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Research Paper

High-dose pharmaceutical grade biotin (MD1003) in amyotrophic lateral sclerosis: A pilot study

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ABSTRACT

Background: Oligodendrocytes (OGs) provide metabolic support to motor neurons (MNs) and are implicated in the pathophysiology of amyotrophic lateral sclerosis (ALS). MD1003, or high-dose Pharmaceutical grade Biotin (hdPB), may improve disability in progressive multiple sclerosis patients via augmentation of OG or MN energy levels. Here, we assessed the safety and efficacy of MD1003 in ALS patients.

Methods: This single centre, randomised, double-blind, placebo-controlled trial included patients aged 25–80 years with probable or definite ALS. Patients were assigned (2:1), using a computer-generated randomisation list, to receive oral MD1003 (300 mg/day) or placebo treatment for 24 weeks. The primary outcome, safety, was analysed in all patients who received at least one dose of study drug. This study, registered with ClinicalTrials.gov, NCT03114215, has been completed.

Findings: Between June and December 2016, 30 patients were enrolled (MD1003, n = 20; placebo, n = 10). Baseline characteristics were representative of the ALS population. MD1003 and placebo groups were not well balanced at screening, with the MD1003-treated group having a higher rate of ALSFRS-R decline prior to screening versus placebo (-6·0 IQR [-8·5, -5·0] vs. -5·0 IQR [-5·0, -3·0]) and a predominance of ALS with upper limb onset compared to placebo (35% vs. 10%). MD1003 had a favourable safety profile and was well tolerated. The occurrence of adverse events was similar in both groups (60%). Two deaths occurred in the MD1003 group versus 1 in the placebo group. ALSFRS-R median change from baseline to month 6 was not significantly different between the two groups (p = 0·49); the mean difference between groups was -1·6 (SEM=3·3).

Interpretation: MD1003 treatment was safe and well tolerated. It was not possible to establish MD1003 efficacy in this relatively small study. Given the favourable safety profile of MD1003 and an imbalance between treatment groups favouring placebo, additional, larger studies in ALS are warranted.

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1. Introduction

Amyotrophic lateral sclerosis (ALS), the most frequent and severe motor neuron disease, is characterised by progressive death of upper and lower motor neurons (MNs). The incidence of ALS is about 2–5 per 100,000 individuals, however, prevalence is low, as the median patient survival is 3 years following onset of weakness [1,2].

The cause of ALS is unknown, except in familial cases, which has allowed for the development of the transgenic superoxide dismutase 1 (SOD1) mouse model of ALS [3]. Several lifestyle factors, including physical activity (strenuous work, certain professional sports, i.e., football, marathon running, cross-country skiing) and smoking, are associated with an increased risk of ALS [4–6]. Biological factors, such as vitamin D deficiency, low creatinine blood levels, high ferritin blood levels, hyperlipidaemia and weight loss, are associated with a worse ALS prognosis [7–11]. These risk factors highlight the importance of metabolism in both the onset and progression of the disease. Indeed, ALS patients frequently exhibit a hyper-metabolism that increases the risk of weight loss [12]. Changes in metabolism in ALS may be linked to mitochondrial alterations or to dysregulation of the response to hypoxia, a process implicated in MN death [13,14].

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Research in context

Evidence before this study

Finding a cure for ALS patients is an important and an unmet need. Studies of ALS risk factors have highlighted the importance of metabolic changes in both the onset and progression of the disease. Moreover, ALS patients often exhibit a hyper-metabolism that is associated with a worse prognosis. These studies and observations suggest that modulation of metabolism could be a therapeutic approach in ALS.

Oligodendrocytes (OG), one of the brain-resident cells that provide metabolic support to neurons, have recently been implicated in ALS pathogenesis, with suppression of mutant SOD1 expression in OGs improving survival of transgenic SOD1 mice.

Biotin is a cofactor for several carboxylases that are involved in key neurometabolic pathways, including energy production and myelin synthesis. Severely reduced levels of endogenous biotin, as is observed in patients with biotinidase deficiency, leads to OG death and axonal degeneration. Supplementation of low doses of biotin in these biotinidase-deficient patients resulted in clinical improvement. High-dose Pharmaceutical grade Biotin (MD1003) is being investigated as a treatment in progressive multiple sclerosis, a phase of the disease that is accompanied by metabolic perturbations and neurodegeneration, including degeneration of the corticospinal motor pathway. In a previous Phase III study in progressive MS, MD1003 was shown to improve clinical disability in a subset of patients.

Added value of this study

This study was the first randomized, placebo-controlled trial of MD1003 for the treatment of patients with ALS. The safety profile of MD1003 in ALS was good. We did not find evidence of efficacy as measured by the decline of ALSRFRS-R score and the ALSFRS-R severity score. There was a trend towards less worsening of SNIP favoring MD1003. At screening, placebo and MD1003 groups were not well balanced, with a significantly higher rate of decline in the MD1003 group.

Implications of all the available evidence

To date only a single drug, riluzole, is approved for the treatment of ALS but this drug only modestly extends lifespan. The present trial showed that MD1003 is well tolerated. Taking into account: 1) the unmet needs for ALS patients; 2) the good safety profile; 3) the trend in favour of MD1003 for less worsening of SNIP; and 4) faster disease progression at screening that did not favour MD1003; further studies with MD1003 in ALS may be warranted.

Cells surrounding MNs play a key role in the degenerative processes that occur in ALS. Oligodendrocytes (OGs) provide metabolic support to neurons via cytoplasmic “myelinic” channels and monocarboxylate transporters (MCTs). In the SOD1 transgenic mouse model of ALS, OG degeneration in the spinal cord grey matter precedes visible impairment [15]. In addition, newly formed OGs fail to reach maturity, leading to progressive demyelination associated with down-regulation of MCT1. Suppression of SOD1 mutation expression in OGs greatly improves survival of these mice [15,16]. In ALS, substantial demyelination is present in the motor cortex and the spinal cord grey matter. Although activated oligodendrocyte progenitor cells can be found in these areas, there are few or no functional OGs, due to reduced expression of myelin basic protein and MCT1 [15,16].

Biotin, or vitamin B8, is a cofactor for carboxylases that function within the tricarboxylic acid cycle to produce ATP, which is particularly important in energy-demanding cells such as neurons and OGs [17,18]. In individuals with biotinidase deficiency, low biotin levels lead to central nervous system (CNS) degeneration associated with OG death and axonal degeneration [18]. The resulting clinical consequences are dramatic, but the supply of low doses of biotin (5 to 10 mg/day) leads to clinical improvement with a time-dependent therapeutic effect [19]. Biotin’s role in the CNS was recently emphasized when treatment with high-dose Pharmaceutical grade Biotin (hdPB; MD1003) at 300 mg/day led to a slowing down of clinical worsening and, in some cases, an improvement of disability in progressive multiple sclerosis (MS) patients [19,20]. One important clinical characteristic of progressive MS is the degeneration of the upper MNs in the corticospinal motor pathway. The action of biotin in progressive MS is likely mediated through an effect on cellular energy levels, both in MNs and OGs [20,21]. Since the metabolic and cellular targets in progressive MS are also implicated in ALS pathogenesis, we sought to evaluate MD1003 in a pilot study in ALS patients, treated with the same regimen as patients in the progressive MS study (300 mg/day).

2. Methods

2.1. Study design

This single centre, double-blind, randomized, placebo-controlled study was performed in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki [22]. ALS patients were recruited between June and December 2016 in the Amyotrophic Lateral Sclerosis center, Centre Hospitalier Universitaire Gui de Chauliac, Montpellier, France. The Ethics Committee CPP Sud-Méditerranée IV and French Agency for the Safety of Health Products (ANSM) approved the protocol.

2.2. Patients

Patients aged 25−80 years with a diagnosis of probable or definite ALS according to the revised international El Escorial criteria [23] were recruited for this study. Main inclusion criteria were: (1) ALS onset at inclusion < 3 years; (2) a loss of at least 5 points on the ALS Functional Rating Scale-Revised (ALSFRS-R) during the 12 months preceding screening visit or at least 2 points in the previous 6 months [24]; (3) stable dose of riluzole for at least 3 months at inclusion; 4) slow vital capacity (SVC) $> 60\%$. Main exclusion criteria were: (1) non-invasive ventilation more than 10 h/day; (2) ALSFRS-R score < 20; (3) gastrostomy; (4) weight loss $> 15\%$ of the reference weight (eg, before disease onset); (5) dyspnea at rest or with the least effort; (6) dementia. Eligible patients were identified in the clinic at the study site and recruited in the study according to the protocol. All patients provided written informed consent.

2.3. Randomisation and masking

All eligible patients were randomised within 4 weeks of enrolment to receive MD1003 or placebo at the second study visit (M0). Patients were not matched or stratified for treatment assignments. Treatment randomisation was performed centrally using a computer-generated allocation schedule. The hospital pharmacy received randomisation blocks totalling 30 units (1 unit per patient). The randomisation blocks contained 2 active treatment units for 1 placebo unit in a random order (randomisation scheme 2:1). The hospital pharmacy was provided with the randomisation list for treatment number allocation in a specific order. The investigator or his/her delegate assigned a treatment to a patient following the randomisation list. A randomisation number was assigned to each patient and was retained for the whole study. The composition, appearance, and packaging of placebo treatment were identical to those of active treatment. Treatment kits were labelled with batch number, expiry date,

randomisation number, storage/administration conditions, and a kit identification number. Patients, physicians (those caring for patients and those assessing outcomes), pharmacists, and all investigational staff were blinded to the treatment allocation for the entire duration of the trial.

2.4. Procedures

Patients were treated with MD1003 or placebo for 24 weeks. Treatment was an oral capsule containing 100 mg of MD1003 or placebo, administered three times a day (tid). Concomitant medications were medications that were started before the first dose of MD1003 or placebo and continued after the first dose of blinded treatment. Study visits were V1 (screening, month [M]−1), V2 (baseline, M0), V3 (M3), and V4 (M6). A phone call was made at M1 for adverse event (AE) reporting and compliance assessment. At M0 and M3, patients received a kit to self-administer orally a capsule tid during the 24-week double-blind treatment period. Clinical assessments were done at all study visits starting at baseline and comprised: ALSFRS-R, SVC, maximum inspiratory pressure (MIP), and sniff nasal inspiratory pressure (SNIP). Quality of life was assessed by EQ5D-3L at baseline and M6. Clinical Global Impression, clinician- (CGI) and patient-assessed (SGI), was determined at M6. Screening and baseline laboratory tests evaluated haematology, renal and liver functions to corroborate inclusion and exclusion criteria. All measures were performed centrally.

2.5. Outcomes

The primary objective of the study was to assess the safety of MD1003 in ALS patients via monitoring of serious AEs (SAEs), AEs, disease-related events, routine laboratory tests, and vital signs. AEs and disease-related AEs were coded according to the Medical Dictionary for Regulatory Activities coding system. Routine lab tests were performed at each visit. Values for clinical laboratory assessments were compared with both the appropriate normal ranges and ranges of potential clinical concern. Any abnormal test result or other safety assessment judged by the investigator to be clinically significant was recorded as an AE or SAE. Secondary outcome measures were parameters of clinical progression and quality of life: handicap score evaluated using ALSFRS-R; ALSFRS-R severity score, defined as the rate of ALSFRS-R score decline (number of points lost / number of elapsed months); respiratory function (SVC, MIP and SNIP); weight; quality of life (EQ-5D3L); and CGI [24,25].

2.6. Statistical analysis

This was a pilot exploratory study with the aim to confirm the safety profile of MD1003 and determine the most relevant clinical efficacy parameter(s) for a potential larger study.

2.6.1. Analysis set

The Full Analysis Set (FAS), which consisted of all the randomised patients who received at least one dose of study medication and with at least one assessment at screening or baseline, was the primary population used for safety and efficacy analyses. Supportive and *post hoc* analyses were conducted on per protocol (PP) set (excluding patients without an assessment of ALSFRS-R at baseline and M6 and/or with major deviations).

2.6.2. Main endpoint

We compared the two treatment groups for the number of treatment emergent AEs (TEAEs) and the total number of patients with at least one TEAE. We also recorded suspected disease-related AEs (DRAEs), serious DRAEs (SDRAEs), and deaths.

2.6.3. Secondary endpoints

The change in ALSFRS-R score over the 24-week treatment period was compared between the two treatment groups using a Mann-Whitney U test as the primary analysis. An ANCOVA based on the change from baseline (quantitative variable) was performed using two covariates: the baseline value of ALSFRS-R and the log of time from first weakness to randomisation. The response was the change from baseline to M6 and included the following covariates: treatment, severity score between screening and baseline, log of time from the start of symptoms, and the interactions between treatment and each of the other two covariates. A likelihood-based mixed-effects repeated measures (MMRM) model was used to compare the variation of ALSFRS-R score between treatment groups. The Kenward-Roger approximation was used to estimate denominator degrees of freedom and adjust standard errors, with an unstructured variance-covariance matrix. The change in respiratory parameters from baseline to month 6 was analysed using a MMRM model adjusted for two covariates: baseline value and treatment group.

Statistical analyses on primary efficacy criterion were performed on imputed data. Only the use of the worst score (ALSFRS-$R = 0$) in case of death was necessary for handling missing data. For the other criteria, we performed comparisons of proportions between groups using the Chi-square test or Fisher's exact test, as appropriate, comparisons of quantitative parameters between groups using the Mann-Whitney U test, and comparisons between paired series (intra-group comparisons) using the Wilcoxon test for paired data.

This study was analysed in compliance with standard Good Clinical Practice regulations (ICH-E9). The database and statistical analysis plan were locked prior to unblinding. [26] All tests were two-sided and significance was assumed at $p < 0{\cdot}05$. SAS package release 9.4 (Institute INC, Cary, NC, USA) and R release 3.3.2 were used. This study is registered with ClinicalTrials.gov, number NCT03114215.

2.7. Role of the funding source

The sponsor designed the study with input from the authors and monitored its conduct. Data were obtained by investigators and analysed by the sponsor. The funder of the study was also involved in funding the participating centre and writing the manuscript. All authors had full access to all the data. All authors, including those employed by the funder, were involved in manuscript preparation and had control over the content, for which they take full responsibility and have given final approval for submission and publication.

3. Results

Study enrolment commenced in June 2016 and was completed in December 2016. We recruited a total of 30 patients from the Amyotrophic Lateral Sclerosis center, CHU Gui de Chauliac, Montpellier, France. Randomisation was performed for all the recruited patients. Patients were allocated (2:1) to MD1003 ($n = 20$) or placebo ($n = 10$). These 30 patients comprised the FAS, of which 27 completed the 6-month double-blind phase (ended June 2017). Fig. 1 provides a flowchart of the study. Three patients died before completing the double-blind phase of the study. One patient in the MD1003 group interrupted treatment for more than 10 consecutive days, which was a condition for exclusion from the PP set as a major deviation. However, this patient was included in the FAS population. Table 1 presents the baseline characteristics of the FAS, which were similar across treatment groups except for site of disease onset, with a predominance of upper limb onset in the MD1003-treated group.

Reported AEs, SAEs, and AEs leading to permanent discontinuation of study drug or withdrawal from the study were similar in nature and frequency between the treatment groups (Table 2). Two deaths occurred in the MD1003-treated group, one following percutaneous gastrostomy and the other due to respiratory failure; both deaths were judged by investigators to be due to ALS. One death, due to cardiac arrest, occurred in the placebo group. A total of 17 treatment emergent

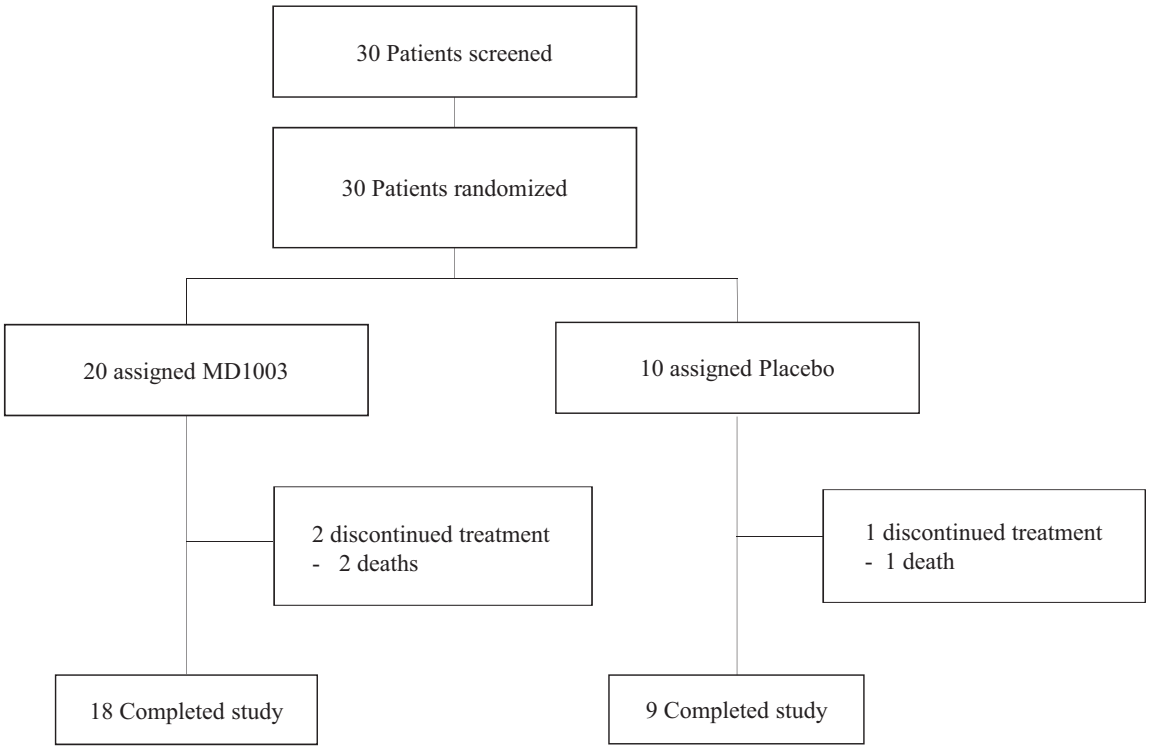

Fig. 1. Study flow chart.

Table 1
Baseline demographics and clinical characteristics.

	MD1003 group (n = 20)	Placebo group (n = 10)
Age at onset (years)	60·5 [46·0, 64·0]	61·5 [48·0, 66·0]
Sex ratio	14 / 6 (2·33)	7 / 3 (2·33)
Men	14 (70·0%)	7 (70·0%)
Women	6 (30·0%)	3 (30·0%)
ALS duration (months)	21·2 [15·5, 25·3]	22·3 [19·4, 25·0]
Weight loss*	−1·5 [−6·3, 2·7]	1·0 [−1·3, 2·9]
Body mass index (kg/m^2)	24·2 [23·1, 27·6]	25·1 [22·3, 27·3]
Type of disease		
Sporadic	18 (90·0%)	10 (100%)
Familial	2 (20·0%)	0
Site of onset		
Bulbar	3 (15·0%)	2 (20·0%)
Cervical	2 (10·0%)	2 (20·0%)
Upper Limb	7 (35·0%)	1 (10·0%)
Lower Limb	8 (40·0%)	5 (50·0%)
ALS diagnostic criteria		
Definite ALS	7 (35·0%)	3 (30·0%)
Probable ALS	12 (60·0%)	5 (50·0%)
Probable ALS-LS	1 (5·0%)	2 (20·0%)
ALSFRS-R total score	35·0 [29·0, 37·5]	36·0 [33·0, 38·0]
Slow Vital Capacity**	84·5 [71·0, 99·0]	97·0 [84·0, 106·0]
MIP (cmH2O)	59·0 [49·0, 84·0]	95·0 [47·0, 109·0]
SNIP (cmH2O)	52·0 [40·0, 78·0]	73·0 [61·0, 100·0]

Value are medians [IQR] or n (%). ALSFRS-*R*=Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised. MIP=Maximal Inspiratory Pressure. Probable ALS-LS=probable ALS laboratory-supported. SNIP=Sniff Nasal Inspiratory Pressure.
* Values are% of weight loss related to reference weight.
** Values are% of predictive value.

Table 2
Summary of adverse events.

	MD1003 group (n = 20)	Placebo group (n = 10)
Total number of TEAE	17	7
At least one TEAE		
All	12 (60·0%)	6 (60·0%)
Disease-related	4 (20·0%)	4 (40·0%)
At least one Serious TEAE		
All	4 (20·0%)	2 (20·0%)
Disease-related	1 (5·0%)	1 (10·0%)
At least one TEAE leading to death		
All	2 (10·0%)	1 (10·0%)
Disease-related	0 (0%)	1 (10·0%)
Most frequent adverse events		
Surgical and medical procedures	3 (15·0%)	0 (0%)
Infections and infestations	2 (10·0%)	1 (10·0%)
Injury and procedural complications	2 (10·0%)	1 (10·0%)
Nervous system disorders	2 (10·0%)	1 (10·0%)
Skin and subcutaneous tissue disorders	2 (10·0%)	0 (0%)
Vascular disorders	2 (10·0%)	0 (0%)
Cardiac disorders	0 (0%)	2 (20·0%)

Data are n (%). TEAE=Treatment Emergent Adverse Event.

AEs (TEAEs) were recorded in the MD1003 group, compared to 7 in the placebo group. Six (6) of the TEAEs were classified as serious and were equally distributed between treatment arms. Disease-related AEs, both serious and non-serious ones, were more frequently reported in the placebo group. Overall, AEs varied in type, without any predominance of a specific event. Median diastolic and systolic blood pressure remained within normal values and did not differ between the groups.

Median heart rate was 74·0 IQR [67·0, 83·5] bpm in the MD1003 group at baseline and raised to 79·0 IQR [69·0, 88·0] bpm at M6, while in the placebo group it was 81·5 IQR [74·0, 85·0] bpm at baseline and decreased to 80·0 IQR [76·0, 85·0] bpm at M6. Clinically significant values and clinically significant abnormalities were rare in both treatment groups throughout the study. At month 3, 1 patient in the placebo group, had a high triglyceride value 2·8-fold above the upper normal limit. At M6, the triglyceride value had returned to normal without further treatment.

Secondary outcome measures were parameters of ALS progression, including change from ALSFRS-R during the double-blind period (ie, M3 vs. M0 and M6 vs. M0). We compared these values to the change in ALSFRS-R during the 6 to 12 months before screening (pre-study period), which was also an inclusion criterion.

At screening, treatment groups were not completely balanced in terms of the rate of progression and disease severity. Worsening of ALSFRS-R score during the pre-study period (ie, between M-6/M-12 and screening) was significantly higher in the MD1003 group than in the placebo group (−6·0 IQR [−8·5, −5·0] vs. −5·0 IQR [−5·0, −3·0], respectively, p = 0·03). In addition, the severity score calculated at screening (M-1) showed a faster rate of disease progression in the MD1003 group compared to the placebo group (median severity score: −0·94 IQR [−1·37, −0·63] and −0·66 IQR [−0·81, −0·41], respectively, p = 0·04). This slight imbalance in the 2 study groups is most likely due to the randomisation of a small number of patients.

Between baseline and M6, the median change of ALSFRS-R score was −4·0, IQR [−10·0, −2·0] in the MD1003 group compared to −2·5, IQR [−8·0, −1·0] in the placebo group; the difference between the two groups was not significant (Table 3, p = 0·49). Additional analyses using a MMRM model showed that the adjusted mean (SE) difference between MD1003 and placebo at M6 was −3.6 (2.9); p = 0·23. Median change of ALSFRS-R severity score from pre-study slope to M6 was lower in the MD1003 group (−0·13 IQR [−1·12, 0·35]) compared to the placebo group (0·09 IQR [−0·64, 0·49]); the difference was not significant (Table 3, p = 0·18). Using a MMRM model, the adjusted mean (SE) difference between MD1003 and placebo at M6 of the ALSFRS-R severity score was −1.0 (0.8); p = 0·22.

Respiratory parameters are important for monitoring ALS progression, as respiratory failure is the main cause of death among patients with the disease. Median SVC declined from 84·5% (IQR [71·0, 99·0]) to 74·0% (IQR [56·0, 90·0]) between M0 and M6 in the MD1003-treated group; this decline was sharper in the placebo-treated group, from 97% (IQR [84·0, 106·0]) to 80·5% (IQR [69·2, 96·0]) (Table 4). The main effect of treatment on SVC over time was not significant (p = 0·45; MMRM). No significant difference between the two treatment groups was observed at M6 on the adjusted mean change from baseline (−13·5% ± 4·4% for MD1003 and −17·8% ± 6.0% for placebo, p = 0·58; MMRM); the adjusted mean (SE) difference between these two groups at M6 was 4·2 (7·5) [−11·2; 19·7]. Similar changes were observed for MIP; the adjusted mean change from baseline at M6 for the MD1003 group was −4·3 cm H2O ± 4·8 and −9·2 cm H2O ± 6·5 for the placebo group. The adjusted mean (SE) difference in MIP between the two treatment groups was 4·5 (8·1) [−11·8; 21·6] and was not significant (p = 0·55; MMRM). A trend towards less worsening of SNIP in the MD1003 group was observed: −7·6 cm H2O ± 4·4 vs. −20·4 cm H2O ± 6·0 for placebo, p = 0·10; MMRM. The adjusted mean (SE) difference in SNIP between MD1003 and placebo at M6 was 12·8 (7·5) [−2·6; 28·1].

At M6, clinical global impression (CGI) was evaluated by the investigator and subject global impression (SGI) was evaluated by the patients. These two evaluations, using the same scale independently, were similar. In 40% of the cases the impression was “much worse” for the MD1003 group compared to 25% in the placebo group, but the differences of global impressions between treated groups were not significant (CGI p = 0·25, SGI p = 0·26, Fig. 2).

4. Discussion

This phase II study evaluated the safety of MD1003 treatment in ALS patients. MD1003 was safe and well tolerated over the six-month double-blind treatment period. The number and type of AEs were similar between MD1003-treated patients and placebo-treated patients. Serious TEAEs were similar in incidence between treatment groups.

Secondary efficacy measures, focused on ALS disease progression, were not significantly different between MD1003-treated and placebo-treated groups. Importantly, the results did not show any evidence that MD1003 worsened disease progression or outcome. This is an encouraging point for potential future trials with this drug.

This trial was robust, as the study population was representative of the overall ALS population. The functional decline observed over 6 months in both groups was similar to that reported in other studies in ALS patients [24,25]. The absence of an effect of MD1003 on ALS disease progression was not surprising as this was a relatively small pilot study not powered to properly assess efficacy. Results on SNIP-measured respiratory muscle strength, one of the secondary outcome measures, showed a statistical trend in favour of MD1003. Although encouraging, this trend regarding SNIP data should be considered with caution. Previous work on ALS therapies have demonstrated inconsistencies between short term phase I/II trials and phase III trials. For example, in a phase IIb trial, the troponin activator tirasemtiv was associated with a 50% reduction of SVC worsening compared to

Table 3
ALSFRS-R global score and severity score.

	MD1003 group (n = 20)	Placebo group (n = 10)	MD1003 vs. Placebo*	p value
ALSFRS-R score				
Baseline median [IQR]	35·0 [29·0, 37·5]	36·0 [33·0, 38·0]		
M3 median [IQR]	32·5 [28·0, 36·0]	35·0 [25·0, 39·0]		
Median change from baseline [IQR] at M3	−1·0 [−3·0, 0·0]	−0·5 [−4·0, 0·0]		0·90**
M6 median [IQR]	29·5 [24·0, 33·5]	32·0 [25·0, 39·0]		
Median change from baseline [IQR] at M6	−4·0 [−10·0, −2·0]	−2·5 [−8·0, −1·0]		0·49**
Mean change from baseline				
Mean change (SD) at M3	−2·2 (3·8)	−4·1 (8·4)	1·9 (2·9)	
Mean change (SD) at M6	−7·1 (8·3)	−5·5 (8·0)	−1·6 (3·3)	
Adjusted mean change from baseline (MMRM)				
Adj. mean change (SE) at M3 [95% CI]	−2·8 (1·2) [−5·2, −0·4]	−2·8 (1·7) [−6·3, 0·8]	−0·1 (2·2) [−4·6, 4·5]	0·98***
Adj. mean change (SE) at M6 [95% CI]	−7·8 (1·6) [−11·1, −4·5]	−4·2 (2·3) [−9·0, 0·6]	−3·6 (2·9) [−9·6, 2·4]	0·23***
ALSFRS-R severity score				
M0, pre-study slope# median [IQR]	−0·8 [−1·2, −0·6]	−0·7 [−0·9, −0·5]		
M3 vs. M0 median [IQR]	−0·3 [−1·0, 0·0]	−0·2 [−1·2, 0·0]		
Median change from pre-study slope [IQR] at M3	0·4 [−0·1, 0·8]	0·3 [−0·2, 0·8]		0·50**
M6 vs. M3 median [IQR]	−0·9 [−1·7, −0·4]	−0·5 [−1·3, 0·0]		
Median change from pre-study slope [IQR] at M6	−0·1 [−1·1, 0·4]	0·1 [−0·6, 0·5]		0·18**

* MD1003 vs. placebo: mean difference (SEM) or adjusted mean difference (SE) in the treatment group versus control group and 95% CI.
** P-value of Mann-Whitney U test.
*** P-value from MMRM: Response = treatment + ALSFRS-R severity score between screening and baseline + visit + visit by treatment interaction + Log of time from first weakness to randomization.
\# Pre-study slope = slope between pre-study assessment (M-6/M-12) and randomisation (M0).

Table 4
Evolution of respiratory parameters.

	MD1003 group (n = 18)	Placebo group (n = 10)	MD1003 vs. Placebo*	p value**
Slow Vital Capacity (% predicted value)				
M0 median [IQR]	84·5 [71·0, 99·0]	97·0 [84·0, 106·0]		
M3 median [IQR]	85·0 [68·0, 92·0]	85·0 [75·0, 99·0]		
M6 median [IQR]	74·0 [56·0, 90·0]	80·5 [69·2, 96·0]		
Adjusted mean change from baseline (MMRM)				
Adj. mean change (SE) at M3 [95% CI]	−7·1 (4·3) [−15·8, 1·7]	−13·7 (5·7) [−25·5, −1·9]	6·6 (7·2) [−8·2, 21·4]	0·37
Adj. mean change (SE) at M6 [95% CI]	−13·5 (4·4) [−22·6, −4·4]	−17·8 (6·0) [−30·1, −5·4]	4·2 (7·5) [−11·2, 19·7]	0·58
Maximal Inspiratory Pressure (cmH2O)				
M0 median [IQR]	58·5 [48·0, 84·0]	88·0 [38·0, 109·0]		
M3 median [IQR]	66·5 [39·0, 92·0]	78·5 [31·0, 111·0]		
M6 median [IQR]	68·0 [36·0, 91·0]	57·0 [35·0, 97·0]		
Adjusted mean change from baseline (MMRM)				
Adj. mean change (SE) at M3 [95% CI]	−1·4 (4·3) [−10·3, 7·5]	−0·8 (5·8) [−12·7, 11·2]	−0·6 (7·3) [−15·6, 14·4]	0·94
Adj. mean change (SE) at M6 [95% CI]	−4·3 (4·8) [−14·3, 5·7]	−9·2 (6·5) [−22·6, 4·2]	4·5 (8·1) [−11·8, 21·6]	0·55
Sniff Nasal Inspiratory Pressure (cmH2O)				
M0 median [IQR]	52·0 [40·0, 78·0]	68·0 [27·0, 100·0]		
M3 median [IQR]	54·0 [42·0, 83·0]	51·5 [34·0, 103·0]		
M6 median [IQR]	48·0 [37·7, 56·0]	40·0 [16·0, 78·0]		
Adjusted mean change from baseline (MMRM)				
Adj. mean change (SE) at M3 [95% CI]	1·6 (4·3) [−7·2, 10·4]	−5·4 (5·7) [−17·2, 6·4]	7·0 (7·2) [−7·8, 21·7]	0·34
Adj. mean change (SE) at M6 [95% CI]	−7·6 (4·4) [−16·7, 1·5]	−20·4 (6·0) [−32·7, −8·1]	12·8 (7·5) [−2·6, 28·1]	0·10

* MD1003 vs. Placebo: adjusted mean difference in the treatment group versus control group and 95% CI.
** P-value from MMRM: Response = treatment + baseline value + visit + visit by treatment interaction.

placebo over 3 months. However, a subsequent, longer phase III trial did not confirm the initial results of the phase IIb trial [27]. In ALS studies, differences between phase I/II and phase III results may be amplified by the short duration of phase I/II studies as disease progression may be highly variable between patients. The very short-term, 3-month evaluation of tirasemtiv in the phase IIb trial, is the likely explanation for discrepancies between this trial and the subsequent Phase III study. The MD1003 in ALS trial evaluated treatment safety and efficacy over a longer period of 6 months.

This study has several limitations. This was an exploratory trial with a 6-month duration and a relatively small number of patients. Consequently, we cannot exclude the occurrence of adverse reactions with MD1003 over a longer time period or in a wider population of ALS patients. However, safety data collected here were consistent with the safety profile of MD1003 in multiple sclerosis trials; [19,20] in these trials, MD1003 was observed well tolerated up to 24 months.

In the present study, there were several differences at baseline between the MD1003-treated group and the placebo-treated group. In the placebo group, patients with upper limb onset were underrepresented, and those with a diagnosis of probable ALS laboratory-supported were overrepresented. At screening, the rate of ALSFRS-R decline was significantly lower in the placebo group. These differences are likely due to the small number of patients but may potentially have led to an underestimate of therapeutic effect. An additional caveat arises from the natural history of the recruited patients and may have amplified the disequilibrium described above. ALS worsening is highly variable from patient to patient, ranging from a few months to over 40 years. More than 20% of the patients may progress/worsen over more than 5 or sometimes 10 years, and this is an important concern in ALS trials [28]. Those patients with slow progression are less likely than other patients to exhibit changes in ALSFRS-R score, especially during a rather short, 6-month, trial. For

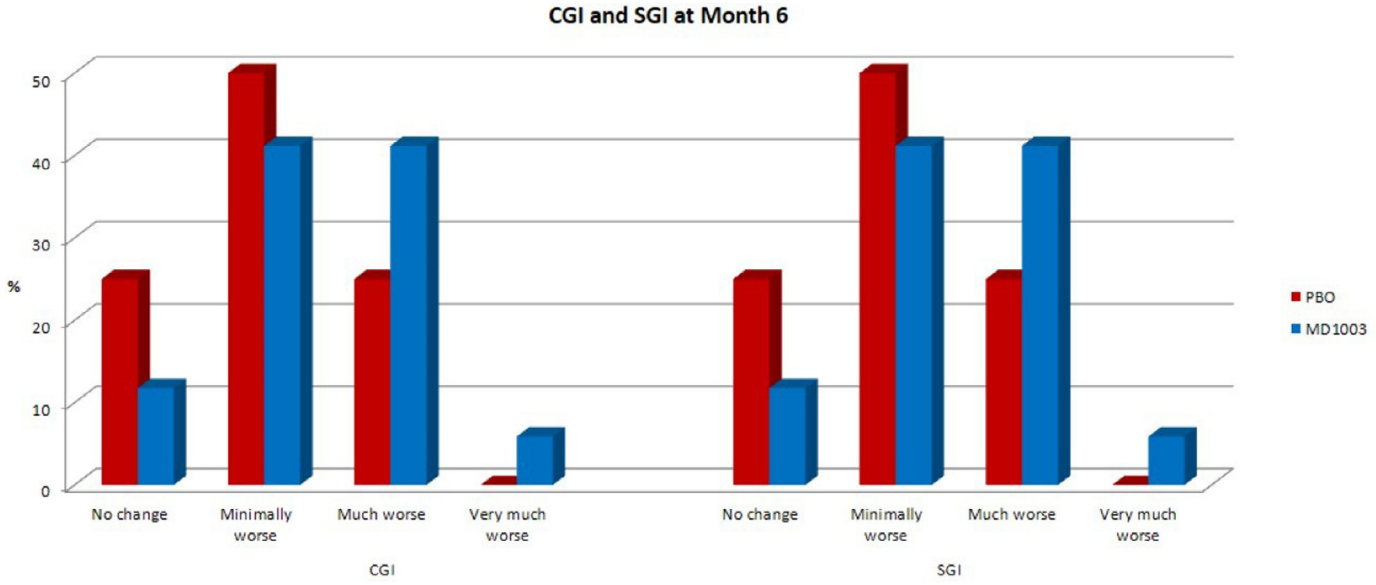

Fig. 2. Clinical global impression assessed by the clinician (CGI) and by the patient (SGI), at month 6. Placebo group (red) and MD1003 group (blue) were compared by Mann-Whitney test. For CGI and SGI, p values were 0·25 and 0·26, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

this reason, the ALSFRS-R slope has been used to help identify slow and fast progressors [28]. This has been taken into account in our trial and inclusion criteria were designed to avoid as far as possible recruiting slowly evolving patients, requiring a minimum of ALSFRS-R score decline of 5 points in the preceding year. Despite this cautious criterion, 5 patients in the total cohort were in fact slow evolvers. All but one of these patients with slow-progressing ALS were randomised in the placebo group (n = 4). We believe these elements are important ones to consider when analysing the effect MD1003 on ALS parameters of progression in the present trial. The recently published positive results with edaravone illustrate this point. The choice of inclusion/exclusion criteria, strictly selecting subjects with recently diagnosed ALS and significant motor impairment together with a clear worsening of the ALSFRS-R score before entry, allowed the study to demonstrate, over 6 months, a positive and significant effect on ALSFRS-R [29].

One method to control for highly variable disease worsening during the course of a trial would be to utilize biomarkers of ALS progression to select study participants. For example, polymorphisms in UNC13A have been reported to be associated with a more severe ALS prognosis and may be useful biomarkers of progression [30,31]. However, the influence of these variants on ALS disease course seem to be population-dependent, i.e., present in certain groups but not others. Additionally, the weakness of the associations between the UNC13A variants and ALS survival makes it difficult to use as a reliable selection parameter. Another biomarker, the serum concentration of neurofilament light chain (sNfL), may be useful for the inclusion of progressing (and exclusion of non-progressing) patients in an ALS trial. When measured using a highly sensitive single molecule array assay (Simoa), sNfL levels have been shown to be predictive of ALS prognosis, with low levels being associated with a median survival of over 5 years [32]. Several groups now routinely measure sNfL levels, but this assay was not available nor validated at the start of this trial. The sNfL marker should be considered for future trials, particularly in pilot trials with small groups of patients.

Finding new treatments in ALS is an unmet need. This study showed that MD1003 had a favourable safety profile in ALS patients and was well tolerated. However, the present trial was focused on safety and was not designed to demonstrate an effect on parameters of ALS disease progression; consequently, the trial was inconclusive about the efficacy of MD1003 in ALS. Importantly, no increased worsening of ALS was observed with MD1003 treatment. The imbalance in baseline clinical characteristics between treatment groups impeded the ability to discern differences between MD1003 and placebo in this small study of 30 patients. However, a trend towards lower progression of SNIP was observed in MD1003-treated patients. Although not conclusive, these are encouraging results that may help in designing future trials with MD1003 in ALS patients.

Declaration of competing interest

WC received personal fees from Actelion, Effik, Merck, MedDay Pharmaceuticals, Novartis Pharma, Roche, and Sanofi. AB and FS are employees of MedDay Pharmaceuticals. RJM, NP and SA have nothing to disclose. SS was funded by MedDay Pharmaceuticals.

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Data sharing statement

Individual anonymised participant data and relevant clinical study documents will be available during a period beginning 9 months and ending 36 months following article publication. Data will be shared with qualified scientific and medical researchers as necessary for conducting legitimate research. To request access to the data and submit a research proposal, please send a request to: dataresquest@meddaypharma.com. Research proposals will be reviewed and approved by MedDay Pharmaceuticals depending on the qualifications of the researchers and the legitimacy of the research. Approved requestors will need to sign a data-sharing agreement to gain access to the data.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2019.100254.

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