Bioorganic & Medicinal Chemistry Letters 30 (2020) 126942

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Digest

Emerging small-molecule therapeutic approaches for amyotrophic lateral sclerosis and frontotemporal dementia

Dean G. Brown[a,*], James Shorter[b], Heike J. Wobst[c,*]

[a] Hit Discovery, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Boston, United States
[b] Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States
[c] Neuroscience, BioPharmaceuticals R&D, AstraZeneca, Boston, United States

ARTICLE INFO

Keywords:
Amyotrophic lateral sclerosis
Frontotemporal dementia
PARP
TDP-43
Stress granules

ABSTRACT

Novel treatments are desperately needed for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In this review article, a survey of emerging small-molecule approaches for ALS and FTD therapies is provided. These approaches include targeting aberrant liquid-liquid phase separation and stress granule assembly, modulation of RNA-protein interactions, inhibition of TDP-43 phosphorylation, inhibition of poly(ADP-ribose) polymerases (PARP), RNA-targeting approaches to reduce RAN translation of dipeptide repeat proteins from repeat expansions of C9ORF72, and novel autophagy activation pathways. This review details the emerging small-molecule tools and leads in these areas, along with a critical perspective on the key challenges facing these opportunities.

Introduction

Amyotrophic lateral sclerosis (ALS) is a debilitating motor neuron disease, with an average life expectancy of ~2–5 years following diagnosis.[1] The incidence of ALS is approximately 3–5 in 100,000, but can be higher in selected populations (e.g. 2:1 male to female for sporadic ALS).[1,2] Of the diagnosed cases of ALS, ~90–95% are sporadic (sALS) and ~5–10% are familial (fALS), indicating that both genetic and environmental factors contribute to the disease. ALS is an extreme manifestation of a spectrum disorder, of which the other extreme is frontotemporal dementia (FTD), the second most common form of dementia after Alzheimer's disease.[3,4] This spectrum is evidenced by the overlap in clinical manifestations of these diseases – ~15% of ALS patients develop FTD-like symptoms while ~15% of FTD patients meet ALS criteria[5,6] – as well as by their pathological similarities[7] and common genetic causes.[8] To date, there are no approved interventional drugs for the treatment of FTD, and only two for ALS patients: riluzole, a glutamate release inhibitor approved in 1995,[9] and the recently approved edavarone (FDA approval in 2017),[10] a free radical oxygen scavenger previously investigated for cerebral ischemia (Fig. 1).[11] Both treatments have limited efficacy in slowing down disease progression, highlighting the urgent need for novel therapies. The purpose of this review article is to give an overview of emerging small molecule therapeutic strategies for the treatment of ALS and FTD that have not yet reached the clinic.

TDP-43 pathology in ALS and FTD

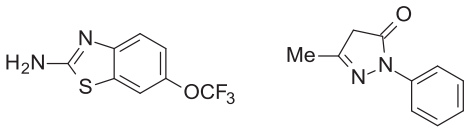
Transactive response DNA binding protein 43 kDa (TDP-43) is an RNA- and DNA-binding protein involved in transcriptional regulation, splicing, miRNA biogenesis and mRNA stability.[12] It has been shown to bind to ~30% of the mouse central nervous system (CNS) transcriptome.[13,14] TDP-43 is a predominantly nuclear protein but is mislocalized to the cytoplasm of degenerating neurons in ALS and FTD patients, where it forms inclusions of aggregated protein.[15–17] These TDP-43 inclusions are found in > 40% of FTD patients as well as ~97% of ALS patients, making them a crucial pathological hallmark for both diseases.[18] Furthermore, TDP-43 mutations are a rare cause of ALS, with ~1% of sporadic and ~5% of familial ALS cases attributed to TDP-43 missense mutations.[17] Given these strong genetic and histological links between TDP-43 and ALS/FTD, therapies aimed at normalizing TDP-43 function and pathology are extensively investigated. Preclinical drug discovery strategies that have been described in the literature include the following: a) modulating liquid-liquid phase separation of TDP-43, b) preventing aberrant phase transition/aggregation of TDP-43, c) modulating RNA binding to TDP-43, d) modulating post-translational modification such as phosphorylation.

Liquid-liquid phase separation and stress granule assembly

Liquid-liquid phase separation (LLPS) is a dynamic, reversible process whereby macromolecules condense to form a liquid phase that

* Corresponding authors at: Jnana Therapeutics, 50 Northern Ave, Boston, MA 02210, United States.
E-mail addresses: dbrown@jnanatx.com (D.G. Brown), hwobst@jnanatx.com (H.J. Wobst).

https://doi.org/10.1016/j.bmcl.2019.126942
Received 28 October 2019; Received in revised form 20 December 2019; Accepted 24 December 2019
Available online 30 December 2019
0960-894X/



1, Riluzole **2**, Edaravone

Fig. 1. Approved drugs for ALS.

exists within a dilute phase.[19] This process is evidenced by the formation of liquid droplets in vitro under permissible conditions. RNA-binding proteins associated with ALS have been shown to undergo LLPS, including FUS,[20] hnRNPA1,[21] and TDP-43.[21,22] LLPS is driven in part by intrinsically disordered regions (IDRs), protein stretches that lack well-defined three-dimensional structures.[23,24] TDP-43 possesses a C-terminal IDR with a glycine-rich low-complexity domain (LCD) responsible for self-association and LLPS.[25] The macromolecular forces that drive the assembly of these structures through IDRs are promiscuous and are thought to consist of non-specific electrostatic, π-π, cation-π and hydrophobic interactions as well as hydrogen bonds.[23] There is increasing evidence that RNA is important for seeding and nucleation in LLPS and that it can influence the size and morphology of condensates.[26] LLPS is hypothesized to drive the formation of membrane-less organelles in the nucleus and cytoplasm, including stress granules (SGs). SGs are transient cytoplasmic structures consisting of non-translating RNAs and RNA-binding proteins formed as a "storage buffer" for components and to conserve cellular energy during states of duress.[27] While LLPS processes such as stress granule formation are reversible, liquid condensates may also undergo irreversible phase transition to a solid state. Among other factors, age has been hypothesized to be an underlying factor in the transition to solid state.[28] It is this liquid-to-solid phase transition that is thought to underlie the formation of insoluble protein aggregates associated with ALS and FTD.[23,25] Thus, modulating LLPS and aberrant phase transition, regulating the material properties of protein phases, or regulating the formation and composition of structures formed through LLPS such as SGs has been an emerging pre-clinical strategy targeting ALS and FTD.[29] Given the limited understanding of the biological significance of SGs and their impact on disease etiology, the development of tool molecules that can selectively interfere with LLPS and stress granule formation, kinetics and composition will be crucial to define which, if any, of these intervention strategies may be therapeutically viable.

Small-molecule approaches to modulating LLPS, SG assembly and aberrant phase transition

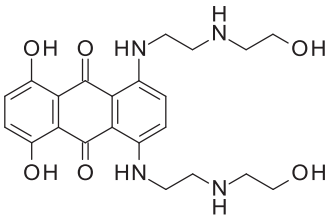
In a recent effort to identify small-molecule modulators of SG formation, Fang et al. have described a screen in HEK293T and neural precursor cells (NPCs) (~5K compounds), induced to form SGs by treatment with $NaAsO_2$.[30] In this screen, ~100 hits were identified that modulated the formation of SGs including digitoxin, anisomycin as well as a number of compounds with planar scaffolds such as daunorubicin, quinacrine and mitoxantrone **3** (Fig. 2). Mitoxantrone is a type II topoisomerase inhibitor approved for the treatment of acute myeloid leukemia as well as certain forms of multiple sclerosis. Further studies revealed that planar compounds such as mitoxantrone, but not non-planar compounds, reduced recruitment of TDP-43 to SGs. In induced pluripotent stem cell-derived motor neurons (iPS-MNs), mitoxantrone **3** also reduced the formation of persistent cytoplasmic TDP-43 puncta induced by long-term (24 h) treatment with puromycin. The authors hypothesized that blocking the recruitment of TDP-43 to stress granules during periods of stress may prevent the subsequent formation of TDP-43 aggregates. Thus, modulation of stress granule composition rather than inhibiting stress granule formation may be a viable therapeutic strategy. In a study by Wheeler et al. mitoxantrone was also found to suppress the recruitment of Fused in Sarcoma (FUS) to SGs and to reduce the number and size of liquid FUS droplets formed in vitro.[31] Like TDP-43, FUS is an RNA-binding protein with broad functions in transcriptional regulation, RNA splicing, mRNA trafficking and miRNA biogenesis.[3] Inclusions of FUS are a pathological hallmark in a subset of FTD cases (FTD-FUS, < 10% of all cases) as well as in familial and sporadic ALS cases caused by mutations in FUS (~5% of fALS, ~1% sALS).[32] In the same study, lipoamide **4** and lipolic acid **5** (Fig. 2) were also shown to suppress FUS-SG formation. However, in contrast to **3**, lipoamide **4** increased the size and number of liquid FUS droplets in vitro, suggesting a different mechanism of action in modifying cellular SG formation to **3**.[31] Lipoamide also prevented axonal dieback in mutant FUS motor neurons and rescued motor dysfunction in a FUS *Drosophila* model of ALS.[31]

Several classes of molecules that modulate SG formation and phase transition of TDP-43 have been described by Aquinnah in a series of patent applications. For example, compound **6** (Fig. 2) was able to modulate TDP-43 inclusions in PC12 cells stably expressing wild-type (WT) TDP-43-GFP (pIC_{50} < 7), and to rescue TDP-43-induced neuron death in embryonic mouse hippocampal neurons.[33] Other structurally distinct series have also been reported (e.g. **7**, Fig. 2), with similar behavior and potency to **6**.[34,35] Finally, a recent example illustrated that the small-molecule "chaperone" trimethylamine *N*-oxide (TMAO) can enhance TDP-43 LLPS but prevent protein fibrillation in vitro.[36] However, while a useful tool to help understand the bulk material processes that regulate LLPS and phase transition, it is unlikely that TMAO would form the basis of a drug discovery campaign given the unspecific nature of the interaction.

Novel assays are being developed to help study the phase transition and trafficking of TDP-43 that may lend themselves to screening for small-molecule modulators. One elegant approach is an optogenetic cell-based model in which TDP-43 inclusion formation is induced by fusing TDP-43 to a blue light-inducible oligomerization domain (optoTDP-43) that undergoes homo-oligomerization in response to blue light, allowing for spatiotemporal studies of the trafficking and phase transition of TDP-43.[37] In this system it was demonstrated that TDP-43 binding to an RNA oligonucleotide with a well-established binding sequence (34 bases with a key UG/GU stretch of 8 bases, K_d = 112 nM) antagonized TDP-43 inclusion formation and prevented neurotoxicity in optoTDP-43-expressing ReN cell cortical neurons.[37] This result is highly encouraging and suggests bait oligonucleotides with TDP-43 binding affinity may be a viable drug discovery approach. While the long RNA nucleotides used in this study do not shed light on whether a small molecule can afford the same outcome, the optoTDP-43 system is easily adaptable to a small molecule screening campaign.

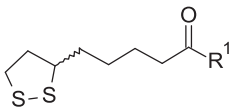
Modulation of TDP-43-RNA interactions

Work by François-Moutal et al. has suggested that a small molecule approach that prevents TDP-43 binding to RNA may reduce neuronal toxicity.[38] There are several published crystal and nuclear magnetic resonance (NMR) structures of partial TDP-43 constructs that have enabled efforts towards inhibiting the binding interaction between TDP-43 and RNA, but there are very few examples of this approach. TDP-43 binds to oligonucleotides via its two RNA-recognition motifs, RRM1 and RRM2, through a series of H-bond interactions to a 10-nucleotide stretch of UG-rich clusters in RNA, and TG repeats for binding DNA (Fig. 3).[25] In their paper, François-Moutal et al. used computational molecular docking models to identify the small molecule rTRD01 **8** (Fig. 2) that binds to RRM1 and RRM2. The binding was confirmed using NMR and microscale thermophoresis measurements (K_d = 89 μM). The compound was able to displace $(G_4C_2)_4$ RNA from TDP-43 with an IC_{50} of ~150 μM, measured using an amplified luminescent proximity homogeneous assay (ALPHA). In the same assay however, **8** was not able to inhibit binding of TDP-43 to $(UG)_6$ repeats. In a TDP-43 *Drosophila* model, **8** reduced locomotor defects in the larval



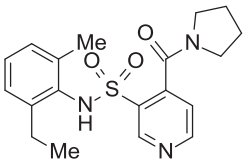
3, Mitoxantrone

Reduces TDP-43 cytoplasmic inclusion in SG pIC_{50} ~5
Reduces FUS recruitment to SG



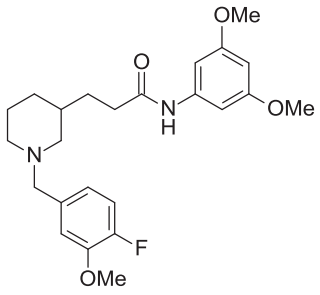
4, $R^1 = NH_2$; (±) lipoamide
5, R^1 = OH; *R* (+) lipoic acid

Reduces FUS SG pIC_{50} ~4.7



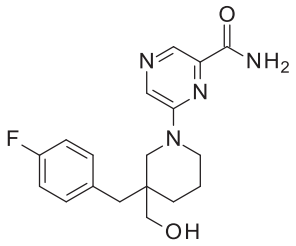
6

TDP-43 SG pIC_{50} <7



7

TDP-43 SG pIC_{50} ~7



8, rTRD01

Binds to TDP-43 RNA recognition motif K_d = 89 μM

Fig. 2. Small molecules which target stress granules (**3–7**) or TDP-43 RNA recognition motif (**8**).

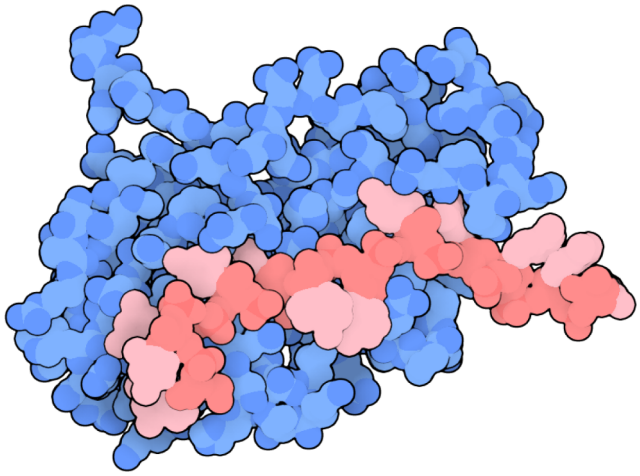


Fig. 3. TDP-43 bound to UG-rich RNA. UG-rich RNA is depicted in red across both RRM domains (RRM1 and RRM2) in blue (PDB: 4BS2). Image created using Illustrate.[39]

turning time assay.[38] Albeit very weak, this is the first reported example of a small molecule that prevents the binding of RNA or DNA to TDP-43. Several key questions regarding this approach are obvious for small molecule drug discovery: Can a small molecule be optimized to effectively compete with TDP-43's 10-base oligonucleotide recognition sequence? If so, will inhibition of RNA binding lead to a therapeutic effect, and will there be any mechanism-based toxicity if TDP-43 is prevented from binding to RNA or DNA? Further elucidation of the structure of TDP-43 and TDP-43-oligonucleotide interactions via novel assay systems is needed to gain more understanding and investigate whether small-molecule drug discovery approaches targeting the interaction between TDP-43 and RNA or DNA may provide therapeutic benefit.

Kinase inhibitors affecting TDP-43 toxicity and pathology

TDP-43 in ALS and FTD pathology shows posttranslational modifications including cleavage, ubiquitination and hyperphosphorylation.[40] TDP-43 contains over 50 potential phosphorylation sites (41 serine, 15 threonine and 8 tyrosine residues), and while it remains unclear whether phosphorylation at S409/410 contributes to aberrant behavior of TDP-43, it is a defining pathological hallmark of TDP-43 inclusions.[41] The impact of phosphorylation on TDP-43 toxicity and aggregation propensity has thus been a particular focus, and kinase inhibitors affecting TDP-43 phosphorylation have been proposed as therapeutic intervention strategies. In two studies, casein kinases 1ε (CK1ε) and 1δ (CK1δ) were shown to increase TDP-43 phosphorylation at S409/410 and promote the formation of TDP-43 aggregates.[42,43] Furthermore, CK1ε enhanced an eye degeneration phenotype in a TDP-43 *Drosophila* model.[43] Inhibition of CK1 with small molecule inhibitors (**9** and **10**, Fig. 4) was shown to reduce TDP-43 phosphorylation and aggregation in tunicamycin-treated NSC-34 cells and ethacrynic acid-treated HEK293T cells, and to increase the lifespan in a TDP-43 *Drosophila* model (**11**, Fig. 4).[44,45] Cell division cycle 7-related protein kinase (CDC7) has also been found to phosphorylate TDP-43 in vitro as well as in cells and in *C. elegans*.[46] Inhibition of CDC7 with PHA767491 (**12**, Fig. 4) was shown to reduce TDP-43 S409/410 phosphorylation and protect against neuronal loss in a TDP-43 transgenic *C. elegans* model.[46]

In addition to querying the impact of specific kinases on TDP-43 phosphorylation, other studies have investigated the impact of kinase inhibitors on TDP-43 recruitment to SGs. Moujalled et al. have

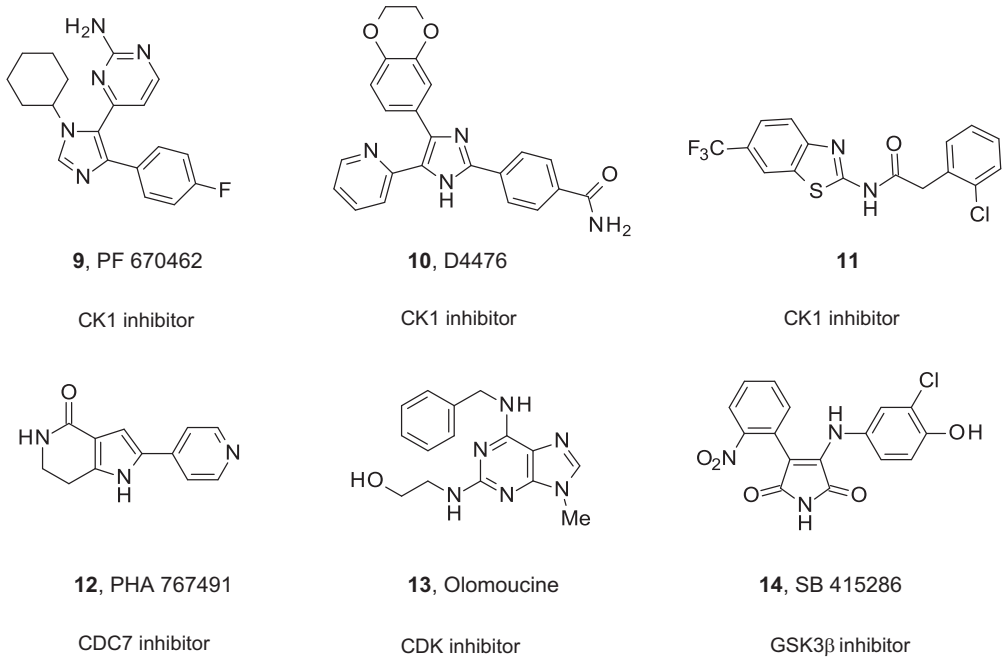


Fig. 4. Example kinase inhibitors involved in TDP-43 phosphorylation.

demonstrated that inhibition of cyclin-dependent kinase 2 (CDK2) with olomoucine (**13**, Fig. 4) and of glycogen synthase kinase 3β (GSK3β) with SB 415286 (**14**, Fig. 4) reduced the accumulation of TDP-43 in SGs.[47,48] However, the effect of CDK2 inhibition on TDP-43 SG localization was not hypothesized to result from changes in TDP-43 phosphorylation status, but rather from its interaction partner hnRNPK, which may act as a recruitment factor for TDP-43 into SGs.

Many key challenges remain concerning the therapeutic potential of kinase inhibitors and their effects on TDP-43 phosphorylation, inclusion formation and LLPS, including: 1.) gaining a clearer understanding of which, if any, phosphorylation sites on TDP-43 directly promote aberrant pathology. Current literature is focused on S409/410, but its impact on TDP-43 pathology remains controversial; 2.) understanding whether kinase inhibitors rescue TDP-43 toxicity directly by reducing TDP-43 phosphorylation or by affecting proteins downstream in toxic TDP-43 pathways; 3.) ensuring that the kinase inhibitors have good CNS penetration, since many of the inhibitors described in the literature are ATP-site binders with physical properties which can diminish CNS penetration, such as high numbers of H-bond donors. However, kinases in general are a very druggable target class with a vast number of tool compounds available commercially and in the literature.

PARP inhibitors

Poly(ADP-ribose) polymerases (PARPs) are enzymes that catalyze the formation of polymers of ADP-ribose (poly(ADP-ribose) or PAR), that can be covalently attached to proteins, a process known as PARylation. Seventeen members of the PARP family have been identified.[49] Of these, four (PARP1, PARP2, PARP5a and PARP5b) catalyze the addition of PAR chains, while the other members of the PARP family are either enzymatically inactive or attach a single ADP-ribose to target proteins.[49] The best-studied PARP enzyme is PARP1, a crucial protein in the repair of DNA damage including single-strand breaks (SSBs), double-strand breaks (DSBs) and replication fork damage.[50] DNA damage triggers the recruitment of PARP1 to the affected DNA stretch where it autoPARylates. PARylated PARP1 in turn recruits and PARylates DNA damage repair proteins. PARP1 is responsible for > 90% of cellular PARylation, with PARP2 providing between ~5 and 10%.[49,51] An abnormal build-up of PAR polymers is toxic to cells, and clearance of these polymers is believed to be an important regulatory mechanism in cellular homeostasis.[52]

PARP inhibitors have become an important new therapeutic class in oncology, with four drugs currently on the market (olaparib **15**, rucaparib **16**, niraparib **17** and talazoparib **18**, Fig. 5)[53] and more compounds in the pipeline. It is believed that these drugs work for oncology indications due to synthetic lethality in cancers with defective homologous recombination (HR), one pathway by which DSBs are repaired. Since mutations in several DNA repair genes such as breast cancer type 1 susceptibility proteins 1 and 2 (BRCA1 and BRCA2) render HR nonfunctional, PARP inhibitors work effectively in patients carrying these mutations.[54]

In addition to its role in cancer treatment, PARP inhibition may also provide a therapeutic avenue for neurodegenerative diseases including Parkinson's disease,[55] Huntington's disease,[56,57] Alzheimer's disease[58,59] and ALS/FTD. Recently, McGurk et al. demonstrated that nuclear PAR is elevated in spinal cord motor neurons of ALS patients without known mutations as well as those carrying ATXN2 mutations and C9ORF72 hexanucleotide repeat expansion mutations, two genes previously linked to ALS.[60] Furthermore, small-molecule PARP inhibitors have demonstrated efficacy in TDP-43 cellular models of ALS. In one study, the PARP inhibitor veliparib **19** rescued TDP-43-induced cell death in rat primary spinal cord cultures.[60] Furthermore, Duan et al. recently demonstrated that either PARP1 knockout or olaparib **15** (at 5 μM screening concentration) reduced PAR levels and rescued TDP-43-induced death of NSC-34 cells.[61] Conversely, knockdown of PAR glycohydrolase (PARG), which catabolizes PAR, increased levels of PAR and exacerbated cell death in TDP-43-expressing cells. In another study, XAV939 **20**, a potent inhibitor of tankyrase 2 (also known as PARP5B) and weaker inhibitor of tankyrase-1 (PARP5A), PARP1 and PARP2 reduced the accumulation of TDP-43 in cytoplasmic foci.[22] Structurally, tankyrases are distinguished from other members of the PARP family by the presence of long repeat ankyrin units, which are involved in protein binding, as well as a sterile alpha motif (SAM). These results suggest that both PARP1/2 as well as tankyrase may be important targets in regulating the TDP-43 pathology in ALS.

The mechanism by which PARP inhibitors protect against TDP-43 toxicity may be through downregulation of PAR. In vitro studies have demonstrated that PAR promotes LLPS of TDP-43 and that the nuclear

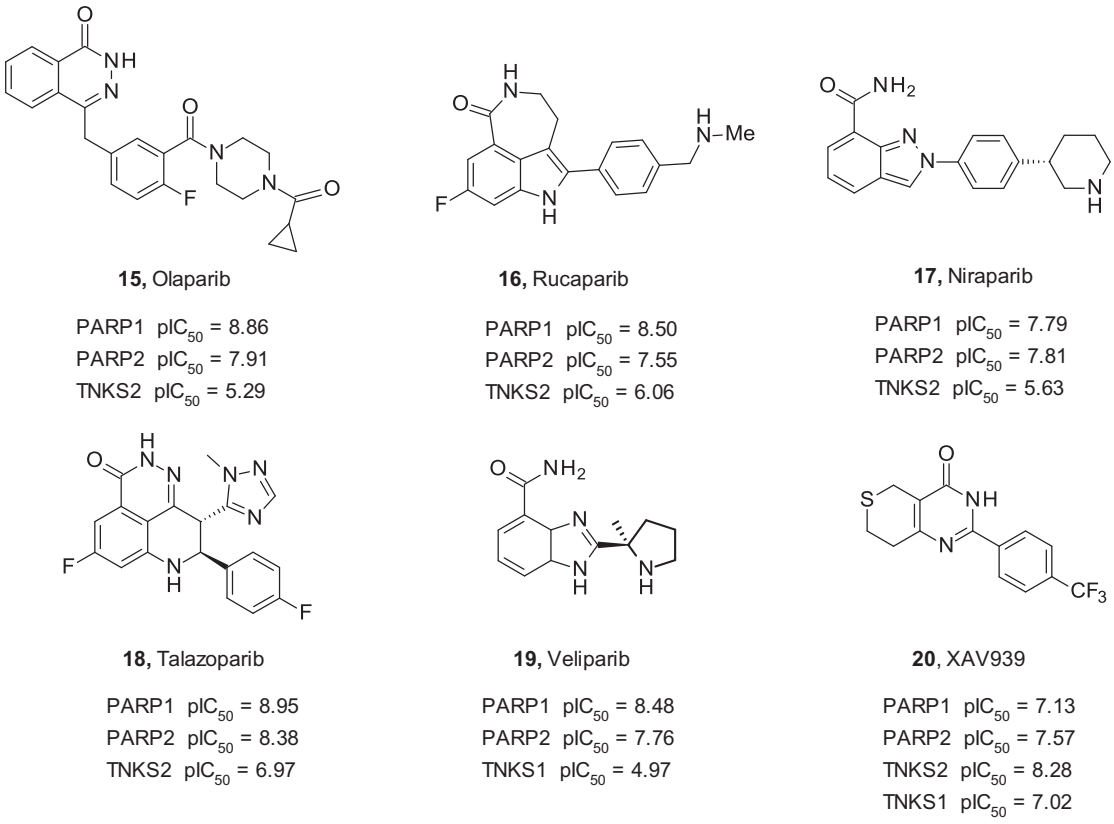


Fig. 5. Approved PARP inhibitors olaparib **15**, rucaparib **16**, niraparib **17**, talazoparib **18,** veliparib **19** and the tankyrase inhibitor **20**, XAV939. TNKS1 = tankyrase 1/PARP5A; TNKS 2 = tankyrase 2/PARP5B.

localization sequence of TDP-43 contains PAR-binding motifs that target TDP-43 to stress granules.[22] Inhibition of PARP1/2 was shown to reduce TDP-43 recruitment to stress granules[61] and both inhibition of PARP1/2 and tankyrase was shown to suppress the formation of cytoplasmic TDP-43 inclusions.[22,60]

The wealth of marketed PARP inhibitors may present an attractive opportunity and the potential for drug-repurposing.[62] However, the currently marketed drugs are approved for oncology indications where the purpose is to stop unregulated cell proliferation. This purpose is not the case for ALS/FTD, where preservation of degenerating neurons is the primary goal. Key fundamental questions will need to be answered to determine whether the currently approved antiproliferative drugs will provide efficacy in ALS clinical trials within acceptable therapeutic margins. At present, the signs are encouraging that several PARP inhibitors can rescue toxicity in ALS cell models; however, the drug concentrations tested in these cellular models greatly exceeded biochemical IC_{50} values for PARP inhibition. For example, veliparib **19** was reported to have low nM potency in PARP biochemical screens (Fig. 5) and an equally low nM potency in a cellular PARylation assay (IC_{50} = 2 nM).[63] However, TDP-43-induced toxicity in mixed rat spinal cord cultures was only rescued at a veliparib concentration of 5 μM. The same is true for olaparib **15**, which demonstrated a rescue of TDP-43 in NSC-34 cell model at 5 μM, but has low single digit nM biochemical activity against PARP1 and PARP2 (Fig. 5) and concentrations ~100 nM have been reported to completely block PARP1 activity in a cellular PARylation assay.[64] These findings raise important questions, such as: How relevant is the PARP inhibitor concentration required to rescue TDP-43-induced cell death in predicting efficacy in an ALS patient population? Is the potency disconnect due to other targets or unknown disease pathways in ALS which are yet to be uncovered? As this field rapidly progress, these questions should be at the forefront of research teams working in the area.

C9ORF72-targeting strategies

The most frequent known cause of familial and sporadic ALS as well as FTD are G_4C_2 hexanucleotide repeat expansions in intron 1 of the C9ORF72 gene.[8,65] While in healthy individuals, the repeat length is typically < 30, repeat lengths of > 1000 can be observed in C9ORF72-linked ALS and FTD.[66,67] C9ORF72 mutations account for approximately 7% and 6% of sporadic ALS and FTD, respectively, as well as for 37% of familial ALS and 24% of familial FTD cases.[68] There are three hypothesized disease mechanisms for this ALS-associated gene:[69] Loss of function of C9ORF72, gain of toxic function from repeat RNA or gain of toxic functions from dipeptide repeat proteins, which form in a process called repeat-associated non-ATG (RAN) translation. RAN translation occurs in all three reading frames both on the sense and antisense strands, leading to the translation of five dipeptide repeats (DPRs) (poly-GA, poly-PA, poly-GP, poly-PR, poly-GR).[70]

The expanded G_4C_2 RNA adopts two folded states that are in equilibrium with each other, a hairpin structure and a quadruplex structure (termed G-quadruplex, formed through stacked layers of four guanine residues stabilized by a central cation) (Fig. 6a and b).[71–73] Several studies have identified small molecules that bind to the hairpin and G-quadruplex structures. Su et al. have described a chemical screen of 132 small molecules against $(G_4C_2)_8$ derived from known CGG binders, using a dye (TO-PRO-1) assay, in which displacement of a fluorescent RNA intercalator from RNA was measured.[73] Compounds **21**, **22**, and **23** (Fig. 7) were found to bind the hairpin form with K_d values of 9.7 μM, 10 μM and 16 μM, respectively. Furthermore, **21** and **22** suppressed the expression of poly-GP and poly-GA as well as nuclear RNA foci in HEK293 cells and motor neurons.[73] In a follow-up study, Wang et al. performed a chemical similarity search to identify 40 compounds with chemical similarity to **21**.[74] Several new and more potent compounds **24**, **25**, **26** (Fig. 7) were subsequently identified that bound to

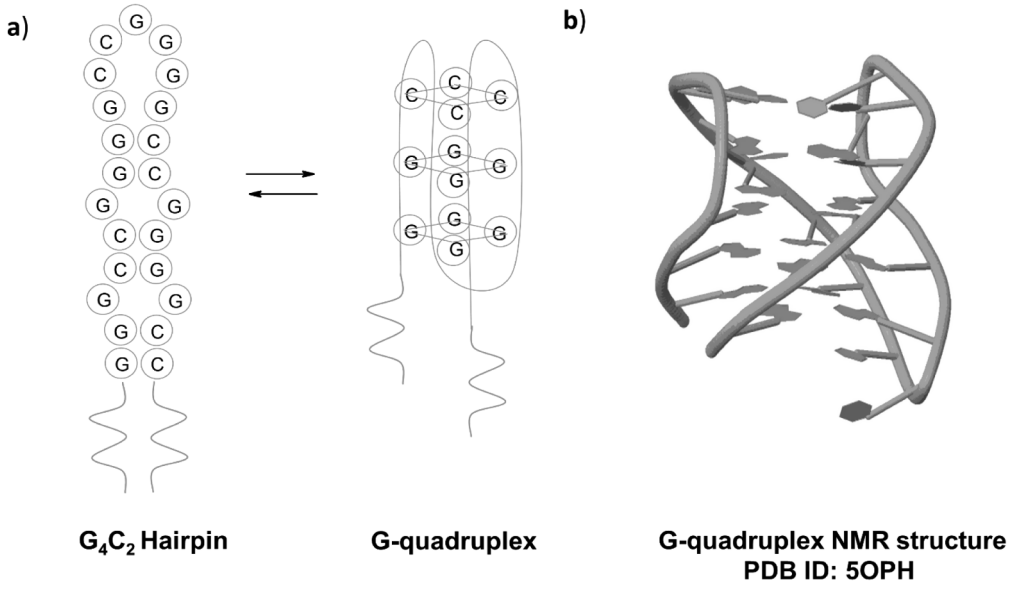
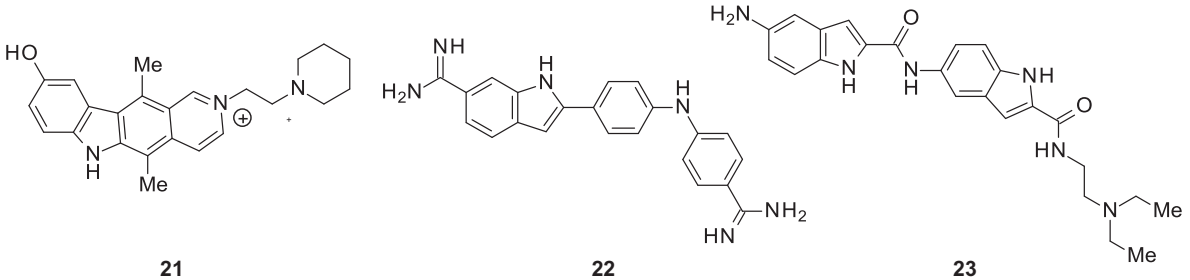


Fig. 6. a) Cartoon depiction of RNA hairpin in equilibrium with RNA G-quadraplex. b) NMR structure of G-quadraplex from C9orf72 PDB ID: 5OPH,[71] rendered with JSMol.



21

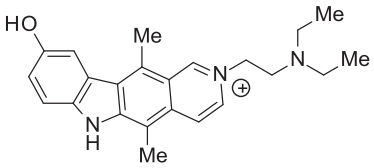
$(G_4C_2)_8$ K_d = 9.7 μM

22

$(G_4C_2)_8$ K_d = 10 μM

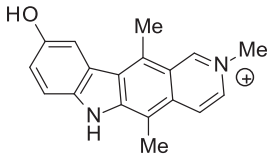
23

$(G_4C_2)_8$ K_d = 16 μM



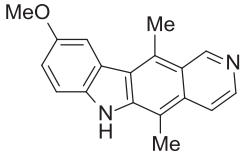
24

$(G_4C_2)_8$ K_d = 1.4 μM
RAN translation IC_{50} >25 μM



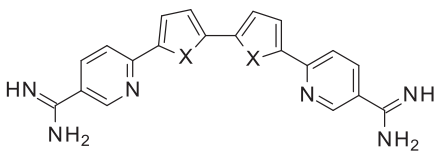
25

$(G_4C_2)_8$ K_d = 0.9 μM
RAN translation IC_{50} = 12 μM



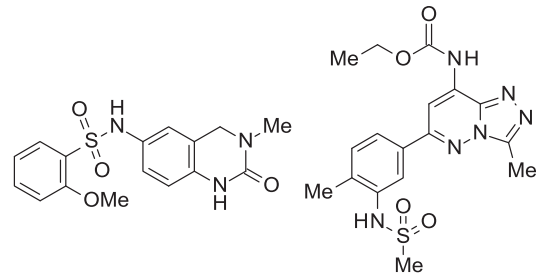
26

$(G_4C_2)_8$ K_d = 8.5 μM
RAN translation IC_{50} = 1.6 μM



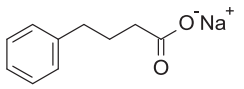
27, X = O, DB1246
28, X = S, DB1247
29, X = Se, DB1273

Fig. 7. Examples of r(GGGCC) hairpin binders **21**–**26**.[73,74] Examples of stabilizers of G-quadraplex structures shown as **27**, **28** and **29**.[75.]

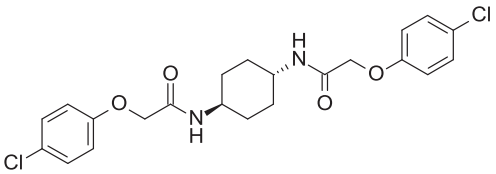


30, PFI-1

31, Bromosporine



32, Sodium phenylbutyrate



33, ISRIB

Fig. 8. Examples of compounds **30–33** found in phenotypic screens of PR_{20}-induced toxicity.

the G_4C_2 repeat hairpin structure, and inhibited RAN translation in a cellular reporter assay.[74] Compound **26** was the most selective for inhibiting RAN translation over canonical translation reported in the paper, and also serves to illustrate that the pyridinium moiety is not required for activity. In a different approach Simone et al. used a fluorescence resonance energy transfer (FRET)-based G-Q melting assay to screen a library of 138 small molecules and identified three compounds **27**, **28** and **29** (Fig. 7) that bound to and stabilized the G-quadruplex.[75] These quadruplex-binding molecules were shown to reduce RNA foci and poly-GP formation in patient-derived iPS-MNs. Furthermore, one molecule **29** was found to reduce the levels of poly-PR and slightly improve survival in a C9ORF72 $(G_4C_2)_{36}$ *Drosophila* model. Compounds such as these may serve as useful tools to help develop the field, but it should be noted that quaternary ammonium compounds that have the propensity to intercalate may have selectivity and safety issues, and thus should be carefully studied to prove their therapeutic viability.

Several papers have focused on identifying modifiers of DPR toxicity. In a phenotypic screening approach of > 4000 compounds Corman et al. treated U2OS cells with synthetic PR_{20} peptide and quantified cell survival by nuclear count.[76]. Three hits were identified in the screen, the bromodomain inhibitors PFI-1 **30** and bromosporine **31**, and sodium phenylbutyrate **32** (Fig. 8). **30** and **32** were further shown to rescue PR_{20}-induced embryonic lethality in zebrafish.[76] In another study utilizing the toxic effects of DPRs, Kramer et al. conducted CRISPR screens in immortalized cells treated with PR_{20} peptide as well as in primary mouse neurons infected with lentiviral PR_{50} to identify modulators of poly-PR toxicity.[77] As the hits in the screen implicated a possible involvement of the endoplasmatic reticulum (ER) stress response, Kramer et al. pretreated cells with Integrated Stress Response (ISR) Inhibitor (ISRIB) **33** (Fig. 8) prior to PR_{20} treatment and found that it protected cells against poly-PR toxicity. In another study, ISRIB ameliorated nucleocytoplasmic transport deficits induced by overexpression of cytoplasmic TDP-43 or PR_{50} and rescued an eye degeneration phenotype in a *Drosophila* G_4C_2 hexanucleotide repeat expansion model.[78] Interestingly, Cheng et al. demonstrated that inhibition of the ISR with ISRIB or a PERK inhibitor also rescued the stress-induced upregulation of RAN translation.[79] Thus, as dipeptide repeats (DPRs) themselves lead to upregulation of the ISR, the authors suggested that RAN translation fuels a feed-forward loop, where RAN-translated DPRs trigger a stress response that in turn causes increased RAN translation. Thus, ISRIB and other small molecules that inhibit the ER stress response may provide a potential therapeutic avenue for C9-ALS/FTD, by breaking this feed-forward loop.

C9ORF72 and DNA damage response

Several studies have shown that DNA damage and the DNA damage response are upregulated in iPSC-MNs and postmortem samples from C9ORF72 ALS patients.[80–82] Patient motor neurons had an increase in the phosphorylated histone member AX (γH2AX) and increased activation of the tumor suppressor p53-binding protein (p53) pathway including ataxia telangiectasia and Rad3-related protein (ATR). In addition, increased phosphorylation of ataxia telangiectasia mutated (ATM) and increased expression of growth arrest and DNA damage gene (GADD45) were detected compared to control neurons. Lentiviral expression of GR_{80} but not GA_{80} increased expression of γH2AX, p53 activation and DNA double strand breaks and induced the production of reactive oxygen species (ROS).[82] The increase in ROS and associated DNA damage was partially rescued when C9ORF72 motor neurons were treated with Trolox, a water-soluble antioxidant related to vitamin E.[82] While no studies have been published to date investigating the effects of inhibiting DNA damage response in C9-ALS/FTD neurons, this therapeutic angle could provide an exciting opportunity. Potent small molecule inhibitors of several key DNA-damage response enzymes exist that could interrogate the therapeutic potential of interfering with DNA-damage signaling such as ATM inhibitors,[83] ATR inhibitors,[83] and PARP1 inhibitors (Fig. 5).

Autophagy mechanisms

Autophagy is an essential cellular mechanism that enables the clearance of damaged organelles, viral particles and aggregated proteins.[84] Well-established activators of autophagy such as rapamycin **34** work as inhibitors of mammalian target of rapamycin complex 1 (mTORC1), resulting in activation of a number of cellular catabolism factors such as Unc-51 like autophagy activating kinase 1 and 2 (ULK1 and ULK2) and transcription factor EB (TFEB). Rapamycin has been shown to rescue cytoplasmic TDP-43 mislocalization in vitro and in vivo[85] and to reduce neuronal loss, cognitive impairment and motor phenotypes in a TDP-43 mouse model, and is currently in a phase 2 clinical trial for the treatment of ALS.[86,87] However, mTOR inhibitors fail to effectively stimulate autophagy in neurons,[88,89] which could limit their efficacy in neurodegenerative diseases.

In addition to mTOR1, novel mechanisms of autophagy activation are being uncovered. Indeed, two phenothiazines and related phenoxazines that promote autophagy of TDP-43 in neurons have emerged.[90] Fluphenazine (**35**), methotrimeprazine (**36**) and 10-(4′-(*N*-diethylamino)butyl)-2-chlorophenoxazine (**37**, NCP) were shown to activate autophagy in a live cell autophagic flux assay (Fig. 9). Furthermore, all three compounds decreased TPD-43 protein levels, inclusions and cytoplasmic mislocalization, and improved neuronal survival in vitro.[90] In another study Nomura et al. discovered EN6 **38** (Fig. 9), a covalent modifier of Cys277 in the ATP6V1A subunit of the lysosomal v-ATPase, by screening a small-molecule library of covalent molecules using an autophagy flux assay in mouse embryonic fibroblasts and HEK293 cells.[91] In an inducible GFP-TDP-43 U2OS cell line, **38** reduced the formation of TDP-43 aggregates by 75%. Mechanistically, the authors demonstrated that inhibition of the ATP6V1A subunit of the lysosomal v-ATPase worked by first decoupling v-ATPase from Ragulator-Rag GTPase, followed by release of mTORC1 into the cytoplasm and

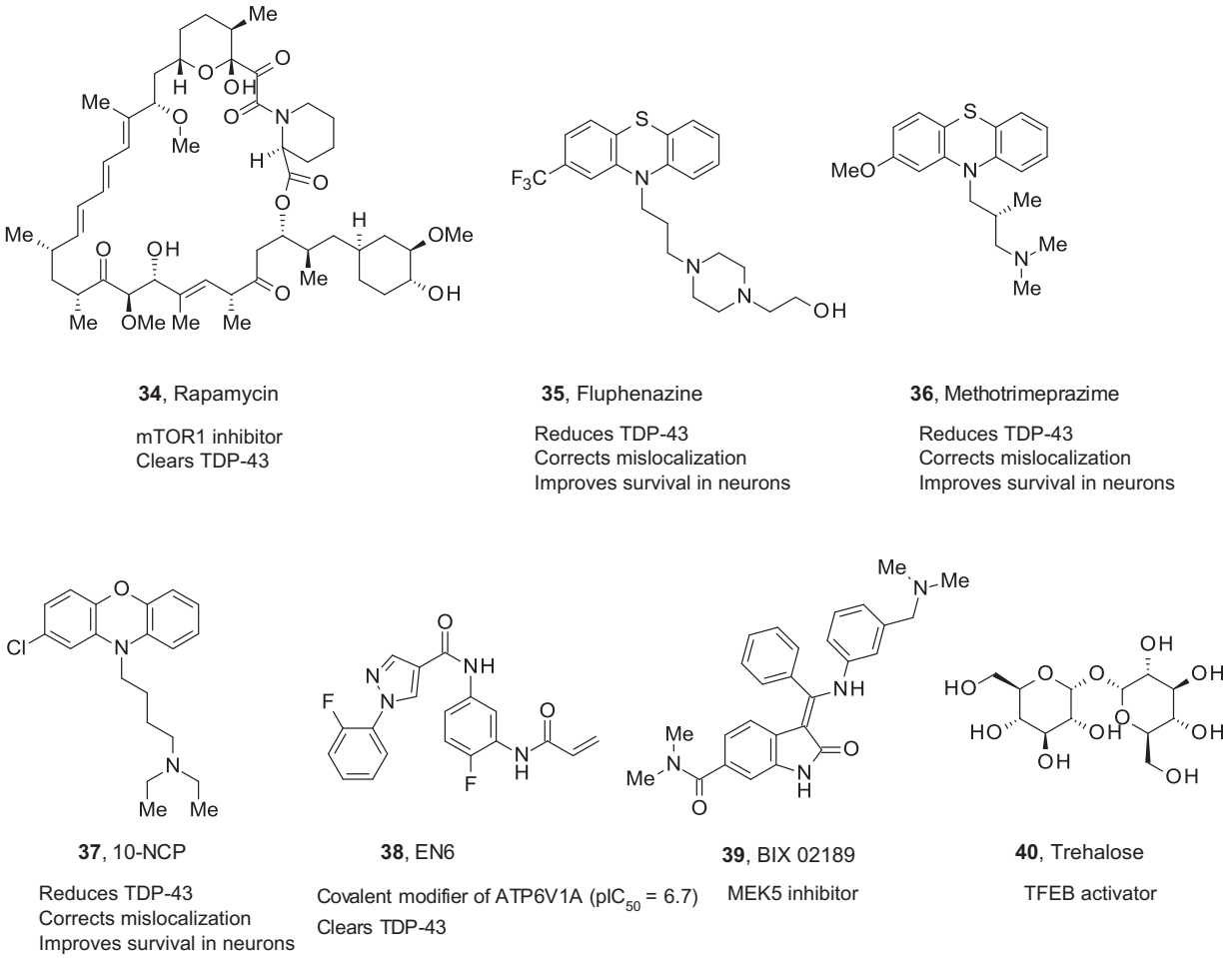


Fig. 9. Small molecules targeting TDP-43 through autophagy mechanisms.

inactivation. Thus, this mechanism represents a mTOR1 selective pathway which works independently from the mTOR1-selective rapamycin/rapalog inhibitors or the ATP-site mTOR1/2 inhibitors.

In addition, several other mTOR-independent activators of autophagy have been recently reported. Jo et al. have described inhibitors of MEK5 (**39**, Fig. 9) that activate the autophagy-lysosome pathway and suppress TDP-43 toxicity in N2a cells.[92] The authors established that this mechanism of autophagy was independent of mTOR signaling, as treatment of cells with MEK inhibitor did not affect phosphorylation of mTOR or ULK1. Furthermore, co-treatment with **39** and rapamycin resulted in a higher LC3-II/LC3-I ratio than treatment with either **39** or rapamycin alone. Another emerging target in the autophagy pathway is the transcription factor TFEB, a master regulator of autophagy that drives expression of autophagy and lysosomal genes.[93] Trehalose **40** (Fig. 9), a protector against cellular stress in yeast,[94] was shown to promote autophagy by inhibiting Akt kinase, which phosphorylates TFEB, thus repressing its nuclear translocation.[95,96] Activation of TFEB with trehalose reduced TDP-43 accumulation in vitro.[97]

Summary

Decades of research have only yielded two therapies for the treatment of ALS, and none for FTD. However, preclinical studies of basic and translational disease biology have yielded an impressive array of novel targets amenable to modulation by small-molecule approaches. Many challenges remain before some of these approaches will make their way into the clinic, from building more in-depth knowledge into the links between fundamental biology and neurodegeneration (e.g. in the case of LLPS) to turning chemical starting points with target affinities into highly potent molecule entities (e.g. in the case of TDP-43/RNA interactions or direct G-quadruplex binders). Many of the approaches outlined above stem from oncology projects, and as such will require experimental evidence demonstrating that the anti-proliferative effects can be separated from any therapeutic benefit to neurodegeneration. While a high rate of attrition from preclinical concept to clinical trial is to be expected, the breadth of different therapeutic approaches provide hope that some of the therapeutic strategies discussed in this review will ultimately provide novel treatments for ALS and FTD patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Brown Jr RH, Al-Chalabi A. Amyotrophic lateral sclerosis. *N Engl J Med.* 2017;377:1602.
2. Longinetti E, Fang F. Epidemiology of amyotrophic lateral sclerosis: an update of recent literature. *Curr Opin Neurol.* 2019;32:771–776.
3. Ling SC, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron.* 2013;79:416–438.
4. van Es MA, et al. Amyotrophic lateral sclerosis. *Lancet.* 2017;390:2084–2098.
5. Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology.* 2002;59:1077–1079.
6. Ringholz GM, et al. Prevalence and patterns of cognitive impairment in sporadic ALS. *Neurology.* 2005;65:586–590.
7. Neumann M, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and

amyotrophic lateral sclerosis. *Science.* 2006;314:130–133.
8. DeJesus-Hernandez M, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron.* 2011;72:245–256.
9. Dharmadasa T, Kiernan MC. Riluzole, disease stage and survival in ALS. *Lancet Neurol.* 2018;17:385–386.
10. Writing G, Edaravone ALSSG. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* 2017;16:505–512.
11. Watanabe T, Yuki S, Egawa M, Nishi H. Protective effects of MCI-186 on cerebral ischemia: possible involvement of free radical scavenging and antioxidant actions. *J Pharmacol Exp Ther.* 1994;268:1597–1604.
12. Ratti A, Buratti E. Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *J Neurochem.* 2016;138(Suppl 1):95–111.
13. Polymenidou M, et al. Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nat Neurosci.* 2011;14:459–468.
14. Tollervey JR, et al. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nat Neurosci.* 2011;14:452–458.
15. Shorter J. Phase separation of RNA-binding proteins in physiology and disease: an introduction to the JBC reviews thematic series. *J Biol Chem.* 2019;294:7113–7114.
16. Palomo V, et al. TDP-43: a key therapeutic target beyond amyotrophic lateral sclerosis. *ACS Chem Neurosci.* 2019;10:1183–1196.
17. Scotter EL, Chen HJ, Shaw CE. TDP-43 proteinopathy and ALS: insights into disease mechanisms and therapeutic targets. *Neurotherapeutics.* 2015;12:352–363.
18. Lee EB, Lee VM, Trojanowski JQ. Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. *Nat Rev Neurosci.* 2011;13:38–50.
19. Gomes E, Shorter J. The molecular language of membraneless organelles. *J Biol Chem.* 2019;294:7115–7127.
20. Murakami T, et al. ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron.* 2015;88:678–690.
21. Molliex A, et al. Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell.* 2015;163:123–133.
22. McGurk L, et al. Poly(ADP-ribose) prevents pathological phase separation of TDP-43 by promoting liquid demixing and stress granule localization. *Mol Cell.* 2018;71 703-717 e709.
23. Boeynaems S, et al. Protein phase separation: a new phase in cell biology. *Trends Cell Biol.* 2018;28:420–435.
24. Protter DSW, et al. Intrinsically disordered regions can contribute promiscuous interactions to RNP granule assembly. *Cell Rep.* 2018;22:1401–1412.
25. Loughlin FE, Wilce JA. TDP-43 and FUS-structural insights into RNA recognition and self-association. *Curr Opin Struct Biol.* 2019;59:134–142.
26. Garcia-Jove Navarro M, et al. RNA is a critical element for the sizing and the composition of phase-separated RNA-protein condensates. *Nat Commun.* 2019;10:3230.
27. Li YR, King OD, Shorter J, Gitler AD. Stress granules as crucibles of ALS pathogenesis. *J Cell Biol.* 2013;201:361–372.
28. Alberti S, Hyman AA. Are aberrant phase transitions a driver of cellular aging? *BioEssays.* 2016;38:959–968.
29. Elbaum-Garfinkle S. Matter over mind: liquid phase separation and neurodegeneration. *J Biol Chem.* 2019;294:7160–7168.
30. Fang MY, et al. Small-molecule modulation of TDP-43 recruitment to stress granules prevents persistent TDP-43 accumulation in ALS/FTD. *Neuron.* 2019;103:e811 802-819 e811.
31. Wheeler RJ, et al. Small molecules for modulating protein driven liquid-liquid phase separation in treating neurodegenerative disease. *bioRxiv.* 2019.
32. Nolan M, Talbot K, Ansorge O. Pathogenesis of FUS-associated ALS and FTD: insights from rodent models. *Acta Neuropathol Commun.* 2016;4:99.
33. Larsen GR, Weigele M, Vacca, J.P. WO2016090317A1. Sulfonamide derivatives, compositions and methods of use in the treatment of neurodegenerative diseases. 2016.
34. Larsen GR, Weigele M, Vacca JP, Burnett DA, Ripka A. WO2017066705A1. Compounds, compositions and methods of use against stress granules. 2017.
35. Burnett DA, Vacca J.P. WO2018195075A1. Compounds, compositions and methods of use. 2018.
36. Choi KJ, et al. A chemical chaperone decouples TDP-43 disordered domain phase separation from fibrillation. *Biochemistry.* 2018;57:6822–6826.
37. Mann JR, et al. RNA binding antagonizes neurotoxic phase transitions of TDP-43. *Neuron.* 2019;102 321-338 e328.
38. Francois-Moutal L, et al. Small molecule targeting TDP-43's RNA recognition motifs reduces locomotor defects in a drosophila model of amyotrophic lateral sclerosis (ALS). *ACS Chem Biol.* 2019;14:2006–2013.
39. Goodsell DS, Autin L, Olson AJ. Illustrate: software for biomolecular illustration. *Structure.* 2019;27 1716-1720 e1711.
40. Kwong LK, Neumann M, Sampathu DM, Lee VM, Trojanowski JQ. TDP-43 proteinopathy: the neuropathology underlying major forms of sporadic and familial frontotemporal lobar degeneration and motor neuron disease. *Acta Neuropathol.* 2007;114:63–70.
41. Neumann M, et al. Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. *Acta Neuropathol.* 2009;117:137–149.
42. Nonaka T, et al. Phosphorylation of TAR DNA-binding protein of 43 kDa (TDP-43) by truncated casein kinase 1delta triggers mislocalization and accumulation of TDP-43. *J Biol Chem.* 2016;291:5473–5483.
43. Choksi DK, et al. TDP-43 phosphorylation by casein kinase Iepsilon promotes oligomerization and enhances toxicity in vivo. *Hum Mol Genet.* 2014;23:1025–1035.
44. Hicks DA, Cross LL, Williamson R, Rattray M. Endoplasmic reticulum stress signalling induces casein kinase 1-dependent formation of cytosolic TDP-43 inclusions in motor neuron-like cells. *Neurochem Res.* 2019.
45. Salado IG, et al. Protein kinase CK-1 inhibitors as new potential drugs for amyotrophic lateral sclerosis. *J Med Chem.* 2014;57:2755–2772.
46. Liachko NF, et al. CDC7 inhibition blocks pathological TDP-43 phosphorylation and neurodegeneration. *Ann Neurol.* 2013;74:39–52.
47. Moujalled D, et al. Kinase inhibitor screening identifies cyclin-dependent kinases and glycogen synthase kinase 3 as potential modulators of TDP-43 cytosolic accumulation during cell stress. *PLoS ONE.* 2013;8:e67433.
48. Moujalled D, et al. Phosphorylation of hnRNP K by cyclin-dependent kinase 2 controls cytosolic accumulation of TDP-43. *Hum Mol Genet.* 2015;24:1655–1669.
49. Schreiber V, Dantzer F, Ame JC, de Murcia G. Poly(ADP-ribose): novel functions for an old molecule. *Nat Rev Mol Cell Biol.* 2006;7:517–528.
50. Pommier Y, O'Connor MJ, de Bono J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. *Sci Transl Med.* 2016;8:362ps317.
51. Ame JC, et al. PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. *J Biol Chem.* 1999;274:17860–17868.
52. Andrabi SA, et al. Poly(ADP-ribose) (PAR) polymer is a death signal. *Proc Natl Acad Sci U S A.* 2006;103:18308–18313.
53. Thorsell AG, et al. Structural basis for potency and promiscuity in poly(ADP-ribose) polymerase (PARP) and tankyrase inhibitors. *J Med Chem.* 2017;60:1262–1271.
54. Bryant HE, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase. *Nature.* 2005;434:913–917.
55. Kam TI, et al. Poly(ADP-ribose) drives pathologic alpha-synuclein neurodegeneration in Parkinson's disease. *Science.* 2018;362.
56. Paldino E, et al. Selective sparing of striatal interneurons after poly (ADP-Ribose) polymerase 1 inhibition in the R6/2 mouse model of Huntington's disease. *Front Neuroanat.* 2017;11:61.
57. Cardinale A, Paldino E, Giampa C, Bernardi G, Fusco FR. PARP-1 inhibition is neuroprotective in the R6/2 mouse model of Huntington's disease. *PLoS ONE.* 2015;10:e0134482.
58. Martire S, et al. PARP-1 modulates amyloid beta peptide-induced neuronal damage. *PLoS ONE.* 2013;8:e72169.
59. Abeti R, Abramov AY, Duchen MR. Beta-amyloid activates PARP causing astrocytic metabolic failure and neuronal death. *Brain.* 2011;134:1658–1672.
60. McGurk L, et al. Nuclear poly(ADP-ribose) activity is a therapeutic target in amyotrophic lateral sclerosis. *Acta Neuropathol Commun.* 2018;6:84.
61. Duan Y, et al. PARylation regulates stress granule dynamics, phase separation, and neurotoxicity of disease-related RNA-binding proteins. *Cell Res.* 2019;29:233–247.
62. Baker NC, Ekins S, Williams AJ, Tropsha A. A bibliometric review of drug repurposing. *Drug Discov Today.* 2018;23:661–672.
63. Penning TD, et al. Discovery of the poly(ADP-ribose) polymerase (PARP) inhibitor 2-[(R)-2-methylpyrrolidin-2-yl]-1H-benzimidazole-4-carboxamide (ABT-888) for the treatment of cancer. *J Med Chem.* 2009;52:514–523.
64. Menear KA, et al. 4-[3-(4-Cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin- 1-one: a novel bioavailable inhibitor of poly(ADP-ribose) polymerase-1. *J Med Chem.* 2008;51:6581–6591.
65. Renton AE, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron.* 2011;72:257–268.
66. van Blitterswijk M, et al. Association between repeat sizes and clinical and pathological characteristics in carriers of C9ORF72 repeat expansions (Xpansize-72): a cross-sectional cohort study. *Lancet Neurol.* 2013;12:978–988.
67. Beck J, et al. Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. *Am J Hum Genet.* 2013;92:345–353.
68. Majounie E, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *Lancet Neurol.* 2012;11:323–330.
69. Balendra R, Isaacs AM. C9orf72-mediated ALS and FTD: multiple pathways to disease. *Nat Rev Neurol.* 2018;14:544–558.
70. Cleary JD, Pattamatta A, Ranum LPW. Repeat-associated non-ATG (RAN) translation. *J Biol Chem.* 2018;293:16127–16141.
71. Brcic J, Plavec J. NMR structure of a G-quadruplex formed by four d(G4C2) repeats: insights into structural polymorphism. *Nucleic Acids Res.* 2018;46:11605–11617.
72. Fratta P, et al. C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. *Sci Rep.* 2012;2:1016.
73. Su Z, et al. Discovery of a biomarker and lead small molecules to target r(GGGGCC)-associated defects in c9FTD/ALS. *Neuron.* 2014;83:1043–1050.
74. Wang ZF, et al. The hairpin form of r(G4C2)(exp) in c9ALS/FTD Is repeat-associated non-ATG translated and a target for bioactive small molecules. *Cell Chem Biol.* 2019;26 179-190 e112.
75. Simone R, et al. G-quadruplex-binding small molecules ameliorate C9orf72 FTD/ALS pathology in vitro and in vivo. *EMBO Mol Med.* 2018;10:22–31.
76. Corman A, et al. A chemical screen identifies compounds limiting the toxicity of C9ORF72 dipeptide repeats. *Cell Chem Biol.* 2019;26 235-243 e235.
77. Kramer NJ, et al. CRISPR-Cas9 screens in human cells and primary neurons identify modifiers of C9ORF72 dipeptide-repeat-protein toxicity. *Nat Genet.* 2018;50:603–612.
78. Zhang K, et al. Stress granule assembly disrupts nucleocytoplasmic transport. *Cell.* 2018;173:958–971.e917.
79. Cheng W, et al. C9ORF72 GGGGCC repeat-associated non-AUG translation is upregulated by stress through eIF2α phosphorylation. *Nat Commun.* 2018;9:51.
80. Walker C, et al. C9orf72 expansion disrupts ATM-mediated chromosomal break repair. *Nat Neurosci.* 2017;20:1225–1235.
81. Farg MA, Konopka A, Soo KY, Ito D, Atkin JD. The DNA damage response (DDR) is

induced by the C9orf72 repeat expansion in amyotrophic lateral sclerosis. *Hum Mol Genet.* 2017;26:2882–2896.
82. Lopez-Gonzalez R, et al. Poly(GR) in C9ORF72-related ALS/FTD compromises mitochondrial function and increases oxidative stress and DNA damage in iPSC-derived motor neurons. *Neuron.* 2016;92:383–391.
83. Brandsma I, Fleuren EDG, Williamson CT, Lord CJ. Directing the use of DDR kinase inhibitors in cancer treatment. *Expert Opin Investig Drugs.* 2017;26:1341–1355.
84. Rubinsztein DC, Codogno P, Levine B. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat Rev Drug Discov.* 2012;11:709–730.
85. Caccamo A, et al. Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability. *J Biol Chem.* 2009;284:27416–27424.
86. Mandrioli J, et al. Rapamycin treatment for amyotrophic lateral sclerosis: protocol for a phase II randomized, double-blind, placebo-controlled, multicenter, clinical trial (RAP-ALS trial). *Medicine (Baltimore).* 2018;97:e11119.
87. Wang IF, et al. Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43. *Proc Natl Acad Sci U S A.* 2012;109:15024–15029.
88. Maday S, Holzbaur EL. Compartment-specific regulation of autophagy in primary neurons. *J Neurosci.* 2016;36:5933–5945.
89. Tsvetkov AS, et al. A small-molecule scaffold induces autophagy in primary neurons and protects against toxicity in a Huntington disease model. *Proc Natl Acad Sci U S A.* 2010;107:16982–16987.
90. Barmada SJ, et al. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat Chem Biol.* 2014;10:677–685.
91. Chung CY, et al. Covalent targeting of the vacuolar H(+)-ATPase activates autophagy via mTORC1 inhibition. *Nat Chem Biol.* 2019;15:776–785.
92. Jo M, et al. Inhibition of MEK5 suppresses TDP-43 toxicity via the mTOR-independent activation of the autophagy-lysosome pathway. *Biochem Biophys Res Commun.* 2019;513:925–932.
93. Settembre C, et al. TFEB links autophagy to lysosomal biogenesis. *Science.* 2011;332:1429–1433.
94. Kandror O, Bretschneider N, Kreydin E, Cavalieri D, Goldberg AL. Yeast adapt to near-freezing temperatures by STRE/Msn2,4-dependent induction of trehalose synthesis and certain molecular chaperones. *Mol Cell.* 2004;13:771–781.
95. Palmieri M, et al. mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. *Nat Commun.* 2017;8:14338.
96. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J Biol Chem.* 2007;282:5641–5652.
97. Wang Y, et al. Autophagic modulation by trehalose reduces accumulation of TDP-43 in a cell model of amyotrophic lateral sclerosis via TFEB activation. *Neurotox Res.* 2018;34:109–120.