From failure to meet the clinical endpoint to US Food and Drug Administration approval: 15th antisense oligonucleotide therapy approved Qalsody (tofersen) for treatment of SOD1 mutated amyotrophic lateral sclerosis

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From Failure to Meet the Clinical Endpoint to U.S. Food and Drug Administration Approval: 15th Antisense Oligonucleotide Therapy Approved Qalsody (Tofersen) for Treatment of *SOD1* Mutated Amyotrophic Lateral Sclerosis

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On April 25 2023, the U.S. Food and Drug Administration (FDA) granted accelerated approval to tofersen (trade name Qalsody) for the treatment of Superoxide Dismutase 1 (SOD1) associated amyotrophic lateral sclerosis (ALS) [1]. The clinical trial failed to meet its primary endpoint; however, approval was granted based on a biomarker response: reduced plasma levels of neurofilament light protein [2]. Although confirmatory studies to assess the impact on disease progression must still be performed, results from the open-label study suggest that tofersen may slow down disease progression.

Tofersen is the first intrathecally delivered RNase H antisense oligonucleotide (ASO) to receive FDA approval and the second approved intrathecal ASO. Nusinersen, an intrathecally delivered splice modulating ASO for the treatment of spinal muscular atrophy, received FDA approval in 2016 [3]. Tofersen is the fifth ASO to be approved based on biomarker results, joining eteplirsen, golodirsen, viltolarsen, and casimersen, all approved for the treatment of Duchenne muscular dystrophy in patients with eligible mutations [4]. With the approval of tofersen, the total number of ASO therapies approved by FDA and/or the European Medicines Agency comes to 15 (Table 1).

Amyotrophic Lateral Sclerosis

ALS is a heterogeneous progressive neurodegenerative disease characterized by the loss of upper and lower motor neurons, resulting in the loss of muscle function [5]. The disease generally has a focal onset, but rapidly spreads to other body regions [6]. Although age of onset and rate of disease progression varies among patients, the symptoms often start between 40 and 60 years, with a survival of 3–5 years after symptom onset.

ALS can occur sporadically or due to hereditary genetic mutations. Currently, there are >30 different genes linked to hereditary forms of ALS [7]. Mutations in the *SOD1* gene account for <2% of all ALS cases and >180 different *SOD1* mutations have been reported. Different *SOD1* mutations are associated with different ages of onset and rates of progression; however, nearly all have an autosomal dominant inheritance pattern [8]. The *SOD1* gene encodes the superoxide dismutase 1 enzyme, and disease pathology is thought to be due to a toxic gain of function rather than a loss of enzymatic function.

As such, reducing concentrations of the mutant protein is anticipated to slow down progression of SOD1-linked ALS, making it an excellent candidate for treatment with RNA lowering oligonucleotide therapeutics, including RNase H inducing ASOs. SOD1 targeting ASOs will induce RNase H mediated cleavage of SOD1 transcripts, thus reducing the amount of toxic superoxide dismutase protein produced. In animal models for SOD1-linked ALS, ASO treatment resulted in reduced SOD-1 transcript, reduced superoxide dismutase 1 protein levels, and functional improvements [9].

Tofersen Development

Tofersen is an RNase H inducing ASO that targets SOD1 transcripts. The ASO contains a phosphorothioate backbone, where the 10 central nucleotides (gap) are not further modified, whereas the 5 nucleotides on each end consist of methoxy-ethyl (MOE) RNA. The RNase H will cleave the transcript in the region targeted by the gap, whereas the MOE flanks promote target binding and increase the stability of the ASO. Tofersen is not allele specific and will reduce the transcripts of both normal and mutated SOD1 alleles.

The clinical development of tofersen was coordinated by Ionis Pharmaceuticals, with support from Biogen. Although ASOs do not cross the blood–brain barrier, since the development of nusinersen, it has become clear that upon intrathecal

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Table 1. Approved Antisense Oligonucleotide Treatments

Drug name	*Mechanism*	*Disease*	*Year of FDA (and EMA) approval*
Fomivirsen	Translation block	Cytomegalovirus induced retinitis	1998 (1999)
Mipomirsen	RNase H	Familial hypercholesterolemia	2013 (no EMA approval)
Inotersen	RNase H	Hereditary transthyretin mediated amyloidosis	2018 (2018)
Valonesorsen	RNase H	Familial chylomicronemia syndrome	No FDA approval (2019)
Tofersen	RNase H	SOD1-linked amyotrophic lateral sclerosis	2023 (no EMA approval)
Nusinersen	Splice modulation	Spinal muscular atrophy	2016 (2017)
Eteplirsen	Splice modulation	Duchenne muscular dystrophy (exon 51 skipping)	2016 (no EMA approval)
Golodirsen	Splice modulation	Duchenne muscular dystrophy (exon 53 skipping)	2019 (no EMA approval)
Viltolarsen	Splice modulation	Duchenne muscular dystrophy (Exon 53 skipping)	2020 (no EMA approval)
Casimersen	Splice modulation	Duchenne muscular dystrophy (Exon 45 skipping)	2021 (no EMA approval)
Patisiran	siRNA	Hereditary transthyretin mediated amyloidosis	2018 (2018)
Givosiran	siRNA	Acute hepatic porphyria	2019 (2020)
Lumasiran	siRNA	Primary hyperoxaluria type 1	2020 (2020
Inclisiran	siRNA	Hypercholesterolemia	2021 (2020)
Vutrisiran	siRNA	Hereditary transthyretin mediated amyloidosis	2022 (2022)

EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; siRNA, small interfering RNA.

delivery, cells in the central nervous system take up ASOs efficiently [10]. Studies in primates treated with nusinersen and posthumous analysis of nusinersen-treated spinal muscular atrophy (SMA) patients revealed that ASOs with a phosphorothioate backbone distribute throughout the brain and the spinal cord [11,12]. Importantly, the clinical studies and subsequent use of nusinersen for SMA patients showed repeated intrathecal delivery of ASOs to be generally well tolerated. In fact, with the expeditious approval of nusinersen, only 91 days after filing for new drug application with the FDA [13], hopes were high that with the favorable ASO distribution and uptake characteristics after intrathecal delivery, more ASOs for neurological and neuromuscular disorders would follow suit.

However, for tofersen, the sailing was less smooth, and the trial had trouble recruiting given the rarity of SOD1-related ALS. The compound was tested in a three-part clinical trial, starting with a single ascending dose study. The first randomized placebo-controlled phase 1 trial was published in 2013 [14], before the clinical development of nusinersen started, and included four cohorts of eight patients. The ASO was administered intrathecally using an external pump, delivering 0.15, 0.50, 1.50, or 3.00 mg of the ASO (then called ISIS 333611) over 11.5 h to six patients, whereas two patients per cohort received placebo.

In all subsequent studies, intrathecal bolus injections were used. It was the first clinical study showing that intrathecally administered ASOs delivered to the central nervous system could be a promising treatment for neurological disorders. The second part was a multiple ascending study, followed by the third, a placebo-controlled efficacy study. Results of the multiple ascending dose and efficacy studies were published in 2020 [15] and 2022 [2], respectively.

In the ascending dose study, 48 SOD-1 related ALS patients received five intrathecal injections of 20, 40, 60, or 100 mg of tofersen or placebo over a 12-week period. In the treatment group, there was a dose-dependent decrease in SOD1 protein levels in the cerebrospinal fluid (CSF). Lumbar puncture-related adverse events were reported by most patients, a subset experienced elevation in white cell counts in the CSF, and three deaths occurred during the trial, although the deaths were disease related rather than tofersen-treatment related.

For exploratory functional outcomes, the 10 patients receiving the 100 mg tofersen dose were compared with the 10 patients who received placebo. Tofersen treated patients had a slower decline in the total ALS functional rating scale-revised (ALSFRS-R), which is a composite outcome measure for residual function in ALS patients. Furthermore, treated patients showed less reduction of percentage predicted forced vital capacity and reduced plasma levels of neurofilament light, a marker for neuronal damage.

After these initial results, the field was cautiously optimistic and anticipating the results of the efficacy trial. The study included 108 SOD1-related ALS patients, of which 72 received 100 mg tofersen and 36 received placebo over a period of 24 weeks. Dosing was done every 2 weeks for 6 weeks and then once every 4 weeks.

Again, the treated group showed a reduction of SOD1 levels in the CSF and plasma neurofilament light levels compared with baseline, whereas both were unchanged in the placebo group. The tofersen treated group showed reduced decline in percentage predicted forced vital capacity compared with the placebo group (14.3 vs. 22.2). However, the primary outcome (reduced decline of ALSFRS-R scores) was not met after 28 weeks of treatment. As progression reduction is best assessed in patients with rapidly progressing disease, a subgroup analysis was done for these individuals.

The subgroup analysis showed that tofersen-treated patients lost 6.98 points compared with baseline, whereas placebo-treated patients lost 8.14 points; however, the difference was not statistically significant. Although efforts were made to randomize patients with rapidly progressing disease over the treatment and placebo groups, authors noticed that the neurofilament light levels were higher at baseline in the tofersen group and that the initial decline was quicker. As such, it is likely that more patients with severe disease were included in the treatment group, possibly affecting the results.

After the study ended at week 28, patients entered an open-label extension study, and all patients received 100 mg tofersen. Analysis after 52 weeks revealed a loss of 6 ALSFRS-R points compared with baseline for the patients who were on treatment the entire time, compared with a loss of 9.5 for those where treatment was initiated after 28 weeks.

Furthermore, the difference between percentage predicted forced vital capacity persisted between the tofersen and delayed-treatment groups (9.4 vs. 18.6). Finally, although no difference was seen between treated and placebo groups for handheld myometry after 28 weeks, at week 52 the delayed treatment group performed worse than those receiving tofersen for 52 weeks. These results highlight the importance of early treatment since lost functionality cannot be recovered.

In hindsight, it is easy to point out that the trial design was not optimal and that 28 weeks was too short of a timeframe to measure impact in more slowly progressing disease. However, as with many rare diseases, complete understanding of the primary endpoint's natural history and variability did not exist, making it challenging to design an optimal trial. Fortunately, the trial included an open-label extension study that showed a clearer pattern for drug efficacy. Importantly, a very clear reduction in CSF SOD1 and plasma neurofilament light levels was observed in both treatment and delayed treatment groups, confirming target engagement.

Ionis filed for accelerated approval and the FDA organized an advisory committee meeting on March 22, 2023. During this meeting Biogen posed that the reduction of plasma neurofilament light levels suggested a treatment effect, and based on the open-label analysis at 52 weeks, was expected to result in later functional benefits. Furthermore, clinical experts and patient advocates stressed both the unmet medical need of ALS and the perceived benefits of longer treatment. The advisory committee members voted 9–0 in favor of accelerated approval of tofersen based on the provided data. As anticipated after this unanimous advice, the FDA formally granted accelerated approval a month later based on the reduced plasma neurofilament light levels. Confirmatory studies to assess the functional efficacy of tofersen were requested as a condition of the accelerated approval.

As mentioned, this is not the first ASO that received approval based on biomarker data. Approval of splice modulating ASOs for the treatment of Duchenne muscular dystrophy were based on restoration of dystrophin, the protein missing in Duchenne patients. These approvals were controversial, especially for the first ASO, eteplirsen, which was based on a 0.4% dystrophin increase in treated patients [16]. There appears to be much less controversy for tofersen, which is unsurprising given the stronger biomarker effect, the higher number of patients in the trial, and the convincing functional effects in the open-label stage. Furthermore, delivery of ASOs to the central nervous system is more efficient than to skeletal muscle, shown by the nusinersen data. Clearly, for patients with spinal muscular atrophy and SOD1-related ALS, earlier treatment results in larger therapeutic effects [2,17], consistent with the progressive and irreversible loss of motor neurons in both diseases.

Toward the Future

Achieving proof of concept for intrathecal delivery of RNase H inducing ASOs provides hope for patients with neurological diseases caused by toxic gain of function mutations. Indeed, a phase 3 clinical trial is currently coordinated by Ionis for Jacifusen, an RNase H inducing ASO that targets fused in sarcoma (FUS) gene transcripts (https://clinicaltrials.gov/ct2/show/NCT04768972?term=NCT04768972&draw=2&rank=1). FUS mutations underlie <1% of sporadic ALS and <5% of familial cases and confer extremely early disease onset and rapid disease progression. Preclinical studies showed that ASOs reduced FUS transcripts and protein levels and delayed motor neurodegeneration in a FUS-ALS mouse model [18].

Although ION363 was put on hold due to challenging clinical development because of the rarity of FUS-ALS, following the positive data, a patient with severe FUS-ALS, Jaci, received Jacifusen (then called ION363). Ionis provided the ASO to Dr. Neil Shneider to treat Jaci under a compassionate use program. Jaci was treated 6 months after onset of symptoms at 25 years of age. Before treatment she lost $\sim$5 points per month on the ALSFRS-R scale, consistent with the severely progressive nature of FUS-ALS. Although the rate of decline slowed after treatment initiation, Jaci sadly passed away 18 months after the onset of symptoms. Analysis of autopsy material revealed reduced FUS messenger RNA and protein in the spinal cord and the brain. After these results, Dr. Shneider treated additional FUS-ALS patients under a compassionate use program, which showed clear changes in disease trajectory. Based on these data, Ionis initiated a phase 3 clinical trial, naming the compound Jacifusen in honor of Jaci.

For Huntington disease the development of tominersen, an RNase H inducing ASO targeting huntingtin transcripts, was initially stopped when it became apparent that disease progressed faster in treated patients than in the placebo group [19,20]. *Post hoc* analysis revealed that younger patients with lower disease burden appeared to respond to treatment [21] and a new clinical trial is currently underway to assess efficacy in this subset of Huntington disease patients (https://clinicaltrials.gov/ct2/show/NCT05686551?term=tominersen&draw=2&rank=1).

A clear theme exists in ASO development for rare progressive brain diseases with unmet medical need. Often there is limited natural history and little clinical trial experience. Although commenting on suboptimal trial design is easy in hindsight, the only way toward disease-modifying therapeutics is to learn from these ''mistakes in hindsight'' and to more optimally design future trials with the newly gained insights [21]. The development and approval of tofersen provides rich scientific data and lessons upon which the community can build in its mission to develop disease-modifying therapeutics for patients and families with huge unmet clinical needs.

Author Disclosure Statement

A.A.R. discloses being employed by Leiden University Medical Center (LUMC), which has patents on exon skipping technology, some of which has been licensed to BioMarin and subsequently sublicensed to Sarepta. As coinventor of some of these patents, A.A.R. is entitled to a share of royalties. A.A.R. further discloses being *ad hoc* consultant for PTC Therapeutics, Sarepta Therapeutics, Regenxbio, Alpha Anomeric, Lilly BioMarin Pharmaceuticals Inc., Eisai, Entrada, Takeda, Spliceseense, Galapagos and Astra Zeneca. Past *ad hoc* consulting has occurred for CRISPR Therapeutics, Summit PLC, Audentes Santhera, Bridge Bio, Global Guidepoint and GLG consultancy, Grunenthal, Wave, and BioClinica. A.A.R. also reports having been a member of the Duchenne Network Steering Committee (BioMarin) and being a member of the scientific advisory boards of Eisai, Hybridize Therapeutics, Silence Therapeutics, and Sarepta Therapeutics. Past Scientific advisory board memberships: ProQR and Philae Pharmaceuticals. Remuneration for these activities is paid to LUMC. LUMC also received speaker honoraria from PTC Therapeutics, Alnylam

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W.v.R.M. discloses being employed by LUMC, which has patents on exon skipping approaches for neurological disorders. In the past, some of these patents have been licensed to ProQR therapeutics. As coinventor on these patents W.v.R.M. is entitled to a share of milestone payments. W.v.R.M. further discloses being *ad hoc* consultant for Accure Therapeutics and Herbert Smith Freehills. Remuneration for these activities is paid to the LUMC. LUMC also received funding for contract research from UniQure and Amylon Therapeutics.

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