A multinational, randomized, double-blind, placebo-controlled Phase 2 study to assess safety and efficacy of olesoxime (TRO19622/RG6083) in patients with Type 2 or non-ambulatory Type 3 spinal muscular atrophy

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Summary

Background

Spinal muscular atrophy (SMA) is a progressive motor neuron disease causing loss of motor function and reduced life expectancy. No approved treatments exist. We investigated the efficacy, safety and tolerability of olesoxime in patients with Type 2 or non-ambulatory Type 3 SMA.

Methods

This randomized, double-blind, placebo-controlled, Phase 2 study (NCT01302600) was performed in 22 neuromuscular care centres in Belgium, France, Germany, Italy, Netherlands, Poland and the UK. Safety and efficacy of olesoxime (TRO19622/RG6083) were assessed in patients aged 3–25 years with genetically confirmed Type 2 or non-ambulatory Type 3 SMA. A centralized, computerized randomization process allocated patients to receive olesoxime 10 mg/kg/day oral liquid suspension or placebo (2:1 ratio) for 24 months. Patients, study personnel and sponsor study personnel were masked to treatment assignment. The primary outcome measure was change from baseline in functional domains 1 and 2 of the Motor Function Measure (MFM D1 + D2) assessed in the full analysis population. Safety was assessed in the intention-to-treat population.

Findings

Of 198 patients recruited between November 2010 and September 2011, 165 were randomized to olesoxime (n=108) or placebo (n=57). The change from baseline to Month 24 on the primary outcome measure was 0.18 for olesoxime and –1.82 for placebo (treatment difference 2.00 points; 96% confidence interval [CI]: –0.25, 4.25; *P*=0.0676). The overall effect of olesoxime on this endpoint across all visits was 2.23 (95% CI: 0.58, 3.88; *P*=0.0084). Further *post hoc* analysis of the primary endpoint in three age groups (<6, 6–15 and >15 years) revealed a consistent significant benefit of olesoxime treatment over placebo in children aged 6–15 years throughout the study period (*P*=0.0107). In addition, patients achieving higher exposure to olesoxime (≥7500 ng/mL) demonstrated improvements in MFM D1 + D2 score compared with baseline at all visits, which were not observed in the lower exposure group or with placebo. Olesoxime appeared safe and generally well tolerated, with an adverse event profile similar to placebo.

Interpretation

Olesoxime was generally safe, well tolerated and demonstrated signs of maintaining motor function in patients with Type 2 and 3 SMA.

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Research in context

Evidence before this study

Approved treatment for spinal muscular atrophy (SMA) is limited to nusinersen, an intrathecal-administered, antisense oligonucleotide intended to restore deficient SMN protein in motor neurons. However, there remains unmet medical need in SMA for additional , potentially complementary therapies that may delay disease progression and/or augment benefit of other therapies; substantial efforts have been invested in identifying the key mechanisms of disease and testing potential compounds. There is growing evidence that mitochondrial dysfunction represents a key mechanism of disease and a valid therapeutic target in neurological diseases, including SMA. *In vitro* studies demonstrated that olesoxime localizes at the mitochondrial membrane, where it increases functional integrity of mitochondria in cortical neurons and protects against apoptosis by preventing release of pro-apoptotic cytochrome C from mitochondria. At the time of study design, only one prior large multicentre efficacy study had been performed in SMA, assessing riluzole in patients with Type 1 SMA, for which results had not been published. Nevertheless, the design of this prior study provided context for the olesoxime trial design, which was developed with the input of experts in the field.

Added value of this study

This study is the largest and longest multinational study performed so far in patients with SMA, and provides the opportunity to assess the efficacy and safety of olesoxime, a promising therapeutic compound, in a broad population of patients with Type 2 and Type 3 SMA. Although the study did not meet the primary endpoint, the results revealed indications of efficacy in terms of maintenance of motor function over 2 years. Recent evidence suggests that maintenance of motor function is a key aspiration for patients with SMA as it allows for preservation of current abilities with regard to activities of daily living. The study also allowed, for the first time, the prospective assessment of motor function using both the Motor Function Measure and Hammersmith Functional Motor Scale, providing important novel data for these measures in a large controlled study setting over a 24-month period.

Implications of all available evidence

The results of this study support the continued development of olesoxime as a therapy for SMA, a progressive, debilitating, disease for which only one approved therapy exists. The clinical development of olesoxime will continue with an open-label extension study (clinicaltrials.gov NCT02628743) and a Phase 3 trial is currently in the planning stages. Given the paucity of approved treatments for patients with SMA, the results of this study provide both invaluable information to investigators for future trial study design and encouraging evidence on the role that olesoxime could play as a novel therapeutic agent for SMA.

Introduction

Spinal muscular atrophy (SMA) is a rare and severely debilitating neuromuscular disease that manifests predominantly in infancy and childhood.[1,2] The clinical phenotype is broadly classified into four types (1-4) according to highest achieved motor function and age at onset of symptoms.[3-5] In the majority of Type 1 SMA cases, the disease leads to severe paralysis and death in the first few years of life.[1,6] Patients affected by the more chronic forms of SMA (Types 2 and 3) have variable degrees of disability, and those at the more severe end of the spectrum may develop respiratory failure that, unless appropriately managed, can lead to premature death.[1,6] The progressive nature of the disease frequently leads to loss of ambulation in initially ambulatory Type 3 patients.[5,7] In Type 2 and Type 3 SMA, the deterioration of motor function, measurable by validated neuromuscular assessments including the Motor Function Measure (MFM) and Hammersmith Functional Motor Scale (HFMS), results in significant disability and impact on patients and their caregivers.[8].

SMA is an autosomal recessive disease caused by loss-of-function mutations in the Survival of Motor Neuron 1 (*SMN1*) gene. The absence of the *SMN1* gene results in insufficient levels of SMN protein in cells; this particularly impacts on motor neurons and neuromuscular junctions (NMJs), leading to muscle weakness, hypotonia and atrophy.[1,9] The variable phenotypes of SMA are associated with the disease-modifying *SMN2* gene, where *SMN2* copy number varies between individuals and SMA types. *SMN2* is always retained in SMA patients, but is only capable of producing residual amounts of functional SMN protein.[5,10]

Although reduced SMN protein levels is the triggering event in all SMA types, the downstream pathological disturbances of atrophy and denervation are related to mitochondrial dysfunction, which affects numerous different cell types throughout the body.[11-14] Given their influence on energy production, mitochondria are vital for cells with a high energy demand, including motor neurons and muscle fibres that are central to the pathophysiology of SMA.[11,13,15,16] Reduced mitochondrial respiration, decreased mitochondrial adenosine triphosphate (ATP) synthesis, decreased mitochondrial membrane potential, and increased oxidative stress level were reported in motor neurons of a mouse model of SMA.[14] In addition, in a study of skeletal muscle biopsies from patients with SMA Types 1, 2 or 3, muscle tissue showed impaired mitochondrial biogenesis, causing decreased mitochondrial DNA and depressed respiratory chain activities.[13]

The majority of current therapeutic strategies in clinical development have aimed to increase SMN production systemically either by replacing SMN1 (e.g. gene therapy with AVXS-101) or by SMN2 splicing modulators (e.g. RO6885247, RO7034067 and LMI070). Recently, a SMN2 splicing modulator for intrathecal administration (nusinersen), targeting motor neuron survival, has been approved by FDA for treatment of SMA .[17] However, there are concerns that therapies which augment SMN levels may not benefit all patients based on evidence from mouse models suggesting a prime role of SMN in neuromuscular system development.[18] Consequently, there may be an important role for systemic, non-SMN therapies that target alternative mechanisms, possibly in a complementary manner, which might maintain motor units, muscle cells and other affected cell types particularly in the slower degenerative phase of the disease.

Olesoxime (TRO19622/RG6083) exerts cytoprotective properties via interactions with the mitochondrial membrane, and was identified through its survival-promoting activity on trophic-factor-deprived primary motor neuron cultures.[19] Olesoxime specifically localizes at the mitochondria across cell types, and has been shown to prevent excessive permeability of the mitochondrial membrane under stress conditions,[19-21] supporting continued function of the cell, preventing apoptosis by reducing the release of pro-apoptotic factors and maintaining energy production.[19-21] Olesoxime demonstrated neuroprotective and neuroregenerative effects in several animal models of motor nerve degeneration, reducing pro-apoptotic factor release from neuronal mitochondria.[19,22] In a transgenic mouse model of severe SMA (SMNF7/F7; NSE-Cre mice), daily olesoxime administration extended survival compared with vehicle-treated mice.[19] Taken together, these data suggest that olesoxime may have a role in maintaining motor neuron function, and has the potential to be a therapeutic agent in SMA.[19,22]

Following a Phase 1 study in SMA that assessed preliminary safety, tolerability and pharmacokinetics (PK),[23] accompanied by excellent safety data in a Phase 2/3 trial in amyotrophic lateral sclerosis,[24] this Phase 2 study was performed with the objectives being to assess the efficacy, safety and tolerability of olesoxime in patients with Type 2 or non-ambulatory Type 3 SMA. We hypothesized that the mitochondrial function improvements associated with olesoxime treatment would at least prevent worsening of, and potentially improve, motor function over a period of 2 years, while decline of motor function in the placebo group would follow the natural history of the disease.

Methods

Study design

This randomized, double-blind, placebo-controlled, multi-national Phase 2 study assessed the efficacy, safety and tolerability of olesoxime over 24 months in patients aged 3–25 years with Type 2 or non-ambulatory Type 3 SMA. The main outcomes were change from baseline on measures of motor function. Patients were recruited from 22 study sites in 7 countries (Supplementary Table 1). All sites were care centres with expertise in treating SMA patients in line with the published standards of care for SMA.[4] The study was approved by local institutional review boards and ethics committees. Analyses of the primary and secondary endpoints of this study have been previously published in abstract form.[25] This study is registered with ClinicalTrials.gov (NCT01302600).

Participants

Patients aged 3–25 years old with Type 2 or non-ambulatory Type 3 SMA were enrolled according to the following key inclusion criteria: weakness and hypotonia consistent with a clinical diagnosis of SMA Type 2 or 3; genetic diagnosis of SMA with homozygous deletion of *SMN1* exon 7, or a heterozygous deletion accompanied by a point mutation on the other allele; Motor Function Measure (MFM) relative score (percentage of the maximum sum of both dimensions) ≥15% (functional domain 1 [D1] + functional domain 2 [D2] score); Hammersmith Functional Motor Scale (HFMS) score at baseline ≥3 and ≤38 (non-ambulatory); onset of symptoms at ≤3 years of age; and ability to take the study treatment (tested at screening after informed consent). Key exclusion criteria are provided in Supplementary Methods. All

patients and/or their parent/guardian provided written informed consent before screening. Patients were recruited mainly via information disseminated through the TREAT-NMD website, patient registries and in the clinics at each site.

Randomization and masking

Patients were randomized in a 2:1 ratio to receive olesoxime or placebo, with stratification by SMA type and centre. All investigators, site personnel, patients and the sponsor study personnel were masked to treatment assignment, and treatment allocations were masked until completion of the study.

Randomization lists were generated centrally by an independent statistician (Business & Decision Life Sciences, Montrouge, France) using validated randomization software (SAS® version 9.2, SAS Institute Inc., Cary, NC, USA). To maintain masking, active and placebo treatments were supplied in brown glass bottles, and randomization details were provided using secure procedures to the clinical research organization performing the packaging of the treatment units and to the laboratory performing the olesoxime PK bioanalysis assay.

Procedures

Patients received oral olesoxime 100 mg/mL liquid suspension formulation (manufactured by Minakem, Beuvry-la-Forêt, France, packaged by CRID PHARMA, Saint-Gély-du-Fesc, France) at a weight-based dose of 10 mg/kg once a day, or matching placebo, with the main daily meal for 24 months. Following screening and baseline visits, follow-up visits were scheduled at Week 4 and Week 13, after which participants were assessed every 13 weeks for a total of nine visits over the 24-month treatment period. The full schedule of assessments is provided in Supplementary Table 2. An interim efficacy analysis was performed by an independent statistician when all patients had been treated for 12 months, to assess the need to continue the study to reach the planned objective. In the event of positive and significant results in favour of olesoxime, the study was to be considered successful and all patients were to be switched to olesoxime to allow assessment of the sustainability of the treatment effect and safety. If the results were significantly in favour of placebo, the study was to be discontinued for failure (futility). The interim efficacy analysis was reviewed by an independent Data Monitoring Committee (iDMC). The final efficacy and safety analysis was performed at 24 months.

Outcomes

The primary outcome measure was the change from baseline at Month 24 in D1 + D2 of the 32-item MFM (MFM32), a valid and reliable scale designed to capture functional abilities of individuals with neuromuscular disorders.[26] A shorter 20-item version (MFM20) specifically adapted for younger children[27] was used to assess children aged <6 years. The MFM assesses standing, ambulation and transfers, and axial, proximal and distal function. The MFM is used in multiple centres globally to monitor the functional ability of individuals with Types 2 or 3 SMA. Longitudinal analyses have identified an MFM total score decline of approximately 0.9 points per year in Type 2 patients, and 0.6 points per year in Type 3 patients.[7] Further information on the MFM is provided in Supplementary Methods.

In 17 patients (olesoxime n=12, placebo n=5) who were <6 years old at enrolment, MFM32 was used at all visits instead of the protocol-defined MFM20. To account for this, two separate analyses of MFM score were performed. In the primary analysis, a score for the MFM20 was calculated from the MFM32 score for these 17 patients by using only the 20 items that are featured in MFM20. A secondary sensitivity analysis instead included data from whichever form of the MFM was used.

Secondary outcomes included analyses of the change from baseline to Month 24 in total MFM score and individual MFM domains (D1, D2 and D3). In addition, the proportion of patients demonstrating maintenance or improvement in scores on MFM D1 + D2 and MFM total score (D1 + D2 + D3) was assessed; this is referred to as a responder analysis. Sensitivity analyses of the primary endpoint, which were pre-specified in the SAP following the interim analysis, included subgroup analyses of MFM D1 + D2 score, to assess the effect of age, country and SMA type. In a further *post hoc* sensitivity analysis, an assessment of the effect of olesoxime exposure on the primary outcome measure was performed (described further in the Supplementary Methods). The change from baseline to Month 21 in HFMS,[6] developed for use in Type 2 and non-ambulatory Type 3 SMA patients, was also included as a secondary motor function outcome. A responder analysis for HFMS scores was also performed.

Non-motor function secondary endpoints included maximum compound muscle action potential (CMAP) and motor unit number estimation (MUNE), clinical global impression of change (CGI-C) assessed by patient/caregiver and physician, forced vital capacity (FVC), Pediatric Quality of Life Inventory (PEDsQL™) Neuromuscular Module,[28] and safety. Safety assessments included adverse events (AEs), standard laboratory assessments, electrocardiograms and vital signs.

Statistical analysis

To test the hypothesis that olesoxime treatment would at least prevent worsening of motor function over a period of 2 years, it was estimated based on natural history studies available at the time of study initiation[7] that a mean decrease of 1.9 points in the MFM D1 + D2 score would be observed over 24 months in the placebo arm, with no worsening in the olesoxime arm, with an assumed standard deviation of 3.32. It was calculated that 150 patients (100 olesoxime patients and 50 placebo patients) would be necessary to reach a power of at least 85% at the final analysis (alpha = 0.04 to take into account the interim efficacy analysis conducted after 12 months [alpha=0.01]), assuming 5% of patients would be lost to follow-up.

All efficacy analyses (including exposure–efficacy exploration) were based on the Full Analysis Set (FAS), which includes all randomized patients who received at least one dose of olesoxime or placebo and who had at least one post-randomization assessment of MFM available. All safety analyses are based on the safety evaluable population (all randomized patients who received at least one dose of the study drug).

As per the pre-determined statistical analysis plan, change from baseline to Month 24 in MFM D1 + D2 was analysed using a mixed-effects repeated measures (MMRM) model. Covariates in the primary model were: MFM score (D1 + D2) at baseline, SMA type, country, treatment group, visit and treatment group by visit interaction. Further detail on the model is provided in Supplementary Methods. Least square means, standard errors and the 96% confidence intervals (CIs) of treatment difference between

olesoxime and placebo were reported. Statistical tests for the primary analysis were performed using a two-sided test with a significance level (α) of 4%.

CGI-C ratings were analysed using a Van Elteren test, a non-parametric test which compares the ranks of responses, stratified by country. A *post hoc* responder analysis of CGI-C was also conducted, mirroring the responder analyses for the MFM and HFMS. Patients rated as 'No change or better ' (i.e., stability or improvement) were considered to be 'responders', and patients rated as 'Minimally worse or worse' (i.e., decline) were considered to be 'non-responders'. The proportion of responders and non-responders were then compared across treatment arms using a log binomial model, controlling for SMA type. Other secondary endpoints were tested using two-sided tests with a significance level (α) of 5%. Further information on statistical analysis of secondary endpoints is provided in the Supplementary Methods. For the *post hoc* analysis of the primary outcome measure according to olesoxime exposure, the time course of the change from baseline (mean ± SEM) of the MFM D1 + D2 score was graphically compared between patients with low and high olesoxime exposure and the placebo group. Further information on this exposure–efficacy analysis is provided in Supplementary Methods.

All analyses were performed using SAS software (Version 9.2, SAS Institute Inc., Cary, NC, USA). The iDMC was responsible for monitoring the safety of patients by review every 13 weeks. The iDMC also reviewed the olesoxime plasma trough concentrations at Weeks 4 and 13, and reviewed the efficacy data at Week 52 to make a recommendation on study continuation.

AEs were reported at each patient visit and traced in source documents, which were then monitored, with all AEs reported in the eCRF by the investigation sites. In addition, a *post hoc* analysis was performed on a series of AE clusters defined as SMA-related complications. Further detail is provided in Supplementary Methods.

Role of the funding source

This study was funded by the French national patient advocacy group AFM-Téléthon, who also contributed to the trial design. JLA was an employee of Trophos SA and responsible for protocol development and study supervision until enrolment was completed. ED was an employee of Trophos SA, who also funded this study, and participated in the study management, data collection, data management and data analysis. Five authors (TB, PF, CR, PSD and EV) are employed by F. Hoffman-La Roche and performed primary, sensitivity and exploratory analyses of this study. The funders of the study had no other role in data interpretation or in the decision to submit the manuscript for publication. Roche also supported reporting of study results by procuring medical writing support. All authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

A total of 165 patients were enrolled and randomized to treatment between 18 November 2010, and 6 September 2011. Of these, 108 patients received olesoxime and 57 patients received placebo (Figure 1). The final patient observation occurred in October 2013. Seventeen patients withdrew prematurely, 10

from the olesoxime group (12.3%) and 7 from the placebo group (9.3%). Of 108 patients allocated to olesoxime, five patients were excluded from the FAS due to the absence of post-baseline assessments. Protocol violations occurred in 30 patients (olesoxime n=19 [18%], placebo n=11 [19%]), including the 17 patients aged <6 years old at enrolment who performed the MFM32 at all visits instead of the protocol-defined MFM20. Other major protocol violations (olesoxime n=7; placebo n=6) included deviation from the inclusion screening criteria (use of forbidden medication, spinal rod or fixation for scoliosis within six months of enrolment), abnormal liver enzymes (ALT or AST >3 x upper limits of normal), treatment compliance ≤50% for at least two visits and delayed visit dates. The FAS comprised 160 patients (olesoxime: n=103; placebo: n=57) (Table 1). All efficacy analyses reported here were based on the FAS.

Patient demographics and baseline characteristics were mainly well balanced between the treatment groups, including proportions of SMA Type 2 and Type 3 patients. However, both mean and median ages were lower in the olesoxime group than in the placebo group, with a difference of 2.1 years in mean ages and a difference of 4 years in median ages across treatment groups (Table 1). In addition, there were slight differences in the proportion of males and females between groups (Table 1). The interim efficacy analysis performed at Month 12 found that treatment effects were in favour of olesoxime but were not statistically significant. Therefore, the study continued with the full 2-year treatment period.

In line with the underlying hypothesis, olesoxime treatment was associated with maintenance of motor function, with a mean change of +0.18 points on the primary outcome variable, change from baseline in MFM D1 + D2 score at Month 24, whereas the placebo group demonstrated a decline in MFM D1 + D2 score (mean change from baseline: –1.82 points). This difference did not achieve statistical significance (2.00 points; 96% CI: –0.25, 4.25; *P*=0.0676, Table 2). The overall treatment difference across all visits was 2.23 points, which was statistically significant in favour of olesoxime (*P*=0.0084, Figure 2). In the key pre-specified sensitivity analysis, taking into account erroneous use of the MFM32 version in some patients younger than 6 years, the difference between the two treatment arms was statistically significant in favour of olesoxime (difference between treatments in change from baseline: 2.20 [95% CI: 0.12, 4.27]; *P*=0.0379). To investigate the effect of differences in development and natural history of disease according to age, we analysed the change from baseline in MFM D1 + D2 score separately in three different age groups: <6 years, 6–15 years and >15 years (Figure 3). In the 6–15-year age group, clear separation was noted between olesoxime and placebo patients over the course of the study, with olesoxime patients showing improvements in scores compared with baseline at all time points and placebo patients showing a consistent decline (>3-point difference between treatments at all visits; overall mean *P*=0.0107). In that *post hoc* analysis, the difference between olesoxime-treated and placebo-treated patients at Month 24 was 3.61 points (*P*=0.036, Figure 3). In the other age groups, no significant differences were observed between the treatment groups.

Results on secondary MFM endpoints are shown in Table 3. For MFM total score (D1 + D2 + D3), the difference between treatment arms did not achieve statistical significance (treatment difference: 2.04; 95% CI: –0.21, 4.28; *P*=0.0755). Analysis of the individual domains of the MFM revealed that the olesoxime group remained relatively stable in D1 and D2, while the placebo group showed decline, and in domain 3 the olesoxime group appeared to improve while the placebo group was stable. However, these differences did not reach statistical significance (Table 3). The proportion of patients who

improved or remained stable over 24 months (response rate) was higher in the olesoxime arm compared with the placebo arm for both the primary outcome measure MFM D1 + D2 (olesoxime 54.4%, placebo 38.6%; *P*=0.0609) and the MFM total score (olesoxime 56.3%, placebo 38.6%; *P*=0.0419) (Table 4). Subgroup analyses of the MFM D1 + D2 score revealed effects of olesoxime across country (data not shown), SMA type, gender, and disease severity at baseline (Supplementary Table 3). Analysis of the primary endpoint including age as a continuous covariate revealed no significant effect of age on MFM D1 + D2 scores (*P*=0.2481, data not shown). The HFMS score declined in both groups from baseline to Month 21, with a non-significant difference favouring the olesoxime group compared with placebo (Table 3). The proportion of patients who improved or remained stable over 21 months (response rate) on the HFMS was higher in the olesoxime arm compared with the placebo arm (olesoxime 49.5%, placebo 28.1%, *P*=0.0091; Table 4). The effects of olesoxime on the HFMS were observed across country (data not shown), age, SMA type and gender (Supplementary Table 3).

Change from baseline to Month 24 was not statistically significant between treatment groups for the electrophysiology endpoints CMAP and MUNE (Supplementary Table 4). There was no difference in the change from baseline to Month 24 in FVC (calculated as a percentage of theoretical capacity based on height and age; see Supplementary Methods) between the two treatment groups (Supplementary Table 4). For CGI-C (patient/caregiver or physician rated), the majority of patients in both treatment arms were rated as 'No change', with no clear differences between the treatment groups. However, in the *post hoc* responder analysis of CGI-C, a statistically significant effect favouring olesoxime over placebo was observed with physician-reported data (relative risk 1.23 [95% CI: 1.01, 1.49]; *P*=0.036). A similar pattern was observed with patient-/caregiver-reported data, with a trend favouring olesoxime over placebo (relative risk 1.19 [95% CI: 0.99, 1.44]; *P*=0.064) (Supplementary Table 5).

Variability in the PEDsQL™ Neuromuscular Module total score was high, with no significant differences observed between the treatment groups in change from baseline to Month 24 (Supplementary Table 6). Two parent-reported PEDsQL™ subscales showed trends for less worsening from baseline to Month 24 with olesoxime (family resources: difference between the means 6.18 [95% CI −0.87, 13.23; *P*=0.0851]; neuromuscular disease: difference between the means 3.75 [95% CI −0.79, 8.29; *P*=0.1050]). For all other subscores, comparisons did not approach statistical significance.

We analysed MFM D1 + D2 score according to olesoxime exposure. MMRM analyses of change from baseline in MFM D1 + D2 score was repeated with systematic, one by one exclusion of patients with the lowest PK exposure values ($C_{average}$; Supplementary Figure 1). The smallest MMRM *P*-value (*P*=0.0088) was obtained for a $C_{average}$ of 7500 ng/mL. This value was reached after exclusion of 37 patients with the lowest exposure. $C_{average}$ values for the overall olesoxime treatment group and the two groups with exposure levels below or above this value (7500 ng/mL) are shown in Supplementary Table 7. Patients with olesoxime exposure $\geq$7500 ng/mL demonstrated improvements in MFM D1 + D2 score at all visits, with a 2.0-point improvement from baseline at Month 24 (Supplementary Figure 2).The group with olesoxime exposure <7500 ng/mL demonstrated a decline in MFM D1 + D2 score.

Olesoxime appeared generally safe and well tolerated, and approximately equivalent proportions of patients in each group experienced at least one AE during the study (Table 5). Two patients died during

the study, with one death in each treatment group (olesoxime: attributed to cardiac arrest; placebo: attributed to increased bronchial secretion), but these deaths were not considered related to treatment by the treating physicians. Further information is provided in Supplementary Information. Several AEs were frequently reported (>5%), with fairly equal frequency in both treatment groups (Supplementary Table 8). A greater proportion of patients experienced serious AEs in the placebo arm than in the olesoxime arm (placebo 50.9%, olesoxime 31.5%, Table 5). The proportion of patients withdrawing from treatment due to AEs was low (olesoxime 3.7%, placebo 3.5%). A *post hoc* analysis investigating selected SMA disease-related AEs (further information on selection of these AEs is provided in Supplementary Information) showed a higher incidence in the placebo arm compared with the olesoxime arm, with a lower frequency of the following disorders with olesoxime treatment: lower respiratory tract infections, gastrointestinal disorders (reflux disorders and constipation) and other joint-related disorders (Table 5).

Discussion

This Phase 2 clinical trial tested the hypothesis that oral administration of olesoxime (10 mg/kg/day) would at least prevent decline of, and potentially improve, motor function in patients with Type 2 and non-ambulatory Type 3 SMA over a treatment period of 2 years, while the placebo group would demonstrate a decline in motor function in line with the natural history of the disease. The trial did not meet the primary outcome measure of improved motor function compared with placebo, as measured by change from baseline to Month 24 in MFM D1 + D2 score. However, we observed trends indicating maintenance of motor function with olesoxime compared with placebo.

First, the overall treatment effect in terms of change from baseline on MFM D1 + D2 across all visits was significantly better with olesoxime than with placebo. In addition, the difference between the two treatment arms was statistically significant in favour of olesoxime at Months 6 and 18, as well as in a sensitivity analysis taking into account erroneous use of MFM32 in some patients aged <6 years. Second, the change from baseline on MFM D1 + D2 scores was significantly better with olesoxime treatment than with placebo in patients aged 6–15 years during the entire treatment period. This is a particularly important finding given that SMA patients in this age group generally experience profound declines in function associated with growth at puberty.[29,30]Given that the expected effect of olesoxime was primarily maintenance of function, and that demonstration of a treatment effect would therefore depend on functional decline in the placebo group, the greatest effect could be expected to be observed in this age group. In fact, the results indicate a change in the trajectory of motor function with olesoxime in 6–15 year olds, from decline to improvement. Such differences between treatment groups were not observed in the youngest (<6 years) and oldest (>15 years) age groups. This may be because children < 6 years may experience improvements in motor function as they develop and achieve motor function milestones,[31] whereas patients aged >15 years may experience periods of fairly stable function over 2–3 years of observation.[29,30]Third, we observed that response on the primary outcome measure was closely associated with olesoxime exposure, with patients that experienced higher olesoxime exposure also demonstrating improved responses. Finally, we also observed positive effects of olesoxime treatment in the responder analyses of the MFM total score and HFMS, where a significantly greater proportion of olesoxime patients than placebo patients demonstrated stable or improved motor function over the

study period, and a trend towards a similar result was observed for MFM D1 + D2 scores. These results were confirmed in the *post hoc* responder analysis of CGI-C, a measure of global change relative to baseline. A significant benefit of olesoxime treatment compared with placebo was observed in the proportion of patients with stable or improved overall status as assessed by physicians. This benefit represents independent evidence supportive of a clinically relevant effect of olesoxime. Furthermore, this was supported by similar, though non-significant, evidence from patient or caregiver ratings.

Given that many of the analyses showed signs of efficacy for olesoxime, it is perhaps surprising that olesoxime did not achieve significance on the primary outcome measure. A potential explanation is the higher than anticipated variability observed on the primary outcome measure in the study population, which caused the study to be underpowered. Our sample size calculation was based on a change of –1.9 points in MFM score in the placebo group over 24 months, with no worsening in the olesoxime group, and an assumed standard deviation of 3.3. In fact, we observed a standard deviation for MFM D1 + D2 of 6.8 points in the placebo arm, with a treatment difference of 2.0, in a population of 160 patients. Two longitudinal multicentre studies of motor function measures in SMA populations across multiple countries have been published. One study used the MFM32 in patients with SMA Types 1, 2 and 3 over a follow-up period of 1.2–66 months, and reported a slope of decline in MFM total score of –0.86 and –0.55 points per year, with standard deviations of 1.45 and 3.99, in patients with Types 2 and 3 SMA, respectively.[7] Another study used both the MFM20 and HFMS in a similar Type 2 and Type 3 SMA population over a 12-month period, and reported a range of changes in MFM20 of –11 to +7 points and in HFMS of –11 to +4 points at 12 months, with standard deviations of 2.94 and 2.74, respectively.[32] The variability observed in our study was greater than the variability reported in these previous studies.

The greater variability observed in our population may have arisen from several causes, including imputation of scores in cases of death or missing data, erroneous use of the MFM32 in some patients aged <6 years, and a relatively large age range with significant variation in stages of development and differences in potential motor function decline.[30] In particular, the inclusion of a large number of children aged <6 years may also have contributed to the variability, as children ≤ 6 years have been shown to continue to improve and achieve motor milestones at these ages.[31] Also, the exposure–efficacy analyses suggest that the variability in treatment effect may have arisen from variations in olesoxime exposure. However, it is possible that the increased variability may simply arise from the differing contexts of the prior studies versus the present one. The prior studies did not include any treatment, and the absence of any expectation of improvement by the patients may in itself produce more homogeneous results. Furthermore, since the overall aim of the prior studies was to validate motor function scales, the methodology would have been more closely focused on demonstrating repeatability and reproducibility, again leading to more homogeneous data.

We further investigated effects of olesoxime treatment on the individual domains of the MFM (described in Supplementary Methods), and observed a trend for a treatment benefit in D2 and non-significant smaller benefits with D1 and D3. This is in accordance with previous work, as the MFM D1 + D2 have previously been shown to be most responsive in patients with Type 3 and Type 2 SMA, respectively.[7] Our study focused on Type 2 and weaker Type 3 patients, who were likely to experience the greatest responses on MFM D2. Conversely, large changes in MFM D1 were not expected in this

cohort of non-ambulatory SMA patients.[32] On the secondary endpoints CMAP, MUNE, PEDsQL™ and FVC, no clear benefit of olesoxime treatment was observed. The correlation between CMAP and MUNE measurements and disease progression in SMA is uncertain and differences have been observed between SMA types,[33] so these measures might not be expected to show any evidence of consistent changes across the study. The use of CMAP and MUNE in clinical trials has been limited thus far, and our data will provide additional information to the field. Further, gaps exist currently in establishing the validation and sensitivity of the PEDsQL™ in SMA patients, as factor analysis to explore construct validity and dimensionality has not yet been performed.[28] For FVC, post-baseline height was not measured, which prevented accurate calculation of predicted FVC after this time, and inter-site variation may have played a role with different equipment used to perform this measure. On CGI-C, the initial analysis may not have been powerful enough to detect differences when the majority of responses reported no change. The results at Month 24 relied on the accuracy of a 2-year recall, which may be too long for optimal assessment.[34] In addition, no instructions were provided to assessors on how to rate clinical change when completing the scale (e.g. what should be considered a minimal improvement or worsening), resulting in a potential lack of consistency amongst respondents. These endpoints will require refinement before use in future trials in SMA, and inter-centre performance would be improved by using standardized methods across study sites.

Olesoxime appeared safe and well tolerated, and a *post hoc* analysis suggested that fewer patients receiving olesoxime experienced disease-related complications including pulmonary, gastrointestinal, and joint-related disorders.

An ongoing difficulty with research in SMA is translating available clinical trial endpoints for measurement of motor function into clinical meaningfulness for patients.[5] Our population of SMA patients was highly heterogeneous and represented young people at varying stages of development and with different needs. Further investigations are ongoing to investigate the treatment effects of olesoxime on specific items of the motor function scales that can be better related to a patient's activities of daily living and translated to clinically meaningful benefits. However, there are strong indications from physician experience of treating patients with SMA and from direct discussions with patients and caregivers that maintenance of function is regarded by patients and their families as a meaningful outcome.[8,35]

There was an age imbalance across the treatment groups, with a lower mean and median age of patients in the olesoxime group compared with the placebo group. As children aged <6 years may improve in motor function as they develop,[30,31] there was potential that the therapeutic effect observed with olesoxime treatment was driven by the younger children in this treatment group. However, *post hoc* analyses demonstrated that there was no significant interaction between treatment and age, and no effect of age on the primary outcome. Furthermore, the effects of olesoxime on motor function appeared to be greatest in patients in the 6–15-years age group, further negating any impact of the age imbalance. The study also lacked an inclusion criterion relating to standard of care, which may have resulted in a heterogeneous population in terms of prior intervention. Evaluation and comparison of non-pharmacological management was not possible. However, all patients were treated in Europe and

the cohort was stratified across countries, with no significant effect of country observed in the subgroup analyses.

A limitation of the study was the wide variability in olesoxime exposure. In the present study, total daily doses approaching 1000 mg have been administered over 2 years in some patients and were well tolerated, suggesting that the therapeutic window may be sufficiently wide for the administration of higher doses of olesoxime in SMA, to maximize benefit. Additional trials exploring higher doses might offer the opportunity to conclusively evaluate the efficacy of olesoxime. A further limitation is the lack of biomarkers to measure disease progression in SMA and the expected biological function of olesoxime.

Currently, disease management in SMA involves supportive care and treating or preventing disease-associated complications.[4] Surveys of SMA patients indicate that avoiding further loss of function is one of the most meaningful benefits that could be achieved with any potential treatment.[8] In the present study, we demonstrate encouraging results that olesoxime treatment may be associated with maintenance of motor function in SMA patients, with particularly notable effects in children aged 6–15 years. Additionally, olesoxime is well tolerated and may be associated with decreases in the frequency of disease-related complications compared with placebo. Despite the acknowledged limitations, this study represents a landmark in SMA, being the longest and largest multinational, controlled study so far to collect prospective assessments with both the MFM and HFMS motor scales. These important, novel data represent a robust baseline for the improved design of future studies in similar SMA populations.

Based on these results, olesoxime offers the potential to provide meaningful clinical benefits for patients with SMA, and given its mode of action, has the possibility of being utilized in combination with other drugs targeting complementary mechanisms of disease.

Contributors

EB, ED, EM, FM, JK, AL, GC, JMC, JLA, BS, WLvdP and CV were involved in study design and data collection, and provided guidance on the data analysis, interpretation and presentation of the data. CR and PSD performed the data analyses, and provided guidance about the interpretation and presentation of the data. EV, JB, KY, PF and TB provided guidance about the data analysis, interpretation and presentation of the data. All authors critically reviewed and edited the manuscript.

Declaration of interests

EB receives grant support from AFM Téléthon and the Italian Ministry of Health; he also served as scientific advisor for AveXis, Biogen, F. Hoffmann-La Roche, Novartis and Edison Pharmaceutics.

FM has served on scientific advisory boards for PTC, GSK, F. Hoffmann-La Roche, Nicox, Italfarmaco, Ashaki and Summit, and the rare disease scientific advisory board for Pfizer. He has received payment for lectures for PTC, Sarepta and Biogen, and receives or has previously received research support at his institution for taking part in clinical trials sponsored by Trophos, F. Hoffmann-La Roche, Biogen/IONIS and PTC. AL and BS received consultant fees from Trophos SA.

CV has received consultancy fees from F. Hoffmann-La Roche. EM has received consultancy fees from Biogen/IONIS, F. Hoffmann-La Roche and Avexis. JK has received consultant fees from Biogen, LabConsult and F. Hoffmann-La Roche. He has received or receives research support for taking part in clinical research by F. Hoffmann-La Roche and Biogen/IONIS.

ED is a former employee of Trophos SA. JLA is a former employee of, and holds stock in, Trophos SA. PF, PSD, TB, CR and EV are current employees of F. Hoffmann-La Roche; PF, TB and PSD also hold stock in F. Hoffmann-La Roche.

GC, LdvP and J-MC have nothing to disclose.

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Tables

Table 1. Patient demographics (FAS)

		Olesoxime n=103	Placebo n=57	Total N=160
Sex, n (%)	Male	55 (53.4)	25 (43.9)	80 (50.0)
	Female	48 (46.6)	32 (56.1)	80 (50.0)
Age at baseline, years	Mean ± SD	9.1 ± 5.5	11.2 ± 6.0	9.9 ± 5.7
	Median	7	11	8
	Range	3–25	3–27	3–27
Age categories, n (%)	<6 years	35 (34.0)	13 (22.8)	48 (30.0)
	≥6 years	68 (66.0)	44 (77.6)	112 (70.0)
SMA type, n (%)	Type 2	74 (71.8)	39 (68.4)	113 (70.6)
	Type 3	29 (28.2)	18 (31.6)	47 (29.4)

FAS=full analysis set; SD=standard deviation.

Table 2. Comparison between treatment groups on change from baseline at Month 24 in MFM D1 + D2 score (MMRM; FAS)

Primary analysis	Olesoxime (n=103)	Placebo (n=57)	Estimate (SE)	96% CI	*P*-value
Baseline, mean (SD)	39.58 (11.701)	38.99 (11.905)			
Change from baseline at Week 104, LS mean (SE)	0.18 (0.717)	–1.82 (0.901)			
96% CI	–1.30, 1.66	–3.68, 0.04			
Difference from placebo			2.00 (1.088)	-0.25, 4.25	0.0676
Overall treatment effect			2.23 (0.835)	0.50, 3.96	0.0084
Sensitivity analysis	**Olesoxime (n=103)**	**Placebo (n=57)**	**Estimate (SE)**	**95% CI**	***P*-value**
Baseline, mean (SD)	39.01 (11.472)	38.69 (11.689)			
Change from baseline at Week 104, LS mean (SE)	0.24 (0.696)	–1.96 (0.872)			
95% CI	–1.14, 1.61	–3.68, –0.24			
Difference from placebo			2.20 (1.050)	0.12, 4.27	0.0379
Overall treatment effect			2.36 (0.817)	0.74, 3.97	0.0044

Primary analysis: for children aged <6 years age who erroneously performed the MFM32, MFM20 score was calculated from MFM32 score. Sensitivity analysis: data as collected from whichever form of the MFM was used. See Supplementary Information for full explanation.

CI=confidence interval; FAS=full analysis set; LS mean=least squares mean; MFM=Motor Function Measure; MMRM=mixed model-repeated measures; SD=standard deviation; SE=standard error.

Table 3. Change from baseline in secondary motor function endpoints (MMRM; analysis; FAS)

MFM total score (to Month 24)	Olesoxime (n=103)	Placebo (n=57)	Estimate (SE)	95% CI	*P*-value
Baseline, mean (SD)	49.32 (10.993)	49.11 (11.432)			
Change from baseline at Week 104, LS mean (SE)	0.59 (0.751)	–1.45 (0.943)			
95% CI	–0.90, 2.07	–3.31, 0.41			
Difference from placebo			2.04 (1.138)	–0.21, 4.28	0.0755
MFM D1 (to Month 24)					
Baseline, mean (SD)	6.76 (7.933)	7.28 (7.543)			
Change from baseline at Week 104, LS mean (SE)	0.07 (0.554)	–0.90 (0.706)			
95% CI	–1.02, 1.16	–2.29, 0.49			
Difference from placebo			0.97 (0.854)	–0.72, 2.66	0.2582
MFM D2 (to Month 24)					
Baseline, mean (SD)	74.10 (18.610)	72.64 (18.882)			
Change from baseline at Week 104, LS mean (SE)	0.38 (1.217)	–2.78 (1.524)			
95% CI	–2.02, 2.78	–5.79, 0.23			
Difference from placebo			3.16 (1.838)	–0.47, 6.79	0.0873
MFM D3 (to Month 24)					
Baseline, mean (SD)	85.41 (13.147)	86.05 (15.412)			
Change from baseline at Week 104, LS mean (SE)	2.27 (1.264)	0.15 (1.606)			
95% CI	–0.22, 4.76	–3.02, 3.32			
Difference from placebo			2.12 (1.945)	–1.72, 5.96	0.2773
HFMS (to Month 21)					
Baseline, mean (SD)	16.47 (10.576)	14.86 (10.514)			
Change from baseline at Week 91, LS mean (SE)	–0.78 (0.416)	–1.72 (0.515)			
95% CI	–1.60, 0.04	–2.74, –0.70			
Difference from placebo			0.94 (0.622)	–0.28, 2.17	0.1309

Primary analysis: for children aged <6 years age who erroneously performed the MFM32, MFM20 score was calculated from MFM32 score. Sensitivity analysis: data as collected from whichever form of the MFM was used. See Supplementary Information for full explanation.
CI=confidence interval; D1=MFM domain 1 (standing position and transfers); D2=MFM domain 2 (axial and proximal motor function); D3=MFM domain 3 (distal motor function); FAS=full analysis set; HFMS=Hammersmith Functional Motor Scale; LS mean=least squares mean; MFM=Motor Function Measure; MMRM=mixed model-repeated measures; SD=standard deviation; SE=standard error.

Table 4. Analysis of responders on change from baseline in motor function scores (FAS)

	Olesoxime (n=103)	Placebo (n=57)	95% CI	Relative risk	*P*-value
MFM D1 + D2 (to Month 24), n (%)	56 (54.4)	22 (38.6)	0.98, 2.08	1.43	0.0609
MFM total score (to Month 24), n (%)	58 (56.3)	22 (38.6)	1.01, 2.10	1.46	0.0419
HFMS (to Month 21), n (%)	51 (49.5)	16 (28.1)	1.16, 2.86	1.82	0.0091

Analysis: for children aged <6 years age who erroneously performed the MFM32, MFM20 score was calculated from MFM32 score. CI=confidence interval; D1=MFM domain 1 (standing position and transfers); D2=MFM domain 2 (axial and proximal motor function); FAS=full analysis set; HFMS=Hammersmith Functional Motor Scale; MFM=Motor Function Measure.

Table 5. Overview of adverse events (safety evaluable population)

	Olesoxime (n=108)	Placebo (n=57)	Total (N=165)
Total number of patients with ≥1 AE, n (%)	103 (95.4)	57 (100)	160 (97.0)
Total number of AEs	1104	612	1716
Total number of deaths, n (%)	1 (0.9)	1 (1.8)	2 (1.2)
Total number of patients withdrawn from the study due to an AE, n (%)	4 (3.7)	2 (3.5)	6 (3.6)
Total number of patients with:			
≥1 AE with fatal outcome, n (%)	1 (0.9)	1 (1.8)	2 (1.2)
≥1 serious AE, n (%)	34 (31.5)	29 (50.9)	62 (37.6)
≥1 AE leading to withdrawal from treatment, n (%)	9 (8.3)	2 (3.5)	11 (6.7)
≥1 severe AE, n (%)	18 (16.7)	14 (24.6)	32 (19.4)
Selected disease-related AEs:			
Lower respiratory tract infections, n (%)	13 (12.0)	10 (17.5)	23 (13.9)
Respiratory failure, n (%)	2 (1.9)	2 (3.5)	4 (2.4)
GI disorders: reflux disorders, n (%)	4 (3.7)	4 (7.0)	8 (4.8)
GI disorders: constipation, n (%)	5 (4.6)	4 (7.0)	9 (5.5)
Scoliosis pathology, n (%)	14 (13.0)	6 (10.5)	20 (12.1)
Other joint-related disorders, n (%)	13 (12.0)	17 (29.8)	30 (18.2)
Surgical procedures, n (%)	9 (8.3)	5 (8.8)	14 (8.5)

AE=adverse event; GI=gastrointestinal.

Figures

Figure 1. Trial profile

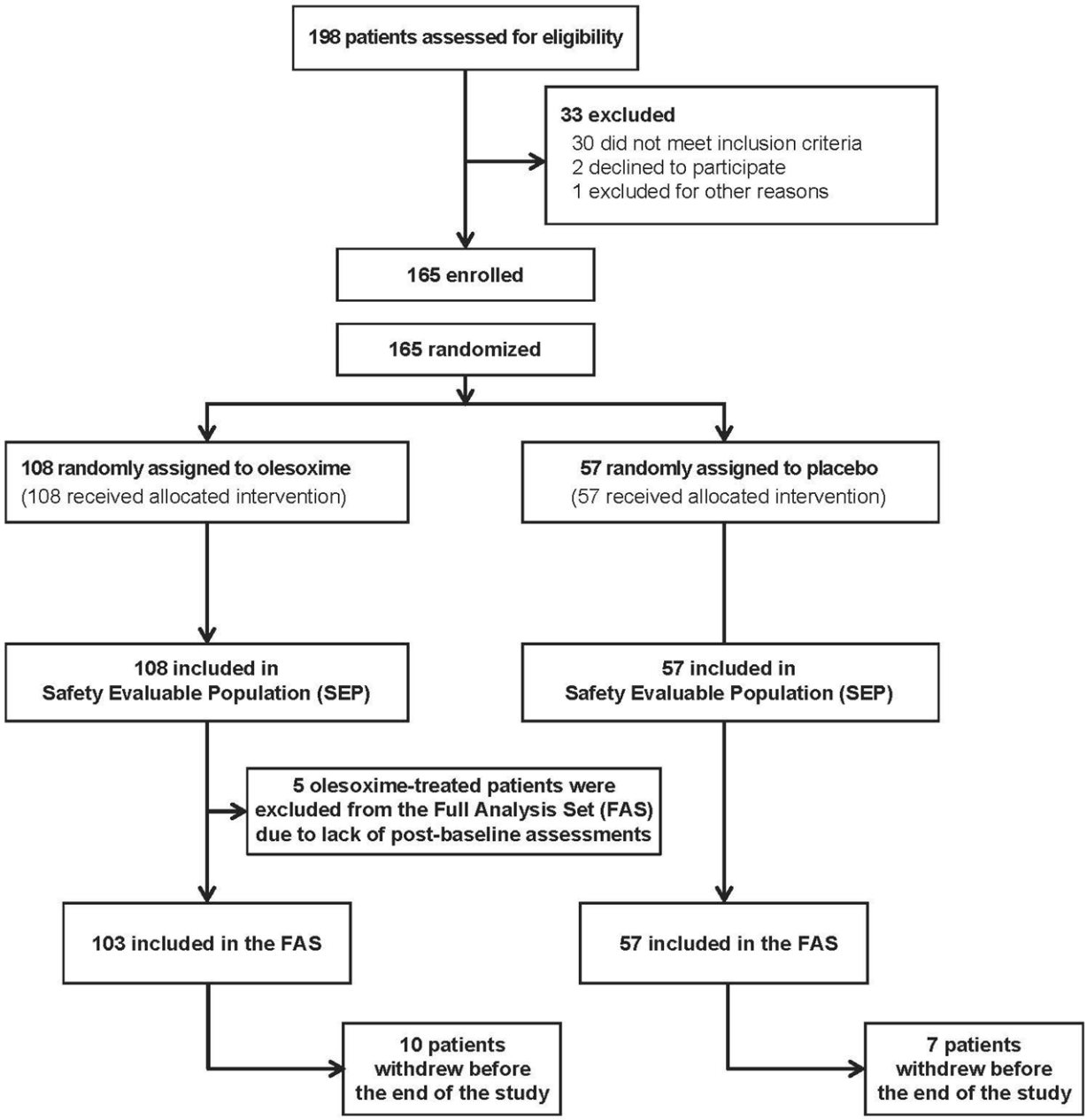
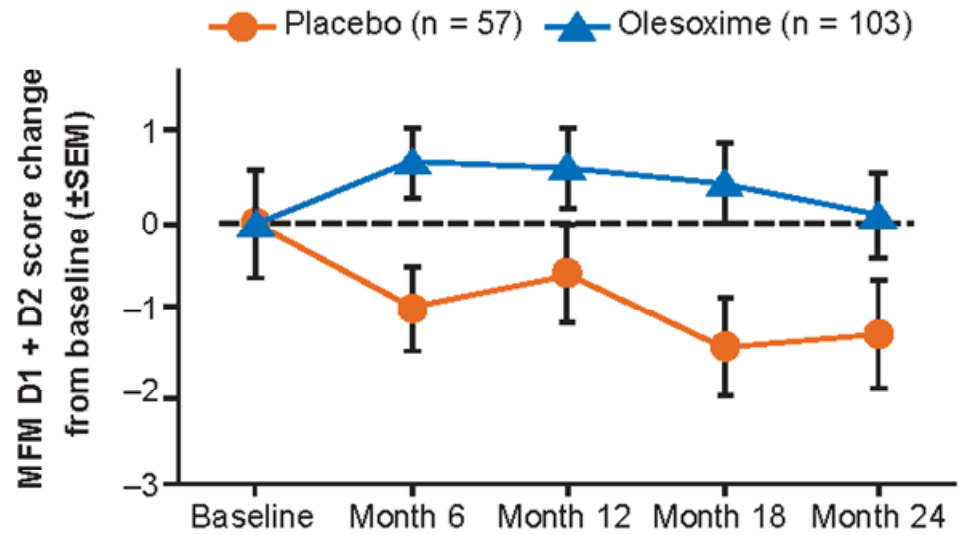


Figure 2. MFM D1 + D2 change from baseline (LS mean ± SEM; FAS, primary analysis) at Months 6, 12, 18 and 24

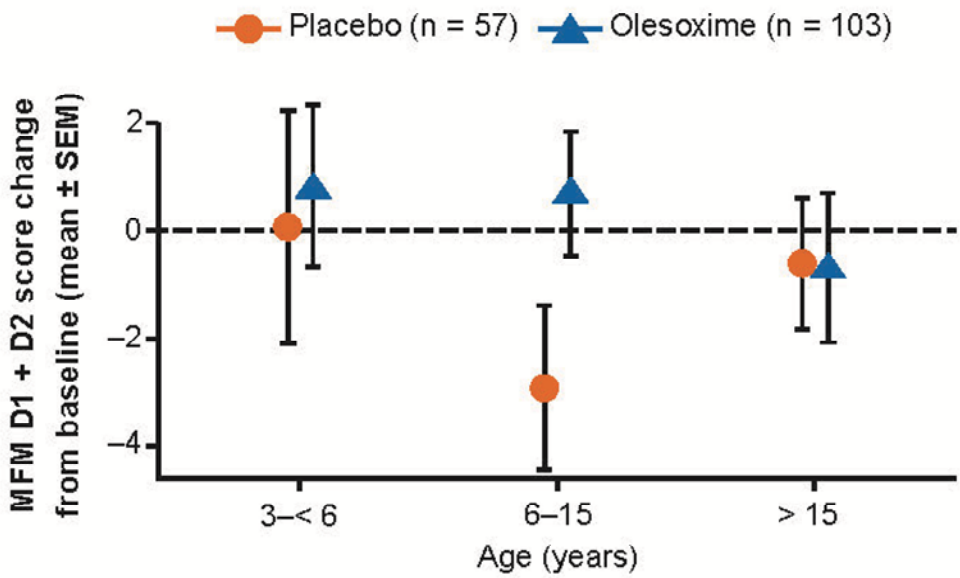


	Month 6	Month 12	Month 18	Month 24
Difference between treatment arms at time point	2.41	1.77	2.74	2.00
P value	0.0039	0.0711	0.0075	0.0676

Overall treatment difference 2.23, *P*=0.0084

Primary analysis: for children aged <6 years age who erroneously performed the MFM32, MFM20 score was calculated from MFM32 score. Error bars represent SEM. MFM=Motor Function Measure; SEM=standard error of the mean.

Figure 3. MFM D1 + D2 mean change from baseline to Month 24 in treatment groups split into three age groups (adjusted mean; FAS; primary analysis)



Placebo, n	13	25	19
Olesoxime, n	35	54	14
P value for treatment difference	0.7459	0.0362	0.9602
Placebo, mean baseline MFM D1 + D2 (SD)	44.07 (10.65)	36.48 (12.14)	38.81 (11.95)
Olesoxime, mean baseline MFM D1 + D2 (SD)	45.89 (10.67)	37.53 (11.07)	31.71 (9.29)

Primary analysis: for children aged <6 years age who erroneously performed the MFM32, MFM20 score was calculated from MFM32 score. See Supplementary Information for full explanation. Error bars represent SEM. MFM=Motor Function Measure; SEM=standard error of the mean.

Supplementary methods

Participants

Key exclusion criteria were evidence of renal dysfunction, blood dysplasia, hepatic insufficiency, symptomatic pancreatitis, congenital heart defect, known history of metabolic acidosis, hypertension, significant central nervous system impairment, or neurodegenerative or neuromuscular disease other than spinal muscular atrophy (SMA), any clinically significant electrocardiogram abnormality, use of medications intended for the treatment of SMA, inability to meet study visit requirements or cooperate reliably with functional testing, and surgical spinal rod or fixation for scoliosis within the past 6 months or anticipated need of rod or fixation within 6 months of enrolment. Patients that received surgical spinal rod or fixations for scoliosis > 6 months before screening and required no further intervention were included in the study. There were no respiratory-related exclusion criteria.

Outcomes

The Motor Function Measure (MFM) consists of 32 items scored on a 4-point Likert scale from 0–3 and grouped by functional domain (domain 1 [D1]: standing position and transfers; domain 2 [D2]: axial and proximal motor function; and domain 3 [D3]: distal motor function).[1] D1 and D2 were selected as they show particular sensitivity in Type 3 and Type 2 SMA patients, respectively. [1] The 20-item MFM includes items representative of all three functional domains and maintains the balance of domains included in the 32-item version.[2]

Trained physiotherapists, using the detailed instructions in the manual, led the patients through a series of 32 physical assessments. The assessment order was designed to reduce the frequency of positional changes. For each item, the physiotherapist scored the ability of the individual to complete the task (e.g., placing both hands on their head) using the detailed description for each score (e.g., 1= raises both hands from the table but the forearms remain in contact with the table). The MFM D1+D2 score is the sum of the 25 items from the D1 and D2 domains, divided by the maximum possible score (75) and multiplied by 100, placing the scores on a 0-100 scale. Due to the training requirements and detailed instructions, subjectivity is limited (as demonstrated by excellent inter-rater reliability).[3] Furthermore, the MFM D1+D2 score has been shown to be a valid and reliable assessment of motor function ability in individuals with Type 2 and non-ambulatory Type 3 SMA.[4]

Statistical analysis

Primary outcome measure

Within the mixed-effects repeated measures (MMRM) model, visit was treated as a repeated variable within a patient (random effects). An unstructured variance–covariance matrix was applied to model the within-patient errors in the primary model. The Restricted Maximum Likelihood method was used for estimates of variance components. The Kenward Roger approximation was used to estimate the denominator degrees of freedom.

Secondary endpoints

Motor function scores

MMRM models described in the main Methods section for the primary outcome measure were used to analyse change from baseline to Month 24 in the MFM Total Score, MFM D1, D2 and D3 scores (primary and sensitivity analyses) and change from baseline to Month 21 for the Hammersmith Functional Motor Scale (HFMS) total score. Changes from baseline in the individual items of the MFM score (to Month 24) and the HFMS (to Month 21), respectively, were analysed using MMRM as described for the primary outcome measure with the exception that missing items were not imputed. In all MMRM analyses of MFM D1+D2 and Total scores, missing scores were imputed using a model based on placebo data and individual MFM items were imputed as follows: If an MFM item was "Not done" or "Not applicable" at baseline, a backward imputation was performed. The value of this item was imputed by the value of the same item at the 6-month visit. If an item was "Not done" or "Not applicable" at 6 months, 12 months or 18 months, the value of the item was imputed by the mean value of the next and previous visits for the same item. If an item was "Not done" or "Not applicable" at 24 months or at the last visit, a forward imputation was performed. The value of this item was imputed by the value of the same item at the previous visit.

Responder analyses

Responders were defined as patients with no worsening compared with baseline in the selected motor function measure. Patients who withdrew prematurely were classed as non-responders. Responder analyses were performed for MFM D1 + D2 at Month 24, MFM total score at Month 24 and HFMS at Month 21 (last scheduled HFMS assessment).

Sensitivity analyses

Subgroup analyses

Subgroup analyses of the MFM D1 +D2 were performed as per the primary outcome measure analysis for the following groups: age (<6 years vs ≥6 years), SMA type (Type 2 vs Type 3), country and baseline severity (< median vs ≥ median). To further investigate the effect of age on the primary outcome, we split the population into three age groups: <6 years, 6–15 years and >15 years at enrolment and repeated the analysis with the MMRM model (as for the primary analyses).

Exploratory exposure–efficacy analysis

Exposure–efficacy *post hoc* analyses were conducted to investigate whether variability in PK exposure could explain part of the variability in MFM D1 + D2 efficacy outcomes in patients treated with olesoxime. One patient included in the Full Analysis Set for the majority of the analyses was excluded from the exploratory exposure–efficacy analysis due to missing post-baseline MFM data (the patient died shortly after randomization). Individual pharmacokinetic (PK) exposure ($C_{average}$) was defined as the average of all the individual plasma trough concentrations measured according to schedule of

assessment (Supplementary Table2). $C_{average}$ reflects steady-state concentrations. The MMRM analysis conducted for the primary outcome measure was conducted repeatedly as subjects with the lowest $C_{average}$ were systematically excluded, one by one (Supplementary Figure 1). The $C_{average}$ above which the MMRM analysis yielded the smallest *P*-value was determined. In a second step, the identified $C_{average}$ was used as a threshold PK exposure to define patients with low and high olesoxime exposure (Supplementary Table 7) and the time course of the change from baseline (mean ± SEM) of the MFM D1 + D2 score between patients with low and high exposure value and the placebo group graphically explored (Supplementary Figure 2).

Other secondary endpoints

Compound muscle action potential (CMAP) measurements were recorded as 1st, 2nd and 3rd increment in mV. Motor unit number estimation was calculated using the formula:
CMAP (mV) *1000 / SMUP (μV)
where SMUP (Single Motor Unit action Potential) is the maximum value of increments for wrist site plus the maximum value of increments for 4 cm proximal to wrist plus the maximum value of increments for ulnar groove), all divided by the total number of increments for 3 sites. The change from baseline to Month 24 was performed on all validated measures on the same nerve over time, assessed using analysis of covariance. The covariance model included the same covariates as specified for the primary outcome measure.

Forced vital capacity (FVC) was performed in all patients at least 5 years old. To adjust the FVC according to individual height, weight and gender, the FVC results were divided by the theoretical capacity (TC, calculated using equations below) and calculated as a percentage.

- For females aged <18 years: TC = 1.4507 + (1.48 + 0.0127A) x H.[5]
- For males aged <18 years: TC = 1.2782 + (1.3731 + 0.0164A) x H.[5]
- For females aged >18 years: TC = 4.43H – 0.026A –4.34.[6]
- For males aged >18 years: TC = 5.76H – 0.026A –4.34.[6]

H=height at baseline(m); A=age at baseline(years).

If height could not be measured accurately, for example in the presence of scoliosis or contractures, ulna length was used to calculate a surrogate height measure. The change in baseline of FVC/TC % was then analysed using analysis of covariance, with baseline score, SMA type, country and treatment group included as covariates.

Pediatric Quality of Life Inventory Neuromuscular Module scores at Month 24 were analysed using analysis of covariance within 6 subgroups defined as young children aged ≤7 years, children aged 8–12 years, teenagers aged 13–18 years, adults aged >18 years, parents of minors and parents of adults, as well as all patient and all parent ratings grouped.

Adverse events

Adverse event (AE) clusters were defined as SMA-related complications based on existing literature,[7] with subsequent refinement by external experts. The following medical conditions were defined as disease-related: lower respiratory tract infections, respiratory failure, gastrointestinal disorders–reflux disorders, gastrointestinal disorders–constipation, scoliosis pathology, other joint-related disorders and surgical procedures.

Supplementary Results

Two patients died during the study, neither death was attributed to study treatment. Further information surrounding these deaths is supplied below.

Patient 1213: Placebo

This 6-year-old girl with SMA Type 2 was generally in good health. She was randomized to placebo. On Day 17, she took a 10-minute fun fair ride and was cyanotic at the end of the ride. She died at hospital from unknown cause, probably suffocation. The investigator assessed the event as unrelated to study medication and unrelated to clinical trial procedure, and considered that the most likely cause of the serious AE was suffocation.

Patient 0107: Olesoxime

This 11-year-old boy was diagnosed with SMA Type 2 in 2000. His health status was weak at inclusion (HFMS 4, MFM 31.25%), with restricted respiratory function (FVC 16%). On Day 553, the boy experienced an acute secretion impaction at home. Respiratory physiotherapy, cough assistance, aspiration and suction remained unsuccessful as well as resuscitation by a rescue team. The boy died at home and no autopsy was performed. The investigator assessed the event as unrelated to study medication and unrelated to clinical trial procedure.

Supplementary References

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Supplementary Tables

Supplementary Table 1. Patient cohort by country (Full Analysis Set)

Country, n	Placebo n=57	Olesoxime n=103	Total N=160
Belgium (2 sites)	5	11	16
France (6 sites)	11	16	27
Germany (3 sites)	7	13	20
Italy (6 sites)	17	33	50
Netherlands (1 site)	4	5	9
Poland (1 site)	5	15	20
UK (3 sites)	8	10	18

Number of patients included in this study by country and treatment group, number of individual study sites included in parentheses after country name.

Supplementary Table 2. Schedule of assessments

Assessments/Visits	Screening V–1	Inclusion V0	V1	T1	V2	V3	V4	V5	V6	V7	V8	V9
Week	–4 to –1	Day 0	4	9	13	26	39	52	65	78	91	104
Month			1	2	3	6	9	12	15	18	21	24
Informed consent	●											
Medical and surgical history	●											
Genotyping if not documented	●											
Genotyping *SMN2* copy number	●											
Inclusion/non-inclusion criteria	●	●										
Randomization		●										
Efficacy												
Motor Function Measure (MFM)		●				●		●		●		●
Hammersmith Functional Motor Scale (HFMS)	●				●		●		●		●	●
Electromyography (CMAP/MUNE)		●				●		●		●		●
Forced Vital Capacity (FVC)		●			●	●	●	●		●		●
Clinical Global Impression (CGI) by physician		●			●	●	●	●	●	●	●	●
CGI by patients/parents		●		●	●	●	●	●	●	●	●	●
Pediatric Quality of Life Inventory (PEDsQL™) Neuromuscular Module		●						●				●
Safety												
Adverse events and concomitant treatments		●	●	●	●	●	●	●	●	●	●	●
Physical examination/vital signs/ECG	●	●	●		●	●	●	●	●	●	●	●
Laboratory assessments	●		●		●	●	●	●	●	●	●	●
Pregnancy test	●						●	●				●
Pharmacokinetic sampling			●		●			●		●		●
Biobank blood and urine sample	●							●				●
IMP dispensation		●	●		●	●	●	●	●	●	●	
IMP return			●		●	●	●	●	●	●	●	●

CMAP=compound muscle action potential; ECG=electrocardiogram; IMP=investigational medicinal product; MUNE=motor unit number estimation; SMN=survival of motor neuron; V=visit.

Supplementary Table 3. Effects of olesoxime on motor function across subgroups (FAS, MMRM analyses)

	Adjusted mean (95% CI)	
Age	<6 years (n=48)	≥6 years (n=112)
MFM D1 + D2	0.75 (–3.86, 5.35)	2.21 (–0.21, 4.62)
HFMS	1.54 (–1.25, 4.33)	0.68 (–0.71, 2.06)
SMA type	2 (n=113)	3 (n=47)
MFM D1 + D2	2.06 (–0.78, 4.90)	2.06 (–0.83, 4.94)
HFMS	0.89 (–0.51, 2.29)	0.72 (–1.72, 3.16)
Gender	Male (n=80)	Female (n=80)
MFM D1 + D2	0.6 (–2.51, 3.70)	3.05 (–0.11, 6.21)
HFMS	1.5 (–0.32, 3.33)	0.72 (–1.02, 2.47)
Baseline severity	< Median (n=79)	≥ Median (n=81)
MFM D1 + D2	2.97 (–0.36, 6.31)	1.25 (–1.64, 4.15)

CI=confidence interval; HFMS=Hammersmith Functional Motor Scale; FAS=full analysis set; MFM=Motor Function Measure; D1=dimension 1 of the MFM (standing and transfers); D2=dimension 2 of the MFM (axial and proximal motor capacity). Analysis: for children aged <6 years age who erroneously performed the MFM32, MFM20 score was calculated from MFM32 score. See Supplementary Information for full explanation.

Supplementary Table 4. Change from baseline to Month 24 for electrophysiology and respiratory function endpoints (FAS; ANCOVA)

CMAP, mV	Olesoxime (n=70)	Placebo (n=34)	*P*-value
Baseline, mean (SD)	3.74 (2.370)	4.02 (2.718)	
Month 24, LS mean (95% CI)	–0.07 (–0.49, 0.36)	–0.16 (–0.74, 0.43)	0.7865
MUNE	Olesoxime (n=58)	Placebo (n=30)	
Baseline, mean (SD)	39.70 (35.096)	36.24 (32.149)	
Month 24, LS mean (95% CI)	–4.51 (–12.21, 3.18)	–6.69 (–16.86, 3.48)	0.7117
FVC/TC %	Olesoxime (n=64)	Placebo (n=38)	
Baseline, mean (SD)	66.53 (28.321)	61.32 (21.863)	
Month 24, LS mean (95% CI)	4.28 (–0.32, 8.88)	6.16 (1.00, 11.33)	0.5655

No differences between treatment groups were observed with electromyography measurements (CMAP and MUNE) or respiratory function assessment (FVC/TC; *P*>0.05). ANCOVA=analysis of covariance; CI=confidence interval; CMAP=compound muscle action potential; FAS=full analysis set; FVC=forced vital capacity; LS mean=least square mean; MUNE=motor unit number estimation; TC=theoretical capacity.

Supplementary Table 5. Responder analysis for CGI-C (FAS; log binomial model)

	Response rate, %		Relative risk (95% CI)	*P* value
	Olesoxime (n=103)	Placebo (n=57)		
Patient/caregiver ratings	85.4%	70.2%	1.19 (0.99, 1.44)	0.064
Physician ratings	86.4%	70.2%	1.23 (1.01, 1.49)	0.036

Qualitative assessment of overall global function using the CGI-C was performed by patients or their caregivers (CGI-C patient/caregiver) and physicians (CGI-C physicians). CGI-C=clinical global impression of change; CI=confidence interval; FAS=full analysis set.

Supplementary Table 6. Change from baseline to Month 24 in PEDsQL™ Neuromuscular Module (FAS; ANCOVA)

Group	Number of patients		Difference between the means (95% CI)	*P*-value
	Olesoxime	Placebo		
Patients				
Young children ≤7 years	27	8	–7.70 (–20.19, 4.79)	0.2163
Children 8–12 years	25	9	–0.48 (–9.90, 8.94)	0.9172
Teenagers 13–18 years	11	17	3.13 (–7.54, 13.80)	0.546
Adults >18 years	8	3	5.12 (–6.07, 16.32)	0.273
All patient ratings (patients >5 years old)	71	37	0.25 (–4.58, 5.08)	0.9185
Parents				
All parent ratings	90	46	3.62 (–0.77, 8.01)	0.1054
Subscore: Family resources				
Parent reports	90	46	6.18 (–0.87, 13.23)	0.0851
Patient reports (≥8 years)	43	29	4.11 (–4.17, 12.40)	0.3427
Subscore: Neuromuscular disease				
Parent reports	90	46	3.75 (–0.79, 8.29)	0.1050
Patient reports	71	37	–0.34 (–5.25, 4.56)	0.8899
Subscore: Communication				
Parent reports	88	46	–0.66 (–8.44, 7.13)	0.8679
Patient reports (≥8 years)	43	29	4.83 (–3.11, 12.78)	0.2288

Differences between means are estimates of the mean difference between the treatment groups (olesoxime - placebo). 95% CIs are the mean differences between the treatment groups (olesoxime - placebo).
ANCOVA=analysis of covariance; CI=confidence interval; FAS=full analysis set; PEDsQL™=Pediatric Quality of Life Inventory.

Supplementary Table 7. Exploratory exposure–efficacy analysis: summary of individual olesoxime exposure $C_{average}$ (PK dataset)

	Olesoxime (all, N = 103)	Low exposure $C_{average}$ <7500 ng/mL (n = 37)	High exposure $C_{average}$ ≥7500 ng/mL (n = 66)
Mean exposure, $C_{average}$, ng/mL (SD)	8590 (2400)	6145 (859)	9960 (1823)
Median, ng/mL	8448	6362	9700
Range, ng/mL	4130–16567	4130–7485	7518–16567

Individual pharmacokinetic (PK) exposure ($C_{average}$) using 7500 ng/mL as a threshold for PK exposure to define patients with low and high olesoxime exposure. See Supplementary Information for details of the exploratory exposure–efficacy analysis. $C_{average}$=mean plasma olesoxime concentration; PK=pharmacokinetics; SD=standard deviation.

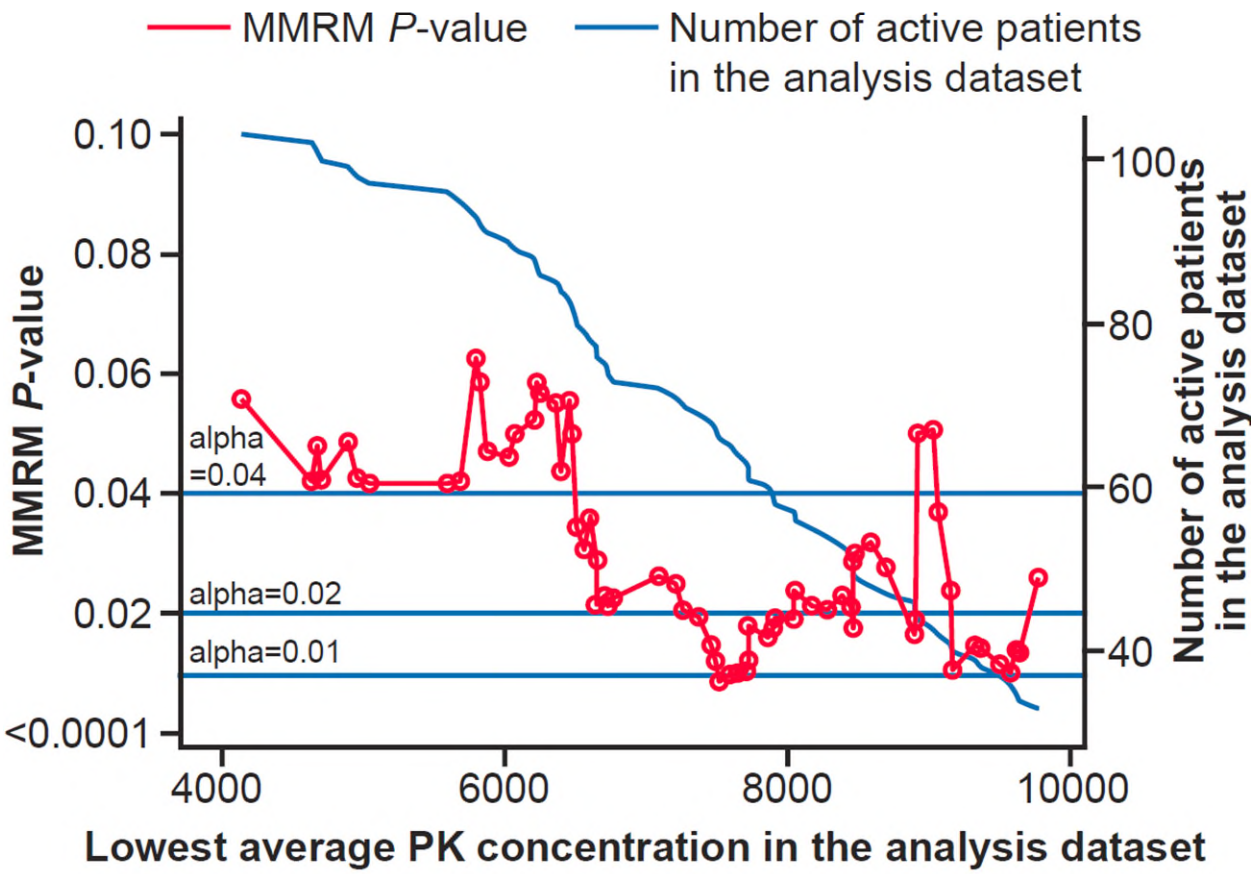
Supplementary Table 8. Adverse events observed in more than 10% of patients (safety-evaluable population)

	Olesoxime, n (%) (N=108)	Placebo, n (%) (N=57)
Nasopharyngitis	25 (23.1)	15 (26.3)
Upper respiratory tract infection	23 (21.3)	13 (22.8)
Bronchitis	17 (15.7)	17 (29.8)
Gastroenteritis	16 (14.8)	10 (17.5)
Respiratory tract infection	17 (15.7)	6 (10.5)
Pharyngitis	15 (13.9)	6 (10.5)
Influenza	11 (10.2)	9 (15.8)
Rhinitis	14 (13.0)	6 (10.5)
Pneumonia	6 (5.6)	6 (10.5)
Vomiting	25 (23.1)	16 (28.1)
Abdominal pain	20 (18.5)	11 (19.3)
Diarrhoea	18 (16.7)	12 (21.1)
Cough	32 (29.6)	16 (28.1)
Oropharyngeal pain	16 (14.8)	9 (15.8)
Pyrexia	34 (31.5)	16 (28.1)
Pain in extremity	14 (13.0)	5 (8.8)
Scoliosis	13 (12.0)	5 (8.8)
Arthralgia	2 (1.9)	7 (12.3)
Fall	10 (9.3)	7 (12.3)
Headache	22 (20.4)	13 (22.8)

Adverse events occurring with >10% frequency within olesoxime and placebo treatment groups, safety-evaluable population.

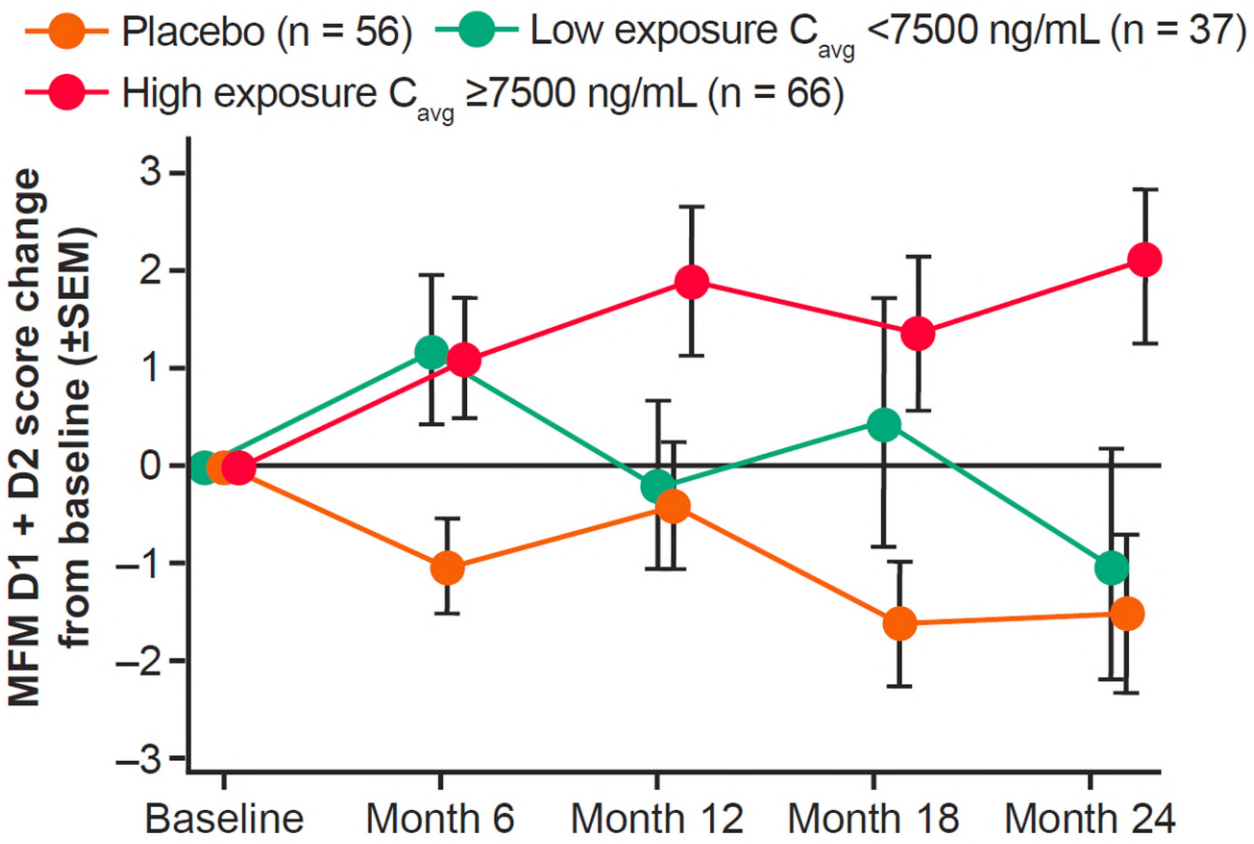
Supplementary Figures

Supplementary Figure 1. MMRM *P*-value versus the lowest $C_{average}$ value in the analysis dataset (PK dataset)



Levels of statistical significance (primary y-axis) achieved on the primary endpoint (MFM D1 + D2) when patients with lower $C_{average}$ plasma olesoxime concentrations are excluded from the analysis (secondary y-axis). The corresponding *P*-value for the MMRM analysis performed is plotted in red. Horizontal blue lines represent alpha 0.01, 0.02 and 0.04 values, indicating the number of patients included in the dataset to achieve these probabilities. $C_{average}$=mean plasma olesoxime trough concentration; D1=MFM domain 1 (standing position and transfers); D2=MFM domain 2 (axial and proximal motor function); MFM=Motor Function Measure; MMRM=mixed model-repeated measures; PK=pharmacokinetic.

Supplementary Figure 2. Time course of MFM D1 + D2 change from baseline (mean ± SEM) for placebo and olesoxime treatment groups with low and high PK exposure (PK dataset)



$C_{average}$=mean plasma olesoxime trough concentration; D1=MFM domain 1 (standing position and transfers); D2=MFM domain 2 (axial and proximal motor function); MFM=Motor Function Measure; PK=pharmacokinetic; SEM=standard error of the mean.