Miller Christopher C, Shaw Christopher E (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science (80-) 323:1208–1211
239. Vucic S, Burke D, Kiernan MC (2007) Diagnosis of motor neuron disease. In: Mot Neuron Dis Handbook Sydney Australas Med Publ Co Ltd 1:89–115
240. Wang Hurng Yi, Wang I Fan, Bose Jayaramakrishnan, Shen CK Jame (2004) Structural diversity and functional implications of the eukaryotic TDP gene family. Genomics 83:130–139
241. Wang Wenzhang, Li Li, Lin Wen-Lang, Dickson Dennis W, Petrucelli Leonard, Zhang Teng, Wang Xinglong (2013) The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. Hum Mol Genet 22:4706–4719
242. Wang Wenzhang, Wang Luwen, Lu Junjie, Siedlak Sandra L, Fujioka Hisashi, Liang Jingjing, Jiang Sirui, Ma Xiaopin, Jiang Zhen, Da Rocha Edroaldo Lummertz, Sheng Max, Choi Heewon, Lerou Paul H, Li Hu, Wang Xinglong (2016) The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. Nat Med 22:869–878
243. Wang Yi Ting, Kuo Pan Hsien, Chiang Chien Hao, Liang Jhe Ruei, Chen Yun Ru, Wang Shuying, Shen James CK, Yuan Hanna S (2013) The truncated C-terminal RNA recognition motif of TDP-43 protein plays a key role in forming proteinaceous aggregates. J Biol Chem 288:9049–9057
244. Watts Giles DJ, Wymer Jill, Kovach Margaret J, Mehta Sarju G, Mumm Steven, Darvish Daniel, Pestronk Alan, Whyte Michael P, Kimonis Virginia E (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 36:377–381
245. Wegorzewska Iga, Baloh Robert H (2011) TDP-43-based animal models of neurodegeneration: New insights into ALS pathology and pathophysiology. Neurodegener Dis 8:262–274
246. Wei Yuanyuan, Lim Liangzhong, Wang Lu, Song Jianxing (2017) ALS-causing cleavages of TDP-43 abolish its RRM2 structure and unlock CTD for enhanced aggregation and toxicity. Biochem Biophys Res Commun 485:826–831
247. Wilcock Donna M, DiCarlo Giovanni, Henderson Debbi, Jackson Jennifer, Clarke Keisha, Ugen Kenneth E, Gordon Marcia N, Morgan Dave (2003) Intracranially administered anti-Abeta antibodies reduce beta-amyloid deposition by mechanisms both independent of and associated with microglial activation. J Neurosci 23:3745–3751

248. Wilcock Donna M, Rojiani Amyn, Rosenthal Arnon, Levkowitz Gil, Subbarao Sangeetha, Alamed Jennifer, Wilson David, Wilson Nedda, Freeman Melissa J, Gordon Marcia N, Morgan Dave (2004) Passive amyloid immunotherapy clears amyloid and transiently activates microglia in a transgenic mouse model of amyloid deposition. J Neurosci 24:6144–6151
249. Winton Matthew J, Igaz Lionel M, Wong Margaret M, Kwong Linda K, Trojanowski John Q, Lee Virginia MY (2008) Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. J Biol Chem 283:13302–13309
250. Woo Jung AA, Liu Tian, Trotter Courtney, Fang Cenxiao C, De Narvaez Emillio, Lepochat Patrick, Maslar Drew, Bukhari Anusha, Zhao Xingyu, Deonarine Andrew, Westerheide Sandy D, Kang David E (2017) Loss of function CHCHD10 mutations in cytoplasmic TDP-43 accumulation and synaptic integrity. Nat Commun 8:15558
251. Wu Chi Hong, Fallini Claudia, Ticozzi Nicola, Keagle Pamela J, Sapp Peter C, Piotrowska Katarzyna, Lowe Patrick, Koppers Max, McKenna-Yasek Diane, Baron Desiree M, Kost Jason E, Gonzalez-Perez Paloma, Fox Andrew D, Adams Jenni, Taroni Franco, Tiloca Cinzia, Leclerc Ashley Lyn, Chafe Shawn C, Mangroo Dev, Moore Melissa J, Zitzewitz Jill A, Xu Zuo Shang, Van Den Berg Leonard H, Glass Jonathan D, Siciliano Gabriele, Cirulli Elizabeth T, Goldstein David B, Salachas Francois, Meininger Vincent, Rossoll Wilfried, Ratti Antonia, Gellera Cinzia, Bosco Daryl A, Bassell Gary J, Silani Vincenzo, Drory Vivian E, Brown Robert H, Landers John E (2012) Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature 488:499–503
252. Xiao Shangxi, Sanelli Teresa, Chiang Helen, Sun Yulong, Chakrabartty Avijit, Keith Julia, Rogaeva Ekaterina, Zinman Lorne, Robertson Janice (2015) Low molecular weight species of TDP-43 generated by abnormal splicing form inclusions in amyotrophic lateral sclerosis and result in motor neuron death. Acta Neuropathol 130:49–61
253. Xu YF, Gendron TF, Zhang YJ, Lin WL, D'Alton S, Sheng H, Casey MC, Tong J, Knight J, Yu X, Rademakers R, Boylan K, Hutton M, McGowan E, Dickson DW, Lewis J, Petrucelli L (2010) Wild-Type Human TDP-43 Expression Causes TDP-43 Phosphorylation, Mitochondrial Aggregation, Motor Deficits, and Early Mortality in Transgenic Mice. J Neurosci 30:10851–10859
254. Yamashita Makiko, Nonaka Takashi, Arai Tetsuaki, Kametani Fuyuki, Buchman Vladimir L, Ninkina Natalia, Bachurin Sergey O, Akiyama Haruhiko, Goedert Michel, Hasegawa Masato (2009) Methylene blue and dimebon inhibit aggregation of TDP-43 in cellular models. FEBS Lett 583:2419–2424
255. Yanamandra Kiran, Jiang Hong, Mahan Thomas E, Maloney Susan E, Wozniak David F, Diamond Marc I, Holtzman David M (2015) Anti-tau antibody reduces insoluble tau and decreases brain atrophy. Ann Clin Transl Neurol 2:278–88
256. Yanamandra Kiran, Kfoury Najla, Jiang Hong, Mahan Thomas E, Ma Shengmei, Maloney Susan E, Wozniak David F, Diamond Marc I, Holtzman David M (2013) Anti-Tau Antibodies that Block Tau Aggregate Seeding In Vitro Markedly Decrease Pathology and Improve Cognition In Vivo. Neuron 80:402–414
257. Yang Chunxing, Danielson Eric W, Qiao Tao, Metterville Jake, Brown Robert H, Landers John E, Xu Zuoshang (2016) Mutant PFN1 causes ALS phenotypes and progressive motor neuron degeneration in mice by a gain of toxicity. Proc Natl Acad Sci USA 113:E6209–E6218
258. Zeineddine Rafaa, Whiten Daniel R, Farrawell Natalie E, McAlary Luke, Hanspal Maya A, Kumita Janet R, Wilson Mark R, Yerbury Justin J (2017) Flow cytometric

measurement of the cellular propagation of TDP-43 aggregation. Prion 11:195–204
259. Zhang Yong-Jie, Caulfield Thomas, Xu Ya-Fei, Gendron Tania F, Hubbard Jaime, Stetler Caroline, Sasaguri Hiroki, Whitelaw Ena C, Cai Shuyi, Lee Wing Cheung, Petrucelli Leonard (2013) The dual functions of the extreme N-terminus of TDP-43 in regulating its biological activity and inclusion formation. Hum Mol Genet 22:3112–3122
260. Zhang Yong-Jie, Gendron Tania F, Xu Ya-Fei, Ko Li-Wen, Yen Shu-Hui, Petrucelli Leonard (2010) Phosphorylation regulates proteasomal-mediated degradation and solubility of TAR DNA binding protein-43 C-terminal fragments. Mol Neurodegener 5:33
261. Zhang Yong Jie, Xu Ya Fei, Cook Casey, Gendron Tania F, Roettges Paul, Link Christopher D, Lin Wen Lang, Tong Jimei, Castanedes-Casey Monica, Ash Peter, Gass Jennifer, Rangachari Vijayaraghavan, Buratti Emanuele, Baralle Francisco, Golde Todd E, Dickson Dennis W, Petrucelli Leonard (2009) Aberrant cleavage of TDP-43 enhances aggregation and cellular toxicity. Proc Natl Acad Sci U S A 106:7607–7612
262. Zhang Zhijun, Almeida Sandra, Lu Yubing, Nishimura Agnes L, Peng Lingtao, Sun Danqiong, Wu Bei, Karydas Anna M, Tartaglia Maria C, Fong Jamie C, Miller Bruce L, Farese Robert V., Moore Melissa J, Shaw Christopher E, Gao Fen Biao (2013) Downregulation of MicroRNA-9 in iPSC-Derived Neurons of FTD/ALS Patients with TDP-43 Mutations. PLoS One 8:e76055
263. Zhao Weihua, Beers David R, Bell Shaughn, Wang Jinghong, Wen Shixiang, Baloh Robert H, Appel Stanley H (2015) TDP-43 activates microglia through NF-κB and NLRP3 inflammasome. Exp Neurol 273:24–35
264. Zhou Yueqin, Liu Songyan, Öztürk Arzu, Hicks Geoffrey G (2014) FUS-regulated RNA metabolism and DNA damage repair. Rare Dis 2:e29515
265. Zhuo Xiao Feng, Wang Jian, Zhang Jing, Jiang Lei Lei, Hu Hong Yu, Lu Jun Xia (2020) Solid-State NMR Reveals the Structural Transformation of the TDP-43 Amyloidogenic Region upon Fibrillation. J Am Chem Soc 142:3412–3421
266. Zu Tao, Liu Yuanjing, Bañez-Coronel Monica, Reid Tammy, Pletnikova Olga, Lewis Jada, Miller Timothy M, Harms Matthew B, Falchook Annet E, Subramony SH, Ostrow Lyle W, Rothstein Jeffrey D, Troncoso Juan C, Ranum Laura PW (2013) RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. Proc Natl Acad Sci USA 110:E4968

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8. Curriculum Vitae

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