

TITLE: Efficacy and Safety of MD1003 (pharmaceutical grade, high-dose biotin) in progressive multiple sclerosis (SPI2): a phase 3, randomised, double-blind, placebo-controlled trial.

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Research in Context

Evidence before study

We searched PubMed for English language articles published from Jan 1, 1980 to May 1, 2020, using the keyword “progressive multiple sclerosis” for clinical trials of disease modifying treatments in both primary progressive and secondary progressive multiple sclerosis and studies of high dose biotin and relevant outcome measures. A multitude of phase 2 and phase 3 studies were identified. Well-conducted studies of potent anti-inflammatory treatments such as natalizumab (ASCEND) and fingolimod (INFORMS) failed to show convincing benefits in slowing disability worsening in progressive multiple sclerosis (MS). Only three phase 3 clinical trials were used to support commercial registration: MIMS (mitoxantrone), ORATORIO (ocrelizumab) and EXPAND (siponimod). Each of these medications’ proposed mechanism of action is through reducing neuroinflammation. Although all three treatments reduced disability worsening none of these medications was shown to reduce disability that was already present. To date, the MS-SPI study was the only successful phase 3 trial using a medication thought to work through a mechanism other than immune modulation/immune suppression. MD1003 (high dose pharmaceutical grade biotin) was proposed to activate acetyl-CoA carboxylases and pyruvate carboxylase to potentially augment ATP production and/or to enhance myelin repair or synthesis. The MS-SPI study found that, compared to placebo, MD1003 improved a disability endpoint that was a composite of the Expanded Disability Status Scale (EDSS) and the timed 25-foot walk (T25W).

Added value of study

The SPI2 trial was designed to replicate and extend upon the observations made in MS-SPI: to improve disability outcomes in non-relapsing, progressive forms of MS. Although the study

recruited a large, international cohort of persons with either primary or secondary progressive MS who appeared to be representative of the targeted patient population, SPI2 failed to meet its pre-specified primary, secondary or exploratory outcomes. Treatment emergent adverse events were infrequent with MD1003. MD1003 was not associated with increased MS relapse risk.

Implications of all available evidence

Despite the positive results of the MS-SPI study, the larger and more representative SPI2 trial findings do not support use of high dose biotin to promote energy metabolism in progressive MS. Moreover, high doses of biotin are known to generate inaccurate laboratory test results when using immunoassays based on a biotin/streptavidin interaction. High dose biotin when used off-label could lead to deleterious health consequences to patients from misleading laboratory tests that, in turn, could lead to inappropriate medical interventions such as mismanagement of thyroid or cardiac conditions. Therefore, MD1003 and by extension, off-label use of commercially available high dose biotin, do not have a role in treating progressive MS.

SUMMARY

Background:

There is an unmet need to develop therapeutic interventions directed at the neurodegeneration that underlies progression in multiple sclerosis (MS). High-dose, pharmaceutical-grade biotin (MD1003) may enhance neuronal and oligodendrocyte energetics resulting potentially in improved cell function, repair or survival. The MS-SPI study was a randomized, double-blind, placebo-controlled study that found that MD1003 improved disability outcomes over 12 months in progressive MS. The SPI2 study was designed to assess the efficacy and safety of MD1003 in progressive forms of MS in a larger, more representative patient cohort..

Methods:

SPI2 was a randomised, double-blind, parallel-group, placebo-controlled trial of MD1003 (biotin 100 mg, by mouth, three times daily) in participants with primary and secondary progressive MS (PPMS, SPMS) conducted at 90 sites across 13 countries. The randomisation list was generated with a 1:1 randomisation ratio and stratified by study site and disease history (PPMS / SPMS). The treatment numbers were provided at each visit through a website. Participants, investigators and assessors were masked to treatment assignment. Key inclusion criteria were age 18-65, expanded disability status scale (EDSS) 3·5-6·5, timed 25 foot walk (TW25) < 40 seconds, with clinical disability progression and without relapses in the 2 years prior to randomization. Concomitant disease modifying therapies were allowed. The primary endpoint was a composite of the proportion of participants with confirmed improvement in either the EDSS, or the TW25 at month (M) 12 confirmed at M15, compared to baseline. The primary endpoint was assessed on the intention-to-treat analysis set by logistic regression only after all participants in the trial completed the month 15 visit. The safety analysis included all participants who received at least

one dose of investigational product. This trial is registered with ClinicalTrials.gov (NCT02936037).

Findings

From February 22, 2017 and June 8, 2018, a total of 642 participants were randomised, 326 to MD1003 and 316 to placebo. The study completed when the primary endpoint for the last-entered participant was assessed on November 15, 2019. The mean (standard deviation) time on study was 20·1 (5·3) months (range 15 months to 27 months). For the primary outcome, 12·0% (39/326) of the MD1003-treated participants compared to 9·2% (29/316) of placebo-treated participants improved at M12 confirmation at M15 (Odds Ratio 1·35; 95% CI: 0·81, 2·26). MD1003 was well tolerated. Treatment emergent adverse events occurred in 277/331 (83·7%) participants in the MD1003 group and in 264/311 (84·9%) in the placebo group. One death occurred in the MD1003 arm and none in the placebo group. Despite use of mitigation strategies, MD1003 led to interference of laboratory test results that could have caused adverse events.

Interpretation

This rigorously conducted, randomized controlled trial showed that MD1003 did not significantly improve disability or walking speed in progressive MS. Potential limitations include the study-site geographic distribution and recruitment of a predominantly white participant cohort. MD-1003, or other preparations of high-dose biotin are not recommended for treatment of MS due to lack of efficacy and the potential for deleterious health consequences from interference of laboratory tests.

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INTRODUCTION

Both secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS) are characterized by worsening of neurologic disability independent of clinical relapses.¹ All approved MS disease-modifying therapies (DMT) reduce acute inflammatory activity by primarily targeting cellular components of the adaptive immune system.²⁻⁴ Some of these treatments reduce disability worsening in active SPMS and PPMS.^{5,6} However, the progressive disability observed in not-active PPMS or SPMS (i.e. no recent relapses and/or MRI activity) may not be mediated solely by immunologic activity. Randomized controlled trials of natalizumab in SPMS and fingolimod in PPMS failed to find a significant impact of these drugs on disability despite their potent anti-inflammatory effects.^{7,8} In addition to neuroinflammation, the pathophysiology of progressive MS includes: energy depletion through mitochondrial dysfunction, oxidative stress, microglia activation, iron accumulation, impaired remyelination, and apoptosis.⁹⁻¹⁴ These processes are not directly targeted by immunotherapies and could be targets for other treatments.

MD1003 is a high dose oral formulation of pharmaceutical grade biotin.^{15,16} Biotin is a cofactor for both acetyl-CoA carboxylase isoforms, which are expressed in oligodendrocytes, for 3-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase, which are both expressed in neurons, and for pyruvate carboxylase.^{17,18} MD1003 may activate these carboxylases to potentially augment ATP production or to enhance myelin repair or synthesis - pathways that may potentially protect against neuronal degeneration.^{15, 18 - 20}

The safety and efficacy of MD1003 was suggested in an open-label pilot study.¹⁵ This led to the MS-SPI clinical trial: a multicentre phase III study of adults with PPMS, or SPMS with no relapse activity in the previous year.¹⁶ MS-SPI was a 12-month, 2:1 randomised, double-blind, placebo-controlled study in which participants (N=154) received either MD1003 100 mg or placebo thrice daily (tid).¹⁶ The primary endpoint was the proportion of patients with disability improvement as measured by the expanded disability status score (EDSS) or timed 25-foot walk (TW25) at month 9 confirmed at month 12. MD1003 was shown to improve this measure of MS-related disability in 12.6% (13/103) of participants, compared to none (0/51) of the placebo participants ($p=0.005$). Mean change in EDSS, clinician-assessed clinical global score (CGI), and subject-assessed clinical global score (SGI) were also significantly improved in MD1003-treated participants compared to placebo participants. MD1003 was well tolerated and there were no safety signals identified in the study. The SPI-2 study was designed to replicate and extend upon these observations in a larger, international cohort.

Here, we report safety and efficacy results from the multicentre, randomised, double-blind, placebo-controlled, phase 3 trial of MD1003 (SPI2) in progressive MS participants without recent relapse.

METHODS

Study design and participants

SPI2 was a randomised, double-blind, parallel-group, placebo-controlled study. The study was conducted at 90 sites (academic and community MS clinics) in 13 countries (Figure S1, appendix page 8). Parexel International, an independent clinical research organization conducted the trial.

The Institutional review boards or ethics committees approved the protocol and amendments at all 90 study centres. The primary outcome was improvement at month (M) 12 confirmed at M15. All participants remained in the placebo-controlled portion of the study until the last randomised participant reached M15 at which point all participants were switched to active drug on the next scheduled visit while remaining blinded to initial randomization. As a consequence the duration of the placebo-controlled portion of the study ranged from 15 to 27 months. This design element was intended to increase the statistical power for the secondary endpoint of time to confirmed EDSS progression.

An independent Clinical Adjudication Committee confirmed certain eligibility criteria and relapse occurrence. Major protocol deviations prior to the M15 visit occurred in 12.3% (79/642) of participants and were comparable between the treatment groups. Deviations involved: procedures or tests (32), disallowed medications (25), inclusion/exclusion criteria (24), non-compliance with study drug (22), visit schedule (4) and IP administration or safety issues (2). Protocol amendments did not affect recruitment or conduct of the randomized controlled trial. The study protocol is available at: <https://ucsf.box.com/s/3gxbn4igvquu0i7byh7j9y99zgatbcda>

Key study eligibility criteria included: age 18-65 years; diagnosis of PPMS or SPMS fulfilling the revised International Panel criteria (2010) and Lublin criteria (2014)¹, EDSS score 3·5 to 6·5; and documented evidence of clinical disability progression within the 2 years prior to inclusion, as defined by (a) progression of EDSS during the past two years of at least 1 point sustained for at least 6 months (EDSS, 3·5 to 5·5) or at least 0·5 point increase sustained for at least 6 months (EDSS, 6 to 6·5) or (b) increase of TW25 by at least 20% in the past two years sustained for at

least 6 months or (c) other well-documented objective worsening that required confirmation by the Clinical Adjudication Committee (appendix). Concomitant DMTs were allowed. Key exclusion criteria were clinical evidence of a relapse in the 2 years prior to inclusion and concomitant treatment with fampridine. Full inclusion and exclusion criteria are provided in (Table S1, appendix page 4). Written informed consent was obtained from all participants. SPI2 was conducted in accordance with applicable laws and regulations including the International Conference on Harmonization Guidelines for Good Clinical Practice, the Declaration of Helsinki, and privacy laws.

Randomization and masking

At randomization visit (V2/M0), participants were randomised centrally to one of the two treatment arms, MD1003 or placebo, at M0. A Parexel International statistician who had no contact with any study investigators generated the underlying 1:1 randomisation list that was stratified by centre and MS disease history (SPMS/PPMS). An Interactive Web Response System controlled assignment and provided the treatment numbers at each visit. Allocation was not revealed until after the study end. Blinded investigators dispensed coded investigational product to participants after verifying eligibility and obtaining informed consent. MD1003 and placebo were indistinguishably encapsulated. Participants, outcome raters, and treating physicians were blinded to treatment assignment. Treating physicians could not function as raters. Treating physicians and participants were surveyed regarding treatment assignment at M15 to assess blinding efficiency.

Procedures

Participants received either oral MD1003 (100 mg pharmaceutical-grade biotin) or placebo thrice daily throughout the course of the study. An overview of SPI2 evaluations is provided in Figure S1 (appendix page 8). Key evaluations included: EDSS and TW25 every 3 months from M0 to M27; MRI at M0, M6, M15, and M27; SDMT, MSQoL-54, CAREQoL-MS at M0, M15, and M27; CGI/SGI at M15 and M27; ambulatory activity (daily step count) was monitored continuously using a wrist-worn accelerometer (FitBit®).^{21, 22} Biotin blood levels were assessed at M0, M6, M12, M15, and M27. NeuroRx Research (Montreal, Canada) performed the MRI analysis. The NIH-funded Eureka Research Platform (U2CEB021881) enabled step count collection and the STEPS CORE facility interpreted the data (San Francisco, CA, USA). University Hospital Basel (Basel, Switzerland) measured serum neurofilament light (NfL) levels.

Neurostatus-certified raters, who were blinded to both treatment assignment and patient history, performed the primary outcome assessments. For TW25 assessments, participants were permitted to use their own assistive device or the best assistive device that was available. If a device was necessary (EDSS \geq 6) at the inclusion visit, the device was utilized during all the subsequent follow-up visits. Further procedural details and specific information on evaluation scales can be found in the SPI2 Protocol v. 4.0 available at:

<https://ucsf.box.com/s/3gxbn4igvquu0i7byh7j9y99zgatbcda>.

Outcomes

Primary

The primary endpoint was the proportion of participants with improvement of MS-related disability at M12 confirmed at M15. Improvement was defined as either a decrease from

baseline in EDSS of ≥ 0.5 point or ≥ 1.0 point (if baseline EDSS 6-6.5 or 3.5-5.5, respectively), or a decrease from baseline TW25 of at least 20%. Baseline EDSS was defined as the lowest (best) EDSS obtained at M-1 and M0. Baseline TW25 is defined as the lowest mean of two TW25 attempts performed at M-1 and M0.

Secondary and exploratory endpoints

There were 4 hierarchically-ordered secondary endpoints: 1) time to 12-week confirmed EDSS progression where progression was defined as an increase from baseline in EDSS of ≥ 0.5 point or ≥ 1.0 point (if baseline EDSS 6-6.5 or 3.5-5.5, respectively), 2) mean difference between treatment arms in CGI at M15, 3) mean difference between treatment arms in SGI at M15, and 4) percentage change in mean TW25 between M0 and M15.

Exploratory endpoints included MRI volumetric measures, mean change in remote monitoring of ambulatory activity (FitBit), mean change in quality of life (QoL) as measured by MSQoL-54 (patient) and CAREQoL-MS (caregiver), mean change in Kurtzke subscores, mean change in Symbol Digit Modalities Test (SDMT) score, and mean change in sNfl concentration.

Safety

To monitor for any signal of increased inflammatory activity associated with MD1003, all emergent possible relapses were classified as a serious adverse event and reviewed by a blinded Clinical Adjudication Committee using primary documentation. Furthermore, MRI was monitored for new Gd+ T1 lesions and new/enlarging T2 lesions on study. Other safety evaluation including: the recording of adverse events (AEs), laboratory testing,

electrocardiograms (ECG) and assessment of suicidal ideation or behaviour using the Columbia-Suicide Severity Rating Scale (C-SSRS). An external data safety monitoring committee periodically reviewed all safety data during the course of the study.

Statistical analysis

In SPI2, the sample size was determined in terms of superiority of MD1003 versus placebo, according to the primary endpoint of improvement in MS-related disability (either EDSS or TW25). Although there were no improvements in the placebo arm of MS-SPI, the sample size hypotheses for the proportion of participants with clinical improvement in the placebo group was set at 3% and at 12% in the MD1003 group based on results from MS-SPI. These proportions resulted in a sample size of 200 participants in each group for a two-sided Cochran-Mantel-Haenszel test set to 0.05 significance level with 90% power. After discussion with the FDA, the sample size was increased to 300 participants in each group to better assess SPMS and PPMS subgroups.

The intention-to-treat (ITT) analysis set consisted of all randomised participants. Statistical analyses were based on treatment assignment, regardless of the actual treatment received. The safety analysis set comprised all participants who received at least one dose of study treatment. If a participant received any dose of MD1003 or any biotin plasma concentration was above 75 ng/dl, the participant was assigned to the MD1003 treatment group within the safety analysis set; all others were assigned to the placebo treatment group.

For the primary endpoint, participants with missing data precluding evaluation were considered non-responders. For the primary endpoint analysis, a logistic regression model with categorical fixed factors (randomised study treatment, disease history as SPMS or PPMS, and geographical region) was used to estimate and test the study treatment effect. Efficacy was expressed as corresponding response probability odds ratio (a value >1 indicates a favourable effect of MD1003 compared to placebo).

For the first secondary endpoint, time to 12-week confirmed EDSS progression, the hazard ratio (MD1003/placebo) was estimated using a Cox proportional hazard model stratified by disease history and geographic region to estimate and test the effect of MD1003 relative to placebo. Missing data for the secondary analysis was imputed using predefined rules delineated in the Statistical Analysis Plan v 7.0.

To assess the treatment effect of the primary and secondary efficacy endpoints, subgroup analyses defined by the following baseline characteristics were performed: MS disease history; geographical region: North America / Australia or Europe; EDSS at baseline: “up to 5.5” or “6 or above”; age: \leq overall median or $>$ overall median; sex: women or men; concomitant physical therapy (no or yes); BMI: \leq overall median or $>$ overall median; use of rituximab or ocrelizumab at the date of randomization (no or yes); use of other DMTs at the date of randomization (no or yes); use of anti-spasticity drugs at the date of randomization (no or yes). The analyses were replicated in the subgroups separately and interaction tests were not performed. Sensitivity analyses that examined the influence of various analysis populations, handling of missing data

(i.e., imputation with pre-defined rules), and/or pre-specified potential effect modifiers, were undertaken for the primary and secondary endpoints.

The comparison of CGI/SGI scores at M15 and the mean change in TW25 between M0 and M15 were assessed using a non-parametric Van Elteren test, stratified on disease history and geographical region with significance defined as $p < 0.025$ thereby allowing for a one-sided test.

A Parexel International statistician performed all analyses using SAS[®] version 9.3 or later (Cary, North Carolina, USA). An independent Data Safety Monitoring Board reviewed safety data and provided guidance throughout the study. This study is registered with ClinicalTrials.gov (NCT02936037) and the EudraCT database (2016-000700-29).

Role of Funding Source

The sponsor initiated and funded the study and provided the first draft of the manuscript. The sponsor was involved in the study design, data collection, data analysis and interpretation, and reviewed the manuscript. The authors include academic members of the study steering committee and sponsor employees. The authors had full editorial control of the manuscript and approved the final content. The corresponding authors had full access to the study data and had final responsibility for the decision to submit the manuscript for publication. Requests for access to study data should be directed to the corresponding author. Data access requests will be considered by the steering committee and will be granted to suitably qualified investigators.

RESULTS

Between February 22, 2017 and June 8, 2018, a total of 766 persons with PPMS and SPMS were screened. Of those screened, 101 were ineligible, 665 were eligible and 642 were randomised to study treatment (1:1): 326 to MD1003 and 316 to placebo. The primary endpoint was assessed in 261 MD1003 and 267 placebo participants (Figure 1). The Clinical Adjudication Committee confirmed baseline characteristics consistent with worsening MS without clinical evidence of a relapse in the previous 2 years (Table 1). The randomized controlled period ended on November 15, 2019 after the last patient who entered the trial completed the Month 15 visit.

Baseline demographics and clinical characteristics were generally well balanced across treatment arms (Table 1). For the 642 randomised participants, mean age (SD) was 52.7 (7.7) years, 53.7% (345/642) were women, 64.6% (415/642) were diagnosed with SPMS, mean time since initial diagnosis of MS was 12.6 (8.5) years, mean EDSS score was 5.4 (1.0), and mean TW25 was 11.7 (7.3) seconds. Of randomised participants, 57.6% (370/642) required a walking aid (EDSS 6 or 6.5). Overall, 72.0% (462/642) of participants were previously treated with DMTs, 45.8% (294/642) were receiving concomitant DMTs at randomization, and 5.2% (33/642) had at least one gadolinium-enhancing lesion on MRI.

The primary outcome measure at M15 was evaluated in 86.9% (558/642) of participants. For the duration of the entire double-blind portion of the study, 19.9% (65/326) of participants in the MD1003 and 15.5% (49/316) of participants in the placebo group, discontinued treatment (Figure 1). The overall mean duration of the double-blind portion of the study was 20.1 (5.3) months. The most common reasons for discontinuation were withdrawal of consent, discontinuation due to adverse event (AE), and lack of perceived efficacy by the participant

(Figure 1). Mean percentage of overall compliance was 96.4 (12.03) and was similar between MD1003 and placebo groups.

The proportion of participants with improvement in either EDSS or TW25 at M12 confirmed at M15 was 12.0% (39/326) in the MD1003 group and 9.2% (29/316) in the placebo group (odds ratio [OR] 1.35; 95% confidence interval [CI]: 0.81, 2.26) (Table 2; Figure S2, appendix page 9). For the EDSS component of the primary endpoint, the proportion of responders was 6.7% (22/326) and 6.3% (20/316) in the MD1003 and placebo groups, respectively (OR 1.07; 95% CI: 0.57, 2.02) (Table 2; Figure S2, appendix Page 9). The proportion of responders for the TW25 component of the primary endpoint was 6.7% (22/326) versus 3.5% (11/316) in the two treatment groups, respectively (OR 2.02; 95% CI: 0.98, 4.39, Table 2). Five participants in the MD1003 group and two in the placebo group improved on both EDSS and TW25 components of the primary endpoint (Table S2, appendix page 5).

During the double-blind phase of the study, 18.4% (60/326) of MD1003 group and 19.6% of the placebo group (62/316) experienced 12-week confirmed EDSS progression. For the time to 12-week confirmed EDSS progression analysis, MD1003 did not significantly differ from placebo (hazard ratio [HR] 0.97; CI: 0.680, 1.385) (Table 2; Figure S3, appendix page 10). Pre-specified subgroup analyses of time to 12-week confirmed EDSS progression suggested a positive effect of MD1003 in the low BMI subgroup (O.R. 1.76, 95% CI 1.05, 2.95) with a corresponding negative effect in the high BMI group (O.R. 0.60, 95% CI 0.36, 1.00 [O.R.s and 95% CI were calculated separately and an interaction p-value is not available]). Trends for other subgroups were not observed. (Figure S4, appendix page 11).

For the pre-planned analysis of CGI assessed at M15, the difference in scores between MD1003 and placebo groups did not differ (probability of a better outcome with MD1003 was 0.505) (Figure S5 [appendix page 12]; Table 2). For SGI assessed at M15, a difference was not observed between the study arms (probability for better outcome with MD1003 was 0.515) (Figure S5 [appendix page 12]; Table 2).

The percentage change in mean TW25 (SD) between M0 and M15 was 20.7 (52.1) in the MD1003 group and 22.5 (53.4) in the placebo group (Figure S6 [appendix page 13]; Table 2). The between-group difference in the percentage change in mean TW25 (0.30) was not statistically significant.

Analysis of the mean change from baseline to M15 in the average of daily step counts during the 3 weeks prior to each visit (assessed via remote monitoring of ambulatory activity) resulted in less loss of steps per day favouring placebo although the difference was not significant (least square difference in means of -224.42 (CI: -510.32, 61.45) (Table S3, appendix page 6).

Several other exploratory endpoints with direct bearing on the primary and secondary analysis including SDMT, MSQOL-54 (PCHs and MHCS), CAREQoL-MS, serum neurofilament levels, and a range of MRI measures were assessed. There were no significant differences between the two treatment arms in any of the exploratory analyses (Table S3, appendix page 6).

The most frequently reported serious adverse event was MS relapse (Table 2). Relapses occurred in 8·8% (29/331) MD1003 group and 10·0% (31/311) of the placebo. In total 20 adjudicated relapses occurred in the MD1003 group and 25 in the placebo group resulting in an annualized relapse rate of 0·036 and 0·048, respectively (Figure S12-S13, appendix page 19-20).

MRI measures of disease activity were included as safety measures. At M15, there were no notable differences between MD1003 and placebo in the number of participants with new or enlarging T2 lesions or the number of participants with gadolinium-enhancing lesions (Table 3).

During the double-blind phase of SPI2 safety signals associated with MD1003 were not observed. 26·3% (87/331) of MD1003 and to 26·4% (82/311) of placebo participants experienced ≥ 1 serious treatment emergent adverse event (TEAE) during the double-blind phase of the study (Table 3). Serious infections occurred in 3·3% (11/331) of MD1003 and in 6·8% (21/311) of placebo participants. Skin disorders were observed in 13·9% (46/331) of MD1003 and in 10% (31/311) of placebo participants. One seizure occurred in each group. Two malignancies occurred in the MD1003 group versus five in the placebo group. Similarly, suicide risk, complete blood counts, clinical chemistries, and electrocardiogram measurements did not differ between groups. There was one sudden death in the study in an individual with multiple vascular risk factors; this death was in the MD1003 arm and considered as not related to the study drug by the investigator.

Administration of high doses of biotin is known to generate inaccurate and misleading laboratory test results when using immunoassays based on a biotin/streptavidin interaction.^{23, 24} Patients on

high dose biotin are therefore at risk of clinical decisions being made based on misinterpretation of inaccurate laboratory values. SPI2 employed multiple risk mitigation interventions to reduce the occurrence of misinterpretation of abnormal laboratory values including a central laboratory and multiple participant and health care provider educational interventions. Despite these initiatives, 25 inaccurate laboratory results occurred. The most frequent error was mischaracterizing hyperthyroid (TSH spuriously low with T4 spuriously high) in euthyroid participants.

Post-hoc analyses showed apparent improvements in the MD1003 group for the primary endpoint at some time points after M12 and on time to confirmed disability improvement (Figure S7-S10 [appendix page 14-17]).

DISCUSSION

The SPI2 study did not meet the primary or secondary endpoints and could not replicate the MS-SPI study that found a beneficial effect of MD1003 on MS disability. Over the mean study duration of 87 weeks, MD1003 did not significantly influence MS-related disability trajectories compared to placebo. Analyses of secondary endpoints showed no difference between MD1003 and placebo. Moreover, exploratory analyses using biomarkers of neuronal injury (sNFL), MRI markers of axonal integrity (MRS) and a sensitive measure of ambulation (daily step counts) found no differences between the treatment groups and exclude a neural protective role of biotin. Taken together these results did not find a beneficial effect of MD1003 in MS.

That SPI2 did not replicate the observations from the smaller MS-SPI study¹⁶ was unexpected. Given that SPI2 was a substantially larger trial conducted in a more diverse patient population it seems more likely that the favourable observations from MS-SPI were due to a type 1 error (rejection of a true null hypothesis or falsely positive finding) rather than a type 2 error (non-rejection of a false null hypothesis or falsely negative finding) in SPI2. Therefore, we conclude that biotin is ineffective in progressive MS.

To our knowledge, until MS-SPI¹⁶, studies designed to improve disability outcomes in progressive MS had not been conducted; therefore, estimates of the anticipated placebo effect were based on MS-SPI. With respect to the primary outcome SPI2 was initially designed anticipating a 3.0% placebo response based on the 0.0% observed rate in MS-SPI. Zero event rates sometimes reflect an insufficient sample size to ascertain an accurate point estimate of the true rate. The 95% upper confidence interval for the placebo group from SPI was 5.8%. Had this rate been used to plan SPI2 then the sample size would require 442 patients per group at 12 months for the comparison of 5.8% versus 12%. However, in SPI2, the placebo response rate was 9.2%. Therefore, even with a larger sample size SPI2 would not have met its primary endpoint. It appears that the placebo effect was more robust in SPI2 compared to MS-SPI possibly due to expectations for improvement or other factors resulting in somewhat different study populations. That different placebo-treated groups have variable performance in MS clinical trials is a well-recognized phenomenon.

If MD1003 had a small benefit with a ceiling effect, then a stronger placebo effect in SPI2 might reduce measurable differences between the treatment arms and might account for the different

results observed in MS-SPI and SPI2. However, the numeric difference in favour of MD1003 observed for the primary endpoint was driven by the TW25 with no effect of MD1003 on EDSS suggesting that if MD1003 has any effect in MS such an effect may be symptomatic.

Predefined subgroup analyses showed point estimates of odds ratios favouring MD1003 over placebo in 19 of 20 subgroups, although none were significant. Participants with lower EDSS (3·5 to 5·5), lower BMI ($\leq 25\cdot6$), younger age, concomitant DMT treatment, and female gender appeared to have a greater response compared to those in the opposing subgroups. Participants with lower BMI appeared to have a favourable response to treatment for 12-week confirmed disability progression; however, this possible benefit is offset in participants with a higher BMI who appear to benefit from placebo. Taken together these analyses do not provide supportive data for a clinically meaningful effect of MD1003 in any particular subgroup.

The rate of 12-week confirmed disability progression in both arms of SPI2 was slower than in MS-SPI¹⁶. Over the course of the 20.1 mean months of the randomized controlled period, 18·4% and 19·6% of the MD1003 and placebo groups respectively, experienced confirmed disability progression. To help place this finding in context, the rates of confirmed disability progression (CDP) at ~20 months were approximately: 28% in MAESTRO-1 (MBP8298 in SPMS)²⁵, 25% in ORATORIO (ocrelizumab in PPMS)⁶, 15% in ASCEND (natalizumab in SPMS)²⁶ and 33% in EXPAND (siponimod in SPMS).²³ The relatively slower rates of progression in SPI2 might be attributed to concomitant DMTs or could be characteristic of non-relapsing, progressive patients, a population that has not been previously well studied or due to an expectation bias that could have influenced the placebo group. Slower rates of progression could require longer intervals of

observation in order to detect a therapeutic effect of MD1003. Reversal of neuroaxonal pathology, including chronic demyelination and neurodegeneration - core determinants of disease progression in MS - may necessitate longer periods of treatment exposure.^{27, 28} Nonetheless, the absence of beneficial effects on brain atrophy or serum neurofilament levels argue against MD1003 having neural protective properties.

The study enrolled PPMS and SPMS participants with documented, relapse-free disability progression in EDSS over the 2 years before entry. Only 5.2% of (33/642) randomised participants had ≥ 1 gadolinium-enhancing lesion at baseline. This low rate of gadolinium enhancing activity is consistent with successful recruitment of participants in whom progression may not be driven primarily by overt inflammatory activity. SPI2 participants also had a significant burden of disability at baseline reflected by baseline values of EDSS, TW25, SDMT, and MSQoL-54 (quality of life). Those participants enrolled in SPI2 also had an extensive history of prior DMT use and approximately 50% of study participants were receiving DMT treatment at randomization. Taken together, the SPI2 cohort's baseline characteristics are consistent with those of patients with non-active progressive forms of MS.

Overall, MD1003 was well tolerated in the SPI2 study and the observed safety profile was similar to that in previous studies.^{15, 16} No new or unexpected safety signals were observed with MD1003 over the course of this study. Importantly, in contrast to one published study²⁹ and a conference abstract,³⁰ which reported association of disease activity with biotin supplementation, MD1003 was not associated with a higher rate of MS relapse or MRI activity in SPI2, although these observations likely were influenced by concomitant DMT. Despite a mitigation plan,

MD1003 was associated with interference of laboratory studies and this effect can lead to misinterpretation of test results that could have serious deleterious health consequences.

Despite SPI2's rigorous conduct, the study has several limitations. Design limitations include a relatively short duration for assessment of the primary endpoint, inaccurate estimation of a placebo effect and the allowed use of disease modifying therapies that might have confounded the results. Further, structural imaging of the retina was not performed and MRI of the spinal cord was conducted only in a subset of sites as an exploratory endpoint. This multinational study was conducted exclusively in Europe, North American and Australia. Ideally, sites from other geographic areas would be included. Participation of non-white races was limited in part due to study geography.

In conclusion, this study found no significant difference between MD1003 and placebo with respect to the primary or secondary endpoints. Any observations about potential beneficial effects of MD1003 in certain subgroups or time points are outweighed by the potentially harmful impact of laboratory studies falsely altered by high biotin serum concentrations. Therefore, MD1003 and other forms of high dose biotin are not recommended in MS.

ADDITIONAL INFORMATION

Contributors

BC, GC, RM, FS, and FL were involved in study conception and design. BC, GC, JW, MF, GG, GG, HPH, DA, JK, VB and FL were study investigators. MedDay and Parexel International performed the analysis. All authors contributed to data interpretation. BC, GC, RM, FS, and FL drafted the manuscript. All authors reviewed and revised the manuscript.

Declaration of interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article.

BACC has received personal compensation for consulting from Akili, Alexion, Atara, Biogen, EMD Serono, Novartis, Sanofi and TG Therapeutics.

GC has received personal compensation for participation in Data and Safety Monitoring Boards:

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Vivus, NHLBI (Protocol Review Committee), NICHD (OPRU oversight committee) and

consulting or advisory boards from: Biogen, Click Therapeutics, Genzyme, Genentech, GW

Pharmaceuticals, Klein-Buendel Incorporated, Medimmune, Medday, Novartis, Osmotica

Pharmaceuticals, Perception Neurosciences, Recursion/Cerexis Pharmaceuticals, Roche,

Somahlution, TG Therapeutics. Dr. Cutter is employed by the University of Alabama at

Birmingham and President of Pythagoras, Inc. a private consulting company located in Birmingham AL.

JSW has received compensation for consulting, scientific advisory boards, or other activities with Abbvie, Acorda Therapeutics, Actelion, Alkermes, Brainstorm Cell Therapeutics, Celgene, EMD Serono, GeNeuro, GW Pharma, MedDay Pharmaceuticals, NervGen Pharma Corp., Novartis, Otsuka (ends 11/16/20), PTC Therapeutics, Roche/Genentech, Sanofi and royalties received for out licensed monoclonal antibodies through UHealth to Millipore (Chemicon International) Corporation.

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GC has received in the past 36 months consulting and speaking fees from Celgene Group, Novartis, Teva Pharmaceutical Industries Ltd, Teva Italia Srl, Sanofi, Genzyme Corporation, Genzyme Europe, Merck KGaA, Merck Serono SpA, Celgene Group, Biogen Idec, Biogen Italia Srl, F. Hoffman-La Roche, Roche SpA, Almirall SpA, Forward Pharma, Medday, Excemed.

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H-P H received fees for serving on steering committees, data monitoring boards, and speaking at scientific symposia from Bayer Healthcare, Biogen, Celgene BMS, GeNeuro, MedDay Pharmaceuticals, MedImmune, Merck, Novartis, Roche, Teva, TG Therapeutics, and Viela Bio.

DLA has received honoraria from Acorda, Biogen, Genentech, Genzyme, Novartis, F.

Hoffmann-La Roche and Sanofi and has received research support from Novartis and Biogen; and has an equity interest in NeuroRx Research, which performed the MRI analysis for this trial.

JK reports nothing to disclose.

VB reports nothing to disclose.

FEM is/was an employee of MedDay Pharmaceuticals, Boston, MA, USA.

FS is/was an employee of MedDay Pharmaceuticals, Boston, MA, USA and is the inventor on several patents that are held by Assistance Publique Hôpitaux de Paris around the use of high dose of biotin to treat MS for which he has received royalties.

FDL has received personal compensation for consulting from: Biogen, EMD Serono, Novartis, Teva, Actelion/Janssen, Sanofi, Acorda, Roche/Genentech, MedImmune/Viela Bio, Receptos/Celgene, TG Therapeutics, MedDay, Atara Biotherapeutics, Polpharma, Mapi Pharma, Innate Immunotherapeutics, Apitope, Orion Biotechnology, Brainstorm Cell Therapeutics, Jazz Pharmaceuticals, GW Pharma, Mylan, Immunic, Population Council, Avotres and has received speaker fees from Sanofi (non-promotional).

Data sharing

Individual anonymized 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: Amine Tahiri <amine.tahiri@medday-pharma.com>. Research proposals will be reviewed and approved by the SPI2 steering committee based on the qualifications of the researchers and the legitimacy of the research. Approved requestors will need to sign a data-sharing agreement.

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Table 1: Baseline demographic and disease characteristics (Intention-to-Treat population)

Variable	MD1003 (n=326)	Placebo (n=316)
Age , years, mean (SD)	52.6 (7.8)	52.8 (7.6)
Sex , women, n (%)	175 (53.7)	170 (53.8)
men, n (%)	151 (46.3)	146 (46.2)
Race , White, n (%)	306 (93.9)	291 (92.1)
Asian, n (%)	2 (0.6)	3 (0.9)
Black or African American, n (%)	8 (2.5)	13 (4.1)
Not applicable in case of legal requirement, n (%)	4 (1.2)	5 (1.3)
Other, n (%)	6 (1.8)	4 (1.3)
Height , cm, mean (SD)	171.4 (10.0)	171.2 (9.8)
BMI , mean (SD)	26.5 (6.0)	26.2 (4.9)
EDSS* , mean	5.46 (0.97)	5.42 (1.05)
3.5 – 5.5 (n, %)**	133 (40.8)^	136 (43.0)
6 – 6.5 (n, %)	190 (58.3)^	180 (57.0)
TW25[#] , seconds, mean (SD)	11.5 (6.7)	11.9 (7.9)
SPMS , n (%)	209 (64.1)	206 (65.2)
PPMS , n (%)	117 (35.9)	110 (34.8)
Time since initial diagnosis of MS , mean, years (SD)	12.45 (8.72)	12.65 (8.25)
Time since last relapse (SPMS) , mean, years (SD)	8.13 (5.75)	7.93 (5.18)
Participant previously treated with DMTs , n (%)	237 (72.7)	225 (71.2)
DMT usage at randomisation , n (%)	151 (46.3)	143 (45.3)
Gd+ lesion on T1 MRI (≥ 1)^{\$} , n (%)	15 (4.5)	18 (5.8)

* Baseline EDSS is defined as the lowest (best) EDSS obtained at inclusion and randomization visits.

** Participants with baseline EDSS < 3.5 were included in the EDSS category 5-5.5.

^ Three participants in the MD1003 group did not have an EDSS assessment at baseline.

Baseline TW25 is defined as the lowest (best) mean of TW25 attempts performed at inclusion and randomization visits, ie, min (mean of TW25 attempts at M-1, mean of TW25 attempts at M0).

\$ Gd+ lesions on MRI at baseline is based on the safety analysis Set and baseline is defined as M0. When a value is missing at M0, the last non-missing value recorded prior to randomization is used.

BMI = body mass index; DMT = disease modifying therapy; EDSS = Expanded Disability Status Scale; Gd+ = gadolinium enhancing; MS = multiple sclerosis; PPMS = primary progressive multiple sclerosis; SD = standard deviation; SPMS = secondary progressive multiple sclerosis; TW25 = timed 25-foot walk

Table 2: Primary and secondary endpoints (Intention-to-Treat population)

Endpoint	MD1003 (n=326)	Placebo (n=316)	Between-group difference (95% CI)	P value
Primary: Proportion of participants with improvement, i.e., decreased EDSS (of 1 or 0.5) or improved TW25 (of at least 20%), at M12 confirmed at M15* [^]				
Overall, n/total n (%)	39/326 (12.0%)	29/316 (9.2%)	OR 1.35 (0.81, 2.26) [¶]	0.31 [§]
EDSS, n/total n (%)	22/326 (6.7%)	20/316 (6.3%)	OR 1.07 (0.57, 2.02)	0.87
TW25, n/total n (%)	22/326 (6.7%)	11/316 (3.5%)	OR 2.02 (0.98, 4.39)	0.07
Secondary:[#]				
Time to EDSS progression confirmed at 12 weeks [§] , n/total n (%)	60/326 (18.4%)	62/316 (19.6%)	HR 0.97 (0.68, 1.39)	0.43 ^{&}
Difference between treatment arms in CGI at M15 [@]			0.505 (0.49, 0.52)	0.58
Difference between treatment arms in SGI at M15 [@]			0.515 (0.50, 0.53)	0.75
Percentage change in TW25 between M0 and M15, mean (SD) [±]	20.7 (52.12)	22.5 (53.36)	0.30 (-5.89, 6.49)	0.52

* For EDSS: decrease of at least 1 point if baseline EDSS 3.5 to 5.5 and of at least 0.5 point if baseline EDSS 6 to 6.5 compared to the lowest EDSS at inclusion and randomization visits. For TW25: decrease of at least 20% compared to the lowest mean of TW25 attempts at inclusion and randomization visits. For the primary efficacy endpoint: EDSS response or TW25 response.

[^] Asymptotic logistic regression analysis for the primary efficacy endpoint with study treatment group, disease history, and geographical region as factors. An odds ratio > 1 is in favour of MD1003.

[#] Ordered according to the hierarchy in the statistical analysis plan.

[§] 12-weeks confirmed EDSS progression is defined by an increase of at least 1 point if baseline EDSS 3.5 to 5.5 and of at least 0.5 point if baseline EDSS 6 to 6.5 with respective confirmation 12 weeks later (with time windows of ± 10 days up to 1 year after randomization, ±15 days afterwards).

[&] Proportional Hazards model stratified for geographical region and disease history. A hazard ratio < 1 is in favour of MD1003. Wald test one-sided p-value.

[@] Probability for better outcome with MD1003 - (95% CI); A probability of > 0.5 is in favour of MD1003. One-sided Van Elteren test stratified for disease history (SPMS/PPMS) and geographical region (North America-Australia/Europe) comparing the two study treatment groups.

[±] Hodges-Lehmann Point estimate for location shift of distributions (95% CI). One-sided Van Elteren test stratified for disease history (SPMS/PPMS) and geographical region (North America-Australia/Europe) comparing the two study treatment groups.

[¶] Odds ratios and 95% CI derived from asymptotic logistic regression with study treatment group, disease history and geographic region as factors. An odds ratio > 1 indicates a positive effect of MD1003 compared to placebo. If the lower exact confidence interval (CI) limit is larger than 1, the null hypothesis may be rejected.

[§] two-sided p-values derived using chi-square exact

CI = confidence interval; EDSS = Expanded Disability Status Scale; HR = hazard ratio; OR = odds ratio; SD = standard deviation; TW25 = timed 25-foot walk

Table 3: Safety – TEAEs, Serious TEAEs (Safety population)

	MD1003 (n=331)	Placebo (n=311)
Any TEAE, n (%)	277 (83.7)	264 (84.9)
TEAE causally related to study treatment, n (%)	70 (21.1)	57 (18.3)
TEAE causally related to study procedure, n (%)	22 (6.6)	12 (3.9)
Serious TEAE, n (%)	87 (26.3)	82 (26.4)
Serious TEAE causally related to study treatment, n (%)	5 (1.5)	7 (2.3)
Serious TEAE causally related to study procedure, n (%)	1 (0.3)	3 (1.0)
TEAE leading to study discontinuation, n (%)	17 (5.1)	12 (3.9)
TEAE leading to death, n (%)	1 (0.3)	0 (0.0)
Any Serious TEAE*, n (%)	87 (26.3)	82 (26.4)
Nervous system disorders, n (%)	42 (12.7)	49 (15.8)
MS relapse, n (%)	29 (8.8)	31 (10.0)
Trigeminal neuralgia, n (%)	1 (0.3)	4 (1.3)
Infections and infestations, n (%)	11 (3.3)	21 (6.8)
Urinary tract infection, n (%)	2 (0.6)	6 (1.9)
Injury, poisoning and procedural complications, n (%)	9 (2.7)	7 (2.3)
Gastrointestinal disorders, n (%)	6 (1.8)	2 (0.6)
Psychiatric disorders, n (%)	6 (1.8)	3 (1.0)
Musculoskeletal and connective tissue disorders, n (%)	6 (1.8)	4 (1.3)
Respiratory, thoracic and mediastinal disorders, n (%)	4 (1.4)	3 (1.0)
Cardiac disorders, n (%)	6 (1.8)	3 (0.9)
Neoplasms benign, malignant and unspecified (including cysts and polyps), n (%)	2 (0.6)	5 (1.6)
General disorders and administration site conditions, n (%)	4 (1.2)	2 (0.6)
Surgical and medical procedures, n (%)	4 (1.2)	2 (0.6)

* > 0.5% patients per system class and > 1% patients per preferred term (MedDRA Version 19.1 System Organ Class Preferred Term; MedDRA = Medical Dictionary for Regulatory Activities)
TEAE = Treatment Emergent Adverse Event

Table 4: Safety – MRI (Safety population)

MRI*	MD1003/DMT (n=155)		MD1003/no DMT (n=176)		Placebo/DMT (n=139)		Placebo/no DMT (n=172)	
	M6	M15	M6	M15	M6	M15	M6	M15
Proportion of participants with at least 1 new or enlarging T2-weighted lesions, n (%)[^]	17 (12.4)	21 (16.4)	43 (25.9)	48 (31.8)	17 (12.9)	23 (18.3)	34 (21.7)	46 (31.7)
Number of new or enlarging T2-weighted lesions, mean (SD)[^]	0.2 (0.76)	0.6 (2.23)	1.2 (5.47)	1.5 (6.00)	0.3 (0.89)	0.4 (1.12)	0.7 (1.92)	0.8 (0.91)
Proportion of participants with at least 1 Gd+ lesion on T1, n (%)	3 (2.2)	5 (3.9)	11 (6.6)	14 (9.3)	5 (3.8)	4 (3.2)	15 (9.6)	13 (9.0)
Number of Gd+ lesions on T1, mean (SD)	0.0 (0.15)	0.1 (1.02)	0.3 (2.17)	0.4 (2.68)	0.1 (0.29)	0.0 (0.28)	0.1 (0.50)	0.1 (0.47)
Volume of T2-weighted lesions, mean (SD)	20.7 (17.44)	20.7 (17.71)	22.6 (19.74)	22.8 (19.45)	18.5 (17.44)	18.6 (16.91)	17.9 (19.13)	18.3 (19.60)
Volume of non-enhancing T1-weighted lesions, mean (SD)	4.6 (5.56)	4.6 (5.44)	5.4 (7.02)	5.7 (7.01)	4.1 (5.87)	4.2 (6.06)	3.6 (4.71)	3.7 (4.87)

* Based on Safety population; MD1003 n=331 and placebo n=311.

[^] T2 imaging analysis at M6 is M0-M6 and at M15 is M6-M15.

DMT = disease modifying treatment; Gd+ = gadolinium enhanced; MRI = Magnetic Resonance Imaging