Antisense oligonucleotide therapy in amyotrophic lateral sclerosis (ALS)

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Abstract

Purpose of review: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder with few treatment options available. The approval of tofersen, an antisense oligonucleotide, for *SOD1*-ALS by the FDA and EMA may herald a new era of treatment in these patients.

Recent findings: So far, trials against the most common genetic form of ALS, *C9orf72*, have been unsuccessful, but new preclinical data may show a promising new direction to take. Clinical trials targeting other, more rare genetic mutations associated with familial ALS are currently underway. Other research assessing the use of ASOs to target aberrant splicing associated with sporadic forms of ALS has also produced promising results in preclinical models, using patient-derived induced cellular models and animal models. These therapies are focussed largely on alleviating and reversing TDP-43 pathology, opening up the possibility of not only arresting disease progression, but reversing neurodegeneration.

Summary: ASO therapies have made some promising steps towards treating familial ALS, particularly *SOD1*. Ongoing early clinical/preclinical phase research is underway to utilise this technology in other genetic mutations linked with ALS, as well as in sporadic cases.

Key words: amyotrophic lateral sclerosis; antisense oligonucleotides; *SOD1*; *C9orf72*

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive, neurodegenerative disorder characterised by motor neuron injury and cell death, resulting in weakness and muscle atrophy. Progressive failure of the neuromuscular system including respiratory and bulbar muscles has a substantial effect on life expectancy with a median survival time of only 2-3 years following symptom onset. Approximately 10% of ALS cases can be attributed to a mutation in a single gene inherited in an autosomal dominant fashion [1]. The most common of these is a GGGGCC-hexanucleotide repeat expansion in the first intron in the chromosome 9 open reading frame 72 (*C9orf72*). However, over 30 different genes have been implicated in ALS, and some of these present a potential therapeutic target.

Antisense oligonucleotides (ASOs) are a group of drugs that can modulate expression of specific genes without altering a patient's DNA [2,3], by targeting RNA either prior to or post-translation [4]. The common structures of ASOs are shown in **Figure 1**.

ASOs have been in clinical use for a number of years, and their efficacy in neurological diseases was first highlighted by the use of nusinersen in spinal muscular atrophy (SMA) [5,6]. A number of ASOs exert their effects by attracting RNase H, resulting in cleavage of the target mRNA molecule and inactivating it [7]. This is a useful mechanism in disease states where a mutation causes a toxic gain-of-function effect, where reducing the amount of mRNA available for translation and, consequently, the amount of protein present, can alleviate these toxic effects. In other cases, ASOs can exert their effects by modulating the translation of the target mRNA without breaking it down, leading to altered splicing, translation or localisation of the transcript [7] (see **Figure 2**).

ASOs have become a popular method of targeting diseases with single gene mutations, such as familial ALS (fALS). This review discusses the current state of ASO therapy and research in ALS, including clinical and preclinical work in this field. The common genes and their mRNA targets are summarised in **Table 1**.

SOD1

Mutations in the copper/zinc superoxide dismutase 1 (*SOD1*) gene are implicated in around 2% of all cases of ALS [8,9]. This is thought to lead to disease primarily through toxic gain of function, where the mutant SOD1 protein causes direct neurotoxicity [10,11]. This has made *SOD1*-ALS a popular target for ASO therapies, with the first trial of the ASO 33361 published in 2013 [12].

In recent years, tofersen (BIIB067) has been studied with great interest. Following encouraging results from the Phase 1 /2 trial a Phase 3 trial included 108 patients worldwide [13]. However, the Phase 3 trial [14] did not meet its primary end point of slower progression in clinical symptoms as measured by the revised ALS Functional Rating Scale (ALSFRS-R) at the 6-month time point. Despite this, Miller *et al.* did demonstrate the safety of tofersen, and with improved exploratory CSF markers of disease progression (lowering of CSF SOD1 protein concentration and neurofilament light chain [NfL in plasma]). However, after the 6-month experimental period, all patients including the original placebo group were able to access tofersen therapy. At the 12-month time point comparing the patients on tofersen throughout, with those on placebo for the first 6 months, clear significant improvements in clinical parameters were apparent including measured by the ALSFRS-R, slow vital capacity, hand held dynamometry measures of muscle strength and quality of life measures [14]. The

results of the longer open label extension with follow-up for >3 years are awaited (NCT03070119). Furthermore, a recent study in Germany showed that tofersen reduced the rate of decline in ALSFRS-R scores in patients with *SOD1*-ALS, and also reduced the level of blood NfL [15].

Tofersen is also currently being tested in a Phase 3 trial in presymptomatic carriers of the *SOD1* gene (ATLAS, NCT04856982). This study follows people with known *SOD1* mutations and longitudinally monitor NfL levels. If NfL levels rise, in the absence of symptoms of ALS, then these people enter the "presymptomatic" stage, and it is presumed that they would progress to develop ALS. These participants are being recruited into ATLAS and given access to tofersen, to investigate whether it can delay or prevent the development of ALS in this cohort. The study is expected to complete in the summer of 2027.

C9orf72

Approximately 10% of all cases of ALS can be linked to a *C9orf72* hexanucleotide repeat expansion [16,17]. It is the most common genetic mutation causing ALS, and can also cause frontotemporal dementia (FTD): around 5-10% of all cases of FTD can be linked to a *C9orf72* mutation [16,18,19]. *C9orf72* carries a more complicated pathophysiology which is not yet fully understood, but there are considered to be both toxic gain-of-function and loss-of-function mechanisms involved. Gain-of-function toxicity is thought to be caused via two separate mechanisms linked to the bidirectional transcription of the gene. Firstly, by sense and antisense RNA strands of the G_4C_2 repeat clustering into RNA foci, which sequester RNA-binding proteins and thus affecting post-transcriptional processing [20]; secondly, by non-canonical repeat-associated non-ATG-mediated (RAN) translation of the hexanucleotide

expansion producing 5 types of toxic dipeptide repeats (DPRs), which can alter proteostasis and contribute to TDP-43 inclusion production, a hallmark of ALS [21,22]. Loss-of-function is thought to be primarily caused by haploinsufficiency, and thus reduced *C9orf72* transcript and protein which can impair autophagy initiation along with other cellular processes [20,21].

This complexity likely explains the difficulties in finding effective therapies against *C9orf72*-ALS. A recent Phase 1 trial of the ASO BIIB078 by van den Berg and colleagues [23] showed no reduction in NfL or improvement in clinical outcomes when compared with placebo. Though there was a dose-dependent reduction in sense DPRs, at higher doses an increase in NfL levels was observed suggesting possible accelerated motor neuron injury. A later trial of the ASO WVE-004 was discontinued after an increase in CSF NfL was detected in some dose groups, as well as faster clinical deterioration [24]. This is despite reductions in the CSF levels of DPRs reported in both trials, suggesting satisfactory target engagement.

The results of these trials could be explained by the failure of these ASOs to target the antisense DPRs, as both ASOs were designed to target only sense repeat strands. A preclinical study demonstrated that antisense repeat RNA alone was sufficient to induce TDP-43 pathology in induced pluripotent stem cell (iPSC) derived neurons from patients with *C9orf72*-ALS [25]. This study also demonstrated that an ASO targeting the antisense RNA rescued TDP-43 function in these cells, raising the potential importance of targeting antisense repeats in *C9orf72*-ALS.

Furthermore, all ASO therapies in C9-ALS mentioned above are seeking to mitigate the toxic gain-of-function effect of mutant *C9orf72*, but fail to address the potential loss-of-function caused by haploinsufficiency. This could be another important consideration, as

haploinsufficiency has been demonstrated to exacerbate gain-of-function toxicity in mouse models of *C9orf72*-ALS [26,27].

Future ASOs in *C9orf72*-ALS will need to take into account the role of antisense RNA repeats, as well as the role of haploinsufficiency in disease pathology. Furthermore, downstream effects of *C9orf72* mutation may also need to be examined further and the potential for targeting evaluated, including TDP-43 dysfunction [27].

FUS

Fused in sarcoma (*FUS*) is a DNA/RNA-binding protein that is involved in several vital cellular processes, including DNA repair, splicing and translation of RNA [2,28]. Mutations in *FUS* lead to motor neuron damage and loss in the absence of TDP-43 pathology, causing a form of ALS associated with young age of onset and aggressive disease course [2,29,30]. The exact mechanism of *FUS*-ALS is still incompletely understood, but it is thought to be mediated through a mix of gain-of-function (FUS protein aggregation and RNA-binding protein sequestration, impacting RNA metabolism) and loss-of-function mechanisms (*FUS* mislocalisation causing impaired functioning of the protein) [31–33], with the majority of evidence pointing towards a predominantly gain-of-function picture. This makes *FUS*-ALS an ideal target for ASO therapy.

The ASO ION363 (now known as Jacifusen) showed promising results in preclinical work done on mice, showing reduction of insoluble FUS protein aggregates and delayed lumbar motor neuron degeneration [29]. The same group was able to supply ION363 to a 26-year-old female patient with a P525L mutation causing *FUS*-ALS, in a compassionate access programme. The

patient did not experience adverse events, and postmortem examination showed a reduction in wild-type and mutant *FUS*, as well as in FUS protein aggregates [29]. This work formed the basis of a randomised, controlled phase 3 trial investigating ION363 in patients with *FUS*-ALS (FUSION, NCT04768972). Participants are administered with either monthly or bimonthly ASO, or with placebo, for 29 weeks in Part 1 of the trial, which will be followed by a 72-week open-label extension period in Part 2. The study includes a rescue plan, where participants on placebo are transferred into Part 2 of the trial if they show signs of significant clinical deterioration during Part 1. The results are expected in summer 2025, with primary outcome measures of time to rescue, survival time without ventilation and ALSFRS-R.

ATXN2

ATXN2 encodes for Ataxin-2, an RNA-binding protein, that contains 22-23 cytosine-adenosine-guanine (CAG) repeats in healthy individuals. However, a trinucleotide CAG expansion of >34 copies is associated with a severe neurodegenerative disorder known as spinocerebellar ataxia 2 (SCA2) [34]. A group working with yeast found that intermediate length expansions of 27-33 copies is associated with TDP-43 overexpression and reduced cell survival [35], a well-known hallmark of ALS pathology. Intermediate expansion gene changes in *ATXN2* are now well-recognised in ALS, detected in up to 5% of people with ALS [35,36].

A TDP-43 mouse model was treated with an ASO targeting ataxin-2, demonstrating effective reduction of the mutant protein [37]. Furthermore, the team also found a reduction in TDP-43 aggregates as well as improved survival. This prompted the development of an ASO for human clinical trials, BIIB105. However, the Phase 1/2 trial was discontinued after no

improvement in clinical outcomes and no reduction in NfL was found over a 6-month experimental period in the treated cohort when compared with placebo [38].

STMN2

Stathmin-2 is a microtubule-binding protein that plays a key role in axonal stability and regeneration [39,40]. *STMN2* pre-mRNA contains a cryptic exon that is usually repressed by TDP-43; however, in states of TDP-43 mislocalisation, the cryptic exon is included in the *STMN2* mRNA secondary to dysregulated splicing and polyadenylation [39]. This results in a truncated form of mRNA that produces a non-functional transcript. As TDP-43 mislocalisation is a hallmark of ALS [41], stathmin-2 was hypothesised to be downregulated in this disease. This was confirmed to be the case in both sporadic and familial ALS cases [42].

In 2023, Baughn and colleagues used an ASO that binds to the cryptic splice region of *STMN2* pre-mRNA, mimicking the action of TDP-43 [43]. Treatment with this ASO was shown to restore normal splicing of *STMN2* pre-mRNA and stathmin-2 functionality in TDP-43 mice and human iPSC-derived motor neurons. Subsequently, an ASO with a similar mechanism of action, QRL-201, is currently being trialled in ALS patients in a Phase 1 trial to assess its safety and tolerability (ANQUR, NCT05633459). The study is due to complete in 2026, and will administer multiple-ascending doses of intrathecal QRL-201 or placebo to patients with sporadic and C9orf72-ALS.

PIKFYVE

PIKFYVE kinase phosphorylates and downregulates the levels of phosphatidylinositol 3-phosphate (PI3P), which is involved in endosomal maturation into lysosomes and binding with autophagosomes [44,45]. As such, PIKFYVE inhibition was proposed to stimulate removal of toxic protein build-up in cells via autophagosome-lysosome fusion, reducing levels of TDP-43 aggregates in ALS and DPRs in C9orf72-ALS [46].

This led researchers to investigate the effects of PIKFYVE inhibition in induced motor neurons (iMNs) from iPSCs, as well as in a range of animal models. Hung and colleagues used a range of PIKFYVE ASOs, as well as apilimod, a small molecule inhibitor of PIKFYVE, to demonstrate increased clearance of TDP-43 aggregates in these cells, regardless of genotype (sporadic ALS, *C9orf72, FUS, TARDBP)* [47]. This extended cell survival, and the team replicated these results in a range of animal models (*Drosophila melanogaster, Caenorhabditis elegans,* and mice). A recent Phase 1 study demonstrated safety and tolerability of VRG50635, a small molecule inhibitor of PIKFYVE similar to apilimod, in healthy volunteers (ISRCTN14792372) and is now being investigated in proof-of-concept Phase 1b trial (NCT06215755). Apilimod also demonstrated pharmacodynamic target engagement in a trial of *C9orf72*-ALS patients, though no difference was observed in survival and functional outcomes [48].

AS-202, a *PIKFYVE*-ASO, was first demonstrated to be tolerated and safe in mice and primates by AccuraStem in a conference poster presentation [49]. This is currently in further development prior to any clinical trials.

UNC13A

The UNC13A protein is involved in presynaptic coordination and normal synaptic functioning [50]. *UNC13A* pre-mRNA includes a cryptic exon, the inclusion of which is usually repressed by nuclear TDP-43 [51]. However, in ALS, mislocalisation of TDP-43, with depletion from the nucleus and the formation of aggregates has been demonstrated to increase cryptic exon inclusion in *UNC13A* pre-mRNA, which leads to nonsense-mediated decay and loss of the UNC13A protein, leading to impaired neurotransmission and reduced neuronal function [52]. Furthermore, single nucleotide polymorphisms (SNPs) have been shown to make *UNC13A* cryptic exon inclusion especially susceptible to TDP-43 depletion, in particular rs12608932 [53]. Patients with this SNP have a more rapid disease course [54]. This makes the regulation of *UNC13A* cryptic exon inclusion an attractive therapeutic target, modulating the downstream effects of TDP-43 mislocalisation in ALS [55].

Lithium carbonate is currently being investigated in a randomised, controlled trial in *UNC13A*-ALS (NCT06008249). This is after previous work demonstrated it to significantly increase 12-month survival in patients who were homozygous for the C-allele at SNP rs12608932[56]. However, a group has also recently demonstrated use of *UNC13A*-ASOs in human iPSC-derived neurons [57]. This demonstrated that use of these ASOs not only restored cryptic exon splicing and UNC13A protein levels, but also *UNC13A* function and synaptic signalling. Furthermore, AccuraStem has recently applied for funding to develop a Direct to Phase 2 *UNC13A*-ASO (NIH RePORTER ID: 1R44NS132698-01), with other companies such as QurAlis also announcing their intent to develop similar ASOs. No clinical trials have yet been established, but *UNC13A* remains a promising candidate for future ASO targeting.

SYF2

SYF2 is a pre-mRNA splicing factor that regulates splicing through recruitment to the spliceosome. *SYF2* was identified as a potential target in ALS by Linares and colleagues using iMN models from patients with ALS [58]. The team demonstrated that inhibiting *SYF2* using ASOs reversed TDP-43 pathology, increased nuclear TDP-43, and reduced cryptic exon inclusion in *STMN2* in iMNs from patients with *C9orf72*, *TARDBP* and sporadic ALS (no effect on *SOD1*, *FUS* or control lines). Treatment with the *SYF2*-ASO also increased cell survival in these models. Furthermore, in a TDP-43 mouse model, the team demonstrated reduced neurodegeneration, motor neuron dysfunction and neuromuscular junction loss using the *SYF2*-ASO [58].

No clinical trials are currently underway to investigate *SYF2*-ASOs, but this remains a promising future target for ASO therapy. AccuraStem has recently received funding to help develop a Direct to Phase 2 *SYF2*-ASO, under a similar programme to their *UNC13A* and *PIKFYVE*-ASOs (NIH RePORTER ID: 1R44AG085411-01).

Conclusions

Antisense oligonucleotides are a promising therapeutic method of targeting both gene-related and sporadic forms of ALS. While tofersen remains a promising therapy for *SOD1*-ALS, recent clinical trials in *C9orf72*-ALS have been less successful. This may be due to a lack of targeting of the antisense strand, and future work will be required to investigate this further. A Phase 1/2 trial targeting *ATXN2* was discontinued following an intermediate analysis, while clinical trials investigated ASOs against *STMN2* and *FUS* are ongoing. Future work is required to determine the reason for the failure of ASO therapy in *C9orf72*-ALS, especially since it accounts for the majority (40%) of fALS cases.

Preclinical work on other genes involved in mitigating the effects of TDP-43 pathology (*UNC13A, PIKFYVE, STMN2, SYF2*) has shown promise of targeting a disease mechanism found in the majority of ALS cases. This could form a cornerstone of management in sporadic as well as familial disease, giving hope for patients living with ALS.

Key points

- Antisense oligonucleotides are a powerful tool that could be used to treat both familial and sporadic forms of ALS.
- Tofersen has been approved for the treatment of *SOD1*-ALS by the FDA and EMA, with a decision by NICE awaited.
- Trials for ASOs against *C9orf72*-ALS have so far been unsuccessful, likely due to a mix of gain- and loss-of-function effects of pathogenic *C9orf72* hexanucleotide repeat expansions.
- Early-stage trials of ASOs against *STMN2* and *FUS* are ongoing, and ASOs against other genes in preclinical development.

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Figure 1: structures commonly used in developing antisense oligonucleotides (ASOs). Most ASOs in clinical trials for ALS use a phosphorothioate-based design, as this grants high stability and efficient cellular uptake

Figure 2: Different mechanisms of action of ASOs. Most ASOs in trials for ALS employ RNAse H-mediated degradation, including tofersen; QRL-201 an ASO currently in Phase 1 trials, employs cryptic exon splicing modulation.