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Pharmacokinetics and pharmacodynamics of MD1003 (high-dose biotin) in the treatment of progressive multiple sclerosis

Laure Peyro Saint Paul¹, Danièle Debruyne², Delphine Bernard³, Donald M Mock⁴, Gilles L Defer⁵,⁶

Affiliations:

1. Clinical Research, Centre Hospitalier Universitaire de Caen, Caen, France
2. Pharmacology, Centre Hospitalier Universitaire de Caen, Caen, France
3. MedDay Pharmaceuticals, ICM-Brain and Spine Institute-IPEPs, Groupe Hospitalier Pitié Salpêtrière, Paris, France
4. Department of Biochemistry & Molecular Biology and Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA
5. Neurology, Centre Hospitalier Universitaire de Caen, Caen, France
6. INSERM U 919, GIP Cyceron, Caen, France

Corresponding author:
Laure Peyro Saint Paul
Clinical Research Department, Bureau 03-809
CHU de Caen
Caen, F-14000, France
Tel: +33(0)2 31 06 53 42
Fax: +33(0)2 31 06 50 68

Email: peyrosaintpaul-l@chu-caen.fr
Abstract

**Introduction:** Multiple sclerosis (MS) is a chronic, potentially highly disabling neurological disorder. No disease-modifying treatments are approved in the progressive and not active forms of the disease.

**Areas covered:** High doses of biotin were tested in an open-label pilot study involving 23 patients with progressive MS and reported positive results. A randomized, double-blind, placebo-controlled trial in 154 progressive MS patients confirmed the beneficial effect of MD1003 (high-dose biotin) on reversing or stabilizing disability progression, with a good safety profile. It is proposed that MD1003 in progressive MS 1) increases energy production in demyelinated axons and/or 2) enhances myelin synthesis in oligodendrocytes. Biotin is highly bioavailable; absorption and excretion are rapid. The major route of elimination is urinary excretion.

**Expert opinion:** A high oral dose of biotin seems generally well tolerated but a few important safety concerns were identified: 1) teratogenicity in one species and 2) interference with some biotin-based laboratory immunoassays. The animal toxicity data are limited at such high doses. Further preclinical studies would be useful to address the mechanism of action of MD1003. Assessment of clinical benefit duration in responders will be also very important to set. Results of randomized, placebo-controlled trial are reassuring and provide hope for the treatment of progressive MS.

**Keywords:**
Biotin, energy metabolism, pharmacodynamics, pharmacokinetics, progressive multiple sclerosis, remyelination
**Drug summary box**

- **Drug name (generic):** Biotin
- **Phase (for indication under discussion):** III
- **Indication (specific to discussion):** Progressive MS
- **Pharmacology description/mechanism of action:** Hypothetical
- **Dosage and route of administration:** 100 mg tid, oral
- **Chemical structure:**

```
HN
\H
\H
HN
\H
\H
COOH
```

- **Pivotal trial(s):** MS-SPI [75, 76] and MS-ON [77]
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>Acetyl-CoA carboxylase</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate intake</td>
</tr>
<tr>
<td>AMN</td>
<td>Adrenomyeloneuropathy</td>
</tr>
<tr>
<td>BBGD</td>
<td>Biotin-responsive basal ganglia disease</td>
</tr>
<tr>
<td>CGI</td>
<td>Clinical global impression</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>DMT</td>
<td>Disease-modifying therapies</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
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<tr>
<td>FIS</td>
<td>Fatigue impact scale</td>
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<tr>
<td>H-MRS</td>
<td>Proton magnetic resonance spectroscopy</td>
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<tr>
<td>ITT</td>
<td>Intention-to-treat</td>
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<td>LD50</td>
<td>Median lethal dose</td>
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<tr>
<td>LOAEL</td>
<td>Lowest-Observed-Adverse-Effect-Level</td>
</tr>
<tr>
<td>MCC</td>
<td>Methylcrotonyl-CoA carboxylase</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance</td>
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<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
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<tr>
<td>MSWS</td>
<td>Multiple Sclerosis Walking Scale</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No-Observed-Adverse-Effect-Level</td>
</tr>
<tr>
<td>OPC</td>
<td>Oligodendrocyte precursor cells</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PC</td>
<td>Pyruvate carboxylase</td>
</tr>
<tr>
<td>PCC</td>
<td>Propionyl-CoA carboxylase</td>
</tr>
<tr>
<td>PPMS</td>
<td>Primary progressive multiple sclerosis</td>
</tr>
<tr>
<td>RRMS</td>
<td>Relapsing-remitting multiple sclerosis</td>
</tr>
<tr>
<td>SMVT</td>
<td>Sodium dependent multivitamin transporter</td>
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<tr>
<td>SPMS</td>
<td>Secondary progressive multiple sclerosis</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic acid</td>
</tr>
<tr>
<td>THTR2</td>
<td>Thiamine transporter 2</td>
</tr>
<tr>
<td>TW25</td>
<td>Timed 25-foot walk</td>
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</tbody>
</table>
1. Introduction

Multiple Sclerosis (MS) is an inflammatory autoimmune disease that damages the myelin of the central nervous system (CNS) causing neurological impairment and, in many cases, severe disability [1]. MS is a common neurological disease affecting more than 1 million people worldwide. The prevalence rate of MS varies considerably between countries and is highest in North America (140 per 100,000) and Europe (108 per 100,000) [2].

Eighty five percent of all patients present with relapsing-remitting (RR) MS, which is characterized by unpredictable acute episodes of neurological dysfunction (termed relapses), followed by variable recovery and periods of clinical stability [1]. Around 15% of all patients develop a sustained deterioration of their neurological function from the onset, which is termed primary progressive (PP) MS [1]. Within ten years about 50% of patients who presented with the RR form develop sustained deterioration with or without superimposed relapses; this form is called secondary progressive (SP) MS. Conversion from RRMS to SPMS is an age-dependent process; the estimated rate of conversion is 2-3% per year [3]. The term “progressive MS” includes both PPMS and SPMS [4]. In all presentations, the disease can be categorized as active or not active and progressing or not progressing [4].

The clinical manifestations of progressive MS are variable because the manifestations result from degenerative processes involving several different CNS regions (optic nerve, brainstem, cerebellum, cerebral hemispheres, and spinal cord). Symptoms and signs generally reflect the areas being the most demyelinated [5]. They include: 1) motor function and coordination difficulty, 2) cranial nerve dysfunction, 3) autonomic nervous system dysfunction, 4) cognitive problems, 5) pain and 6) paroxysmal symptoms including Uhthoff’s phenomenon. Over the years, symptoms worsen and disabilities accumulate without recovering completely due to continuous and irreversible neurodegeneration.
The etiology of MS remains unknown. Most authorities believe that the pathogenesis of MS involves a complex interplay between host genetic factors and environmental exposures (e.g. viral infections). Two pathophysiologic mechanisms are recognized in MS: inflammatory and degenerative processes. Relapses are considered to be the clinical expression of acute inflammatory focal lesions [6], whereas progression is more associated with impaired remyelination and axonal loss, which might be caused by secondary energy failure [7].

Effective anti-inflammatory and immunomodulatory treatments (i.e., beta interferons, glatiramer acetate, mitoxantrone, natalizumab, fingolimod, teriflunomide, alemtuzumab, and dimethylfumarate) that reduce the severity and frequency of new demyelinating episodes, are available in the treatment of relapsing forms of MS and for patients with secondary progressive multiple sclerosis with active disease, evidenced by relapses [4, 8]. There is still a debate as to whether these drugs are efficient in preventing the transition to a progressive disease: some of these disease-modifying therapies (DMT) may delay, but not prevent, the transition to SPMS [9-13]. So far, no drug was shown to have any impact on reversing or stabilizing the progression of disability in progressive MS [14]. Therefore, patients with PPMS or with SPMS that do not have active disease are not currently eligible for any licensed DMT for MS [15, 16]. During the progressive course of MS, treatments are essentially symptomatic as part of a comprehensive, interdisciplinary, and individualized approach encompassing drug therapy, psychological counselling, and physiotherapy.

2. Overview of the market

While immunosuppressive or immunomodulatory therapies are effective for relapsing MS and for patients with secondary progressive multiple sclerosis with active disease, evidenced by relapses (driven by inflammatory process), an effective disease-modifying treatment for patients with progressive MS with no evidence of inflammatory activity was not identified
yet. So far, the results of clinical trials have failed to demonstrate treatment efficacy in terms of reversing or stabilizing disability progression in progressive MS (driven by neurodegenerative process) (see Table 1).

Update on the INFORMS study evaluating fingolimod efficacy in PPMS patients were recently communicated; the study did not meet its primary endpoint given that no significant difference was found between fingolimod and placebo based on a combination of disability measures [17]. Nonmyeloablative hematopoietic stem cell transplantation also failed to benefit for patients who had progressive MS at the time of the transplant [18]. The results of the placebo-controlled, double-blind, Phase III ORATORIO study in PPMS patients evaluating the efficacy and safety of the selective immunosuppressor ocrelizumab, which is another monoclonal antibody antagonizing the B-lymphocyte antigen CD20 was presented at the ECTRIMS in October 2015 [19]. Results demonstrated a small but significant reduction in the time to progression of disability sustained for at least 12 weeks (n=732 patients, including 25% with active disease). Additionally, the study met other secondary endpoints of reducing the time required to walk 25 feet, the volume of chronic inflammatory brain lesions, and brain volume loss. Safety was comparable to placebo except for an increased cancer incidence in the active arm.

Therapeutic option remains essentially symptomatic including fampridine that has an approved indication in Europe, Canada, and the US for the improvement of walking disability in adults with multiple sclerosis (EDSS 4-7), including progressive MS [20]. No impact on disability progression was demonstrated in these patients.

Neuroprotective and remyelination strategies constitute the unmet medical need for progressive presentations of MS. Two promyelinogenic agents are currently in development for treatment of MS:
1) **A monoclonal antibody BIIB033 (Biogen Idec)** neutralizing the LINGO1 protein (leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein, which inhibits the differentiation of oligodendrocyte precursor cells (OPCs) and consequently inhibits myelination [21]. Phase I data from healthy volunteers and patients with MS indicate that inhibition of LINGO1 with the monoclonal antibody BIIB033 (Biogen Idec) appears to be well tolerated [22]. A phase II trial in patients with RRMS is ongoing (EUDRACT #: 2011-006262-40). First results were presented at the American Academy of Neurology. In the per-protocol (PP) analysis, the anti-LINGO-1 group had significantly improved optic nerve conduction latency of full-field visual evoked potentials after 32 weeks, as compared to the placebo group (n=82 patients). There was no statistical difference in the intent-to-treat (ITT) analysis or for the secondary endpoints including visual acuity at low contrast [23]. In a substudy presented at the ECTRIMS 2015, improvement in VEP latency was greater in the anti-LINGO-1 group versus placebo using the mean multifocal visual evoked potential latency, which allow to evaluate a larger area of the visual field [24]. The mean difference in conduction latencies was more pronounced in the PP analysis than in the ITT analysis. However, statistical significance was not reached probably due to the small size group (n=39 patients).

2) **A recombinant form of a human IgM (rHIgM22; Acorda Therapeutics)** that binds to myelin and the surface of oligodendrocytes promotes remyelination in murine models of MS and other demyelinating diseases [25, 26]. The safety and preliminary efficacy of rHIgM22 are currently being investigated in patients with all forms of MS in a phase I trial (NCT01803867).
3. Introduction to the MD1003

In 1916, Bateman observed that rats fed a diet containing raw egg white as the sole source of protein developed a syndrome characterized by severe dermatitis, loss of hair, and signs of progressive neuromuscular dysfunction. In 1936, Kögl and Tönnis isolated from egg yolk a factor that was essential for growth of yeast, which they called biotin. They demonstrate that biotin protected rats against this “egg-white toxicity” [27]. The critical event in the egg white-induced biotin deficiency is a highly specific and very tight binding ($K_b=10^{15} \text{ M}^{-1}$) of biotin by avidin, a glycoprotein found in egg white [28].

Biotin is present in many foods. Good sources for biotin include organ meats (like liver and kidney), egg yolk, some vegetables and cow’s milk; poor sources include lean meat, cereal and fruits [29]. The adequate intake (AI) is 30 µg/day for an adult [30].

MD1003 (MedDay Pharmaceuticals, Paris, France) is an oral formulation of high-dose pharmaceutical-grade biotin currently in clinical development as a treatment for progressive MS and adrenomyeloneuropathy (AMN).

The daily dose of MD1003 currently being investigated in phase III trials is 300 mg biotin, which is 10,000-fold higher than the AI. At this dose, biotin is considered as an active ingredient.

4. Chemistry

Biotin is a water-soluble molecule usually classified as a B-complex vitamin. “Biotin” is by far the most widely used term for this vitamin. However, discovery of biotin by different approaches has also led to names such as Bios IIB, protective factor X, vitamin H, coenzyme R, W factor, and vitamin B7 [27].
Chemical name: 5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoic acid

Structure:

Formula: $\text{C}_{10}\text{H}_{16}\text{N}_{2}\text{O}_{3}\text{S}$

Molecular weight: 244.31 g mol$^{-1}$

Solubility: 22 mg/100 mL (in water)

Biotin is enzymatically active when covalently joined via an amide bond between the carboxyl group of the valeric acid side chain of biotin and the $\varepsilon$-amino group of a lysine residue of any one of five mammalian apocarboxylases to form the holocarboxylases (see Figure 1) [27].

5. Deficiency and indications

5.1. Symptoms of deficiency

In humans, symptoms and signs of biotin deficiency generally include dermatitis, thinning of hair with loss of color, atrophic glossitis, hyperesthesia, muscle pain, lassitude, anorexia, slight anemia, and change in the ECG [27]. Spontaneous deficiency has been observed in some individuals who have consumed raw eggs over long periods. Biotin deficiency was documented in parenteral nutrition without biotin supplementation in patients with short-gut syndrome and other causes of malabsorption. Inborn errors causing biotinidase deficiency and biotin transporter deficiency also result in biotin deficiency. Patients with severe biotinidase deficiency, if untreated, may suffer from seizures, psychomotor delay, deafness, ataxia, visual pathology, conjunctivitis, and alopecia [31]. The one well documented case of
biotin transporter deficiency presented acutely at 18 months of age with rapid neurologic deterioration after an acute illness consistent with viral gastroenteritis; the child was progressively lethargic and finally completely obtunded [32].

5.2. Current therapeutic indications

Biotin is indicated in the prophylaxis and treatment of biotin deficiency, and in the treatment of alopecia (in France) at doses from 100 µg to 20 mg/day [33]. Biotinidase deficiency typically responds to doses of biotin similar to the AI. Inborn errors of biotin metabolism such as holocarboxylase synthase deficiency and biotin transporter deficiency require larger doses of biotin, usually in the 5 to 20 mg/day range [27, 34].

Higher doses of biotin (5 to 10 mg/kg/day) have also shown efficacy in one disorder of energy metabolism called “Biotin-responsive basal ganglia disease” (BBGD) [35]. This orphan neurological disease is caused by mutations in the thiamine transporter THTR2 [36, 37]. Thiamine defect is expected to block the tricarboxylic acid (TCA) cycle at the level of pyruvate dehydrogenase and of alpha ketoglutarate dehydrogenase that are thiamine-dependent. The TCA cycle block is responsible for a chronic cellular dysfunction and death leading to necrosis of caudate nuclei and putamen of the brain. Neurological manifestations include severe episodes of encephalopathy resulting, if not treated, in residual paraparesis, mental retardation, generalized dystonia or death [35, 38]. In BBGD, because biotin is not a substrate for hTHTR2, the precise mechanism by which biotin rescues the clinical phenotype remains unknown [37]. High doses of biotin are speculated to activate the TCA cycle downstream of the pyruvate dehydrogenase and the alpha ketoglutarate dehydrogenase complexes, which are impaired by functional thiamine deficiency.
6. Pharmacodynamics and mechanism of action

6.1. Physiological functions

Biotin acts as an essential coenzyme for five mammalian carboxylases involved in the metabolism of carbohydrates, aminoacids and fatty acids. The five are pyruvate carboxylase (PC, EC 6.4.1.1), methylcrotonyl-CoA carboxylase (MCC, EC 6.4.1.4), propionyl-CoA carboxylase (PCC, EC 6.4.1.3) and two isoforms of acetyl-CoA carboxylase (ACC, EC 6.4.1.2), denoted I and II. Each catalyzes an essential step in intermediary metabolism [27] (see Figure 2).

Each of the five mammalian carboxylases catalyzes the incorporation of bicarbonate as a carboxyl group into a substrate. Each employs a similar catalytic mechanism and the reaction is driven by the hydrolysis of ATP to ADP and inorganic phosphate. In the normal turnover of cellular proteins, holocarboxylases are degraded to biocytin or biotin linked to an oligopeptide containing at most a few amino acid residues (see Figure 1). Because the amide bond between biotin and lysine is not hydrolyzed by cellular proteases, the specific hydrolase biotinidase [biotin amide hydrolase (EC 3.5.1.12)] is required to release biotin for recycling.

6.2. Putative mechanism of action in progressive MS

Progression in MS is often considered as a consequence of both demyelination and energy failure [7]. The causal neurodegenerative process is suggested to arise from an increased energy demand in demyelinated axons together with mitochondria dysfunction, creating a virtual hypoxia phenomenon (see Figure 3). In demyelinated axons, restauuration of the membrane resting potential on the entire demyelinated membrane, rather than on the nodal membrane only, requires higher quantities of ATP to pump out and in Na\(^+\) and K\(^+\) ions, respectively, via the Na\(^+\)K\(^+\) ATPase [39]. This increased demand in ATP is compensated by...
the recruitment of mitochondria locally within the axons. Demyelinated axons are exposed to soluble inflammatory molecules produced by microglia and other inflammatory cells that induce mitochondrial damages through reactive oxygen species-mediated post-transcriptional modification and nitration of the respiratory chain complexes as well as damage to the mitochondrial DNA [39]. Mitochondrial defects accumulate and progressively render chronically demyelinated axons unable to meet the increase energy demand (virtual hypoxia), leading to neuronal dysfunction and ultimately degeneration. Axonal and neuronal death may also result from glutamate mediated excitotoxicity, microglia activation, chronic oxidative injury, ion channel dysfunction and age-related iron accumulation in the human brain [40].

In humans, some clinical aspects of severe biotin deficiency, such as cutaneous fungal infections, suggest that biotin deficiency causes impaired immune function [41]. Moreover, in children suffering from an inborn error that leads to biotin deficiency or from deficiency of multiple biotin-dependent carboxylases, there are specific T-cells and B-cell defects [42]. Indeed, biotin supplementation (1-2 mg/day) exerts complex effects on human peripheral blood mononuclear cells collected after 14-21 days of treatment [43, 44]. While selected cytokines mRNA levels, including IL-1β, increased following biotin intake in five healthy volunteers in one study [43]; the secretion of IL-1β in medium and the proliferation rate decreased in five different healthy volunteers in another study [44]. Biotin might also be important for the normal function of natural killer cells based on the observation that biotin supplementation alleviates the level of suppression in the activity of these cells in patients with Crohn’s disease [45]. Moreover, moderate to severe biotin deficiency had substantial effects on immune function in rodents [46-50]. However, these effects are not replicated in marginally biotin deficient rats suggesting that fairly severe biotin deficiency must be present to induce major functional immune impairment [51]. Overall, the contribution of immune
dysfunction mediated by functional biotin deficiency to the pathogenesis of MS and the therapeutic effect of biotin remains unclear.

The hypothesized mechanism of action of biotin in progressive MS encompasses therapeutic effects on both demyelination and virtual hypoxia through metabolic activation of biotin-dependent enzymes [52] (see Figure 3).

The anaplerotic replenishment of the TCA cycle intermediates by increase pyruvate carboxylase activity due to very high doses of biotin might theoretically increase the energy production in axons, thus reversing the pathogenesis by rescuing the weakened neurons from the “virtual hypoxia phenomenon”. On the other hand, myelin synthesis may be increased as a consequence of increased activity of acetyl-CoA carboxylase (ACC) producing more malonyl-CoA. Because the supply of malonyl-CoA is rate-limiting in the synthesis of long chain fatty acids, ACC activation may increase myelin synthesis [53].

Overall, high doses of biotin may target the main metabolic processes relative to progressive MS by (1) increasing energy production in demyelinated axons through activation of the TCA cycle, and (2) increasing the production of long chain fatty acids, thereby enhancing myelin synthesis in oligodendrocytes through activation of the TCA cycle and ACC.

7. Pharmacokinetics and metabolism

7.1. Bioavailability

After administration of single tests dosages of 40 mg (oral) and 5 mg (intravenous) biotin, in a crossover test design, to 12 yearling heifers on continuous dietary supplementation with a daily 20 mg dose of biotin (n=6) or not (n=6), the calculated overall bioavailability was 48 % [54]. In rats fed ad libidum with diet containing increasing doses of biotin (0.04 to 0.8 g/100 g of diet) for 28 days, free biotin concentration in serum increased with increased
intake of biotin, signifying bioavailability was not modified by the dose in such an experiment [55].

At doses up to 20 mg in humans, urinary excretion of biotin and its metabolites is similar for intravenous dosing and oral supplementation, suggesting 100% bioavailability of orally administered biotin in humans [56].

The intestinal biotin uptake mechanisms include slow passive diffusion and carrier-mediated process that both occur in the small intestine and colon [29]. As a result saturation of the transporter process may be expected with high doses.

7.2. Distribution

In five pigs receiving either physiological doses of radiolabeled \(^{14}\)C-biotin or tracer doses of \(^{3}\)H-biotin, radioactivity mainly accumulated in the liver. Muscle that accounts for 60% of total body mass contained 10–23% of infused radioactivity. Accumulation of radioactivity varied from 2.8 to 6.4% in the kidney and, was <1% of the administered radioactivity in heart, lung, brain or duodenum [57]. After IP injection to rats and chickens, biotin was also mainly found in liver, kidney and spleen [58].

In 1992, Mock and Malick determined that in human plasma, approximately 81% of total biotin is free, 12% is covalently bound and 7% reversible bound [59].

Biotin is actively transported across the blood–brain barrier by a saturable system, essentially via the Na\(^+\)-dependent multivitamin transporter (SMVT) [60, 61], which has an estimated half-saturation concentration (K\(_{\text{m}}\)) around 40 \(\mu\)M [62] in animal models; this K\(_{\text{m}}\) is several orders of magnitude greater than the concentration of free biotin in plasma with normal dietary intake (approximately 1 nM). This system likely will not be saturated even after administration of very high doses; as our mathematical simulations predicts a mean C\(_{\text{max}}\) of
4 μM following repeated administrations of 100 mg biotin tid [52, 63]. Following repeated oral administrations to rats at doses up to 990 mg/kg/day, biotin concentration increased in all tissues examined including brain [55] consistent with these inferences. At the dose of 38.4 mg/kg/day, which is close to a dose of approximately 300 mg/day in a 70-kg human, when extrapolated on the basis of body surface area as recommended in the FDA guidance on selection of a safe starting dose for an initial clinical trial, mean biotin concentration was measured at around 2.5 nmol/g of brain tissue.

7.3. Metabolism

In rats, after IP injection of 56 pmol/g i.e. 13.7μg/kg $[^{14}C]$biotin, $[^{14}C]$bisnorbiotin [i.e. biotin with 2(CH$_2$) in the lateral chain instead of 4(CH$_2$)] and $[^{14}C]$biotin sulfoxide were the main radioactive metabolites identified in urine [64]. After 24h, $[^{14}C]$native biotin, $[^{14}C]$bisnorbiotin and $[^{14}C]$biotin sulfoxide accounted for 51%, 29%, and 10% respectively; after 48h, $[^{14}C]$bisnorbiotin was the major metabolite. The same two major radiolabeled metabolites were detected in pig plasma with an intermediate in biotin oxidation, the bisnorbiotin methyl ketone after intravenous administration of a physiologic amount of $[^{14}C]$biotin [57].

Similarly, biotin, bisnorbiotin, and biotin sulfoxide are the major metabolites in human urine and plasma, but two additional minor metabolites (bisnorbiotin methyl ketone and biotin sulfone) have also been identified in urine. After oral or IV administration of biotin (0.5-20 mg) to 6 healthy subjects, bisnorbiotin, biotin sulfoxide, bisnorbiotin methyl ketone and biotin sulfone accounted for 13-23%, 5-15%, 3-9%, and 1-3% respectively [56]. Biotin metabolites are usually considered as inactive as vitamins [58, 65]. However, in one study, bisnorbiotin had biotin-like effects on gene expression; authors also suggested that bisnorbiotin might serve as a coenzyme for biotin-dependent carboxylases [66].
7.4. Excretion

In rats, around 60% of the IV administered dose (158 pmol/g [$^{14}$C]biotin, i.e. 38.6 µg/kg) was excreted in urine within 24 hours [67]. In contrast, biliary excretion was negligible with only 1.9% of the dose recovered in the bile, confirming the major excretion of biotin via the urinary tract as the parent compound and its radioactive metabolites.

In humans, single biotin doses (600 and 900 µg) were rapidly eliminated from plasma to urine with an elimination half-life calculated to be approximately 1.8 h indicating a credible total disappearance of biotin in plasma within 12 h [68].

7.5. Blood concentration-time profile and pharmacokinetic parameters

After an IV administration of a physiological dose of [$^{14}$C]biotin (102±2 nmol/kg i.e. 25±0.5 µg/kg body weight) to 3 pigs, the [$^{14}$C]radioactivity and [$^{14}$C]biotin disappearance curves exhibited a rapid triexponential decay [57]. The calculated plasma elimination half-lives of [$^{14}$C] radioactivity were 0.07±0.06h, 0.43±0.15h, 5.5±2.3h for T$_{1/2α}$, T$_{1/2β}$ and T$_{1/2γ}$ respectively. The disposition of [$^{14}$C]radioactivity accurately reflected the disappearance of [$^{14}$C]biotin in pig plasma [57]. The plasma disappearance curve of the total radioactivity after administration of a [$^3$H]biotin tracer dose exhibited the same rapid triexponential decay with quite similar T$_{1/2α}$ and T$_{1/2β}$, while T$_{1/2γ}$ was longer due to the contribution of radioactive artefacts. In a second study, five pigs received an IV administration of [$^{14}$C]biotin (88±19 nmol/kg i.e. 21.5±4.6 µg/kg body weight) and the determined T$_{1/2α}$, T$_{1/2β}$ and T$_{1/2γ}$ were 0.11±0.07h, 1.43±0.42h, 22.0±4.11h respectively. The area under the plasma concentration-time curve (AUC), clearance and distribution volume (Vd$_{ss}$) were 440 ± 158 kBq/L.h, 0.46 ± 0.13 L/h.kg, 8.70 ± 1.6 L/kg respectively.
In MD1003 toxicokinetic studies, after repeated doses of biotin (100-1000 mg/kg/day for 36 weeks) maximum biotin concentrations in plasma (C\textsubscript{max}) were reached 1 to 3 hours following oral administration to male (n=8 per dose) and female (n=8 per dose) rats. T\textsubscript{max} was around 1 hour for females and increased with the dose from 1 hour (100 mg/kg/day) to 3 hours (1000 mg/kg/day) in males. The C\textsubscript{max} and AUC increased less than dose-proportionality between 100 (C\textsubscript{max} = 4091 ng/mL and AUC = 34173 ng/mL.h in males) and 1000 mg/kg/day (C\textsubscript{max} = 10161 ng/mL and AUC = 122117 ng/mL.h in males) following single administration. After repeated administration, C\textsubscript{max} and AUC were of the same order on Day1 and Day181, indicating a lack of accumulation. Following single or repeated oral administration (30, 100, 300, 1000 mg/kg/day for 3 days and 1000 mg/kg/day for 28 days) of biotin to male and female Beagle dogs, no gender difference was observed on plasma exposures at all dose levels. As in rat, C\textsubscript{max} and AUC increased less than dose-proportionality between 30 and 1000 mg/kg/day and no accumulation was noted between Day1 and Day28.

Pharmacokinetics parameters of single doses of biotin (100, 200, and 300 mg) were studied in a randomized, crossover trial in healthy adults (4 men, 4 women; MD1003-PK, EUDRACT #: 2014-000766-22) [69]. Blood samples were collected during 24 hours. Fasted plasma concentrations of biotin, below 10 ng/mL in all pre-dose samples, increased up to 494.9±161.0 ng/mL or to 823.8±303.1 ng/mL (C\textsubscript{max}) after administration of a single dose of 100 mg or 300 mg, respectively. The median time of maximal plasma concentration (t\textsubscript{max}) occurred at 1.25 hours at the 100 mg dose and 1.5 hours at the 300 mg dose, respectively (see Figure 4). Mean exposures (AUC\textsubscript{last}) were 2315±498 ng.h/mL at the 100 mg dose and 4010±1358 ng.h/mL at the 300 mg dose, respectively, consistent with the rapid rise in plasma concentrations previously reported with smaller biotin doses [70]. A slight food effect was observed after intake of a standardized high-fat breakfast with small increases in C\textsubscript{max} and AUC\textsubscript{last} by 6% and 13.5%, respectively at the 100 mg dose. The delay in absorption was
longer in the fed conditions, with a median $t_{\text{max}}$ of 2.50 hours post-dose. At the doses tested, $C_{\text{max}}$ and AUC did not triple when the dose was tripled, suggesting that intestinal transport of biotin may be saturated by these high doses [69]. A bi-compartment model provided the most accurate description of the concentration–time profile. The calculated individual elimination half-lives varied between 7.8 and 18.8 hours.

To summarize, biotin appears a) largely and rapidly absorbed, b) promptly distributed in tissues, c) mainly metabolized in bisnorbiotin and biotin sulfoxide in all species studied, d) mostly and quickly excreted as unchanged biotin and metabolites in urine. No accumulation of biotin in blood is expected in case of repeated administration owing to the short elimination half-life. Plasma exposure increases less than dose-proportionality after single oral administration in humans or after repeated oral administration in animals. This fact could be linked to the saturation of the carrier-mediated process supposed to mediate the intestinal absorption of biotin.

8. Clinical efficacy

8.1. Serendipity in progressive MS

High doses of biotin were recently hypothesized as a potential treatment of progressive MS by serendipity [52]. Patients with BBGD, a disorder of energy metabolism, display severe episodes of Leigh-like encephalopathy leading to death or permanent disability, and show dramatic improvement when high doses of biotin and thiamine are administered (see section 5.2) [71]. Because bilateral optic neuropathy is often caused by energy metabolism disorders [72], five patients suffering from bilateral severe optic neuropathies and leukoencephalopathy were treated with high doses of biotin; these patients responded clinically [73], including one who was diagnosed in retrospect with a secondary progressive MS.
8.2. Pilot study

In a multicentric, open-label, pilot study, 23 consecutive patients (including the above cited one) with primary or secondary progressive MS were treated with high doses of biotin ranging from 100 mg to 600 mg/day (median= 300 mg/day divided in three doses) for a mean duration of 9.2 months (range= 2-36 months) [74]. Fourteen patients suffered from PPMS and 9 from SPMS. Four patients had permanent visual loss following optic neuropathies; one patient had progressive lateral hemianopia caused by involvement of optic radiations and 18 patients had progressive paraparesis or tetraparesis related to spinal cord involvement. Varied objective and assessor-dependent measures were collected in these patients depending on their clinical presentations: in the four patients with chronic optic neuropathies, efficacy was assessed using visual acuity, Goldmann perimetry and/or visual evoked potentials; in the patient with homonymous hemianopia, efficacy was assessed using Humphrey automated perimetry; in the 18 patients with spinal cord involvement, efficacy was assessed using walking distance, EDSS, TW25, muscle strength testing and video-taped clinical examination in a subset of patients. Additional clinical symptoms, i.e. fatigue, swallowing difficulties, dysarthria, Uhthoff's phenomenon and urinary dysfunction, were collected. Overall, 21 of 23 patients (91.3%) exhibited some clinical improvement with high doses of biotin. In all cases, clinical improvement was detected after 2 to 8 months (mean=3 months) following treatment’s onset. Only 2 patients with severe tetraparesis did not show positive response to treatment, possibly related to the short duration of treatment (8 and 7 months respectively). Indeed, in another patient with a severe tetraparesis, treatment benefit was apparent after 8 months of treatment. The dose of 300 mg/day was associated with the best clinical efficacy. Based on these encouraging observations, two phase III studies in patients with progressive MS have been initiated: one study in patients with spinal progressive MS (MS-SPI) and one
study in patients with chronic optic neuropathies related to MS (MS-ON; EUDRACT #: 2013-002113-35 and 2013-002112-27, respectively).

8.3. Phase III studies

Results of the blinded phase of the randomized (2:1), double-blind, placebo-controlled study in adult patients with primary or secondary progressive MS and spastic paraparesis were presented at the American Academy of Neurology 2015 in April held in Washington DC, USA [75] and at the European Academy of Neurology 2015 held in June in Berlin, Germany [76] (MS-SPI; Tourbah et al., submitted). One hundred and fifty four patients with baseline EDSS ranging from 4.5 and 7 were selected from 16 investigational sites. Patients with evidence of inflammatory activity, defined as clinical evidence of a relapse or gadolinium-enhanced lesions on a brain MRI, during the year prior to inclusion, were excluded. Treatment duration was 48 weeks.

The primary outcome chosen of the MS-SPI study was improvement in Expanded Disability Status Scale (EDSS) and/or time-to-walk 25 feet (TW25) measured at M9 and confirmed at M12 compared to the best score of the values measured at the screening and baseline visits. At the end of the placebo-controlled phase of the study, a significant proportion of the patients in the active group achieved clinical improvement, corresponding to a reversal of their disability progression curve, whereas no patient were improved in the placebo group (p=0.0051). Secondary endpoints included mean change in EDSS, MSWS, CGI, proportion of patients with stable or worsened EDSS, and FIS; they confirmed the beneficial effect of biotin observed in this patient population during this study.

The other phase III randomized, double-blind, placebo-controlled study in MS evaluates the efficacy of MD1003 in improvement of patients suffering from chronic visual loss after optic
neuritis related to MS (MS-ON). Ninety-three (93) patients have been enrolled in this study.
The MS-ON study was designed to investigate the superiority of MD1003 over placebo in the visual improvement of patients suffering from chronic visual loss resulting from optic neuritis (MS-ON). Treatment duration was 24 weeks. The primary endpoint was the mean change, in the total study population, in 100% contrast visual acuity (VA) at six months from baseline of the diseased eye defined as the eye with the worst visual acuity and acute or progressive worsening within the 3 years prior to inclusion. Analysis of the primary endpoint did not show a significant difference between active arm and placebo arm [77].

In addition to progressive MS, MD1003 is also being evaluated in a phase IIIB/III randomized, double-blind, placebo-controlled study in patients with adrenomyeloneuropathy (see Table 2 for further details).

9. Safety and tolerability

9.1. Nonclinical experience

Based on the literature, acute and chronic toxicity of biotin appears to be very low for all routes of administration: LD50 > 1000 mg/kg by intravenous route and > 10 g/kg after oral administration in rodents [58, 78, 79]. Similarly, no serious toxicity was observed in rodents following repeated oral administrations of doses ranging from 1 to 350 mg/kg/day for up to 120 days. The reported toxicity was as a small (<10%) decrease in body weight of rats given 5 mg/day per os (p.o.) for 120 days [79] and of gerbils given 1 mg/kg/day p.o. for 6 weeks [58].

Biotin was not mutagenic in an Ames test and in a RK bacterial test, but biotin was mutagenic in a non-standard micronucleus test in the monocotyledon plant Tradescantia [78].
Paul and Duttagupta (1975-1976) have reported that injections of 100 mg/kg body weight in pregnant rats resulted in depressed G 6-PD activity, blocked estrogen production, and caused resorption of fetuses and placentas and other productive irregularities [80, 81]. Considering that 1) only a very high dose was studied (no dose-relationship study), 2) the route of administration was subcutaneous, 3) 0.1M NaOH was used as the vehicle (which is known to be reprotoxic), and 4) an appropriate vehicle only control group was not used, the results from these studies regarding reprotoxicity of biotin are not appropriate for risk assessment of high-dose biotin.

However, during the nonclinical development of MD1003, teratogenicity was observed in one species (rabbits) at a dose close to the therapeutic dose used in MS-ON and MS-SPI clinical trials [69]. Biotin was not teratogenic or foetotoxic at even higher doses in a second species (rats) [69].

In 3 week-old weaning rats (n=4/group) fed on a diet supplemented with biotin for 28 days, food intake and body weight gain were reduced from the dose of 79.2 mg/kg body weight/day (Lowest-Observed-Adverse-Effect-Level, LOAEL). The No-Observed-Adverse-Effect-Level (NOAEL) was 38.4 mg/kg/day [55]. In a second study in which biotin was mixed with the diet and given for 6-8 weeks, the LOAEL was a much higher dose, i.e. 990 mg/kg/day, and the NOAEL was 100 mg/kg/day [82]. In addition, at the very high dose (990 mg/kg/day), biotin inhibited spermatogenesis as assessed by a lower mature sperm count, an increased number of sperm with morphologically abnormal heads, inhibition of seminiferous tubule development, decreased number of spermatogonia and absence of spermatocytes in the tissues as compared with rats pair-fed a control diet equal in energy intake (n=6 animals per group). Further studies are needed using a larger number of animals, longer study duration, and incorporating a reversibility design.
Overall, the high dose of biotin used in the nonclinical studies appears to be safe, but currently some effects related to reproductive parameters and embryo-fetal development cannot confidently be ruled out. The clinical relevance of these findings is not known. On balance, given the seriousness of such adverse effects, we currently recommend that pregnant women and women of childbearing age should not take these high doses of biotin unless using effective contraception. Whether a significant dose of biotin might be transmitted to pregnant women or fetuses through the male reproductive tract and seminal vesicle fluid is not currently known. As a consequence, men treated with high-dose biotin should use condoms if not using effective contraception with female partner either of childbearing potential or pregnant.

9.2. Clinical experience

Biotin serious toxicity has never been reported in humans. We infer that biotin toxicity is likely quite low because infants have tolerated injections of 5-20 mg daily for six months with no adverse effects [58, 83, 84]. In addition, no toxicity was reported with administration of large amounts of biotin (2-10 mg/kg/day) for as long as 4 years (see Table 3) [35, 38, 58, 65, 74, 83-91].

There are no reported cases of harmful adverse effects from receiving high doses of biotin with two possible exceptions: 1) the development of temporary (4-5 days) diarrhea observed in a 5-month-old infant receiving 10 mg of biotin/day [58] and 2) eosinophilic pleuroperticardial effusion (an extremely rare condition) was reported in a single person who was concomitantly consuming both pantothenic acid (300 mg/day) and biotin (10 mg/day) orally [92].
In the first 12-month phase of the MS-SPI study and in the first 6-month phase of the MS-ON study, MD1003 was well tolerated. The overall incidence of adverse events was similar across the treated and placebo groups. One patient died from suicide in the active arm of the MS-SPI study, however this event was not considered as related to the drug.

Importantly, in five reported cases and several additional unreported cases (DMM, personal communication), high doses of biotin likely affected results of thyroid function laboratory tests that used (strept)avidin-biotin technology. Specifically, high plasma or urine biotin saturates the (strept)avidin causing falsely low values with sandwich immunoassays and falsely high values with competitive immunoassays [93, 94]. The tests potentially affected by this biotin-induced interference include anemia, cardiac, fertility, hormonal, oncology, bone metabolism, inflammation biomarkers, infectious disease antigens and antibodies titration. Some immunohistochemistry methods used in diagnostic pathology could also be affected. The time needed to recover biotin plasma concentrations compatible with the tests is not known with precision but may be as long as a few weeks according to simulated data and published data on withdrawal of high-dose biotin therapy [32]. Indeed, immunoassays can be disturbed with a biotin plasma concentration as low as 10 ng/mL, while the mean $C_{max}$ following MD1003 administration tid is estimated to about 1 µg/mL.

10. Regulatory affairs

MD1003 is not authorized for sale in any country.

11. Conclusion

Progression in MS belongs from different complex biological mechanisms. Among them demyelination and energy failure may play a key role. If MD1003 (high-dose biotin) may
rescue energy production in axons and facilitate myelin synthesis, the putative mechanisms of biotin action in MS needs to be better explored. In addition encouraging results of the phase III MS-SPI study needs now to be confirmed in a larger international cohort to confirm the efficacy and safety of MD1003 in progressive MS.

12. Expert opinion

Over the last two decades, we have observed major developments in MS treatments that substantially changed patient care in clinical practice. Unfortunately, all drugs on the market show efficacy in the active forms of disease only, i.e. RRMS and to a lesser extent SPMS with superimposed relapses [95]; except may be for ocrelizumab shown to be active in PPMS patients [19], subgroup analyses are awaited in order to determine if the antibody was similarly efficacious in PPMS patients with and without evidence of activity. This therapeutic progress was hampered by the inability of these drugs to show efficacy in preventing, ameliorating, or reversing disability increases in the progressive forms of the disease. Today finding a treatment for progressive MS represents a great therapeutic challenge to improve care and give hope for these chronically disabled patients. Even if emerging therapies offering new therapeutic perspectives are currently evaluated in this form of MS, they mainly act on specific immunological targets involving the complex inflammatory interplays of the disease. Moreover, the endless debate concerning the outside-in model (peripheral autoimmune disease with dysregulated T cells crossing into the CNS) versus the inside-out model (primary CNS degeneration promoting autoimmune inflammatory response) [7] supports the need for new approaches investigating the non-inflammatory aspects of the disease’s pathophysiology such as the ongoing development of therapeutic strategies promoting remyelination [21-23, 25, 26].
One may understand that, in the context of progressive MS, the ultimate therapeutic goal will be the capacity to slow or even to reverse progression over years in these patients. The results already observed in the pilot study and in the first randomized trial with high-dose biotin (MD1003) are encouraging. If they are confirmed in other and larger studies, this will open a new field for research and therapeutic trials focusing on axonal energy protection or prevention of myelin damage.

One of the present limitations of a possible large use of MD1003 in progressive MS is the lack of preclinical studies and paraclinical data shedding light on the mechanisms driving the clinical therapeutic effect. In the pilot and MS-SPI studies, clinical improvement was observed only after few months of MD1003 treatment, which is consistent with a biological mechanism of metabolic failure partially reversible under treatment. This actually offers a primary question that preclinical studies should address. In addition, the duration of the clinical benefit in responders will be very important to evaluate. Will progressive MS patients continue to improve, will they stabilize after months or years of administration, or will they go back to their baseline disability level despite maintaining MD1003 therapy? Data coming from the extension phase of the MS-SPI study and the other ongoing studies should provide answers to these important questions. Again the mechanisms of action should be evaluated in preclinical studies, even if difficult because there is no validated animal model of progressive MS. The use of different animal models (even possibly nonhuman primates) might allow elucidation these mechanisms.

From a pathophysiological and clinical point of view, evaluation of the clinical effect of the co-administration of MD1003 and treatment against inflammation processes will be important. Of great interest would be exploration of the potential of synergistic effects in
patients who respond to MD1003 because the potential therapeutic effects of drugs targeting simultaneously the “outside-in” and “inside-out” model have never been evaluated.

To further evaluate the therapeutic effects of MD1003, functional evaluations (non-conventional MR and PET imaging and/or neurophysiological studies) should facilitate elucidation of the changes in the brain and spinal cord of patients who responded to MD1003 compared with those who did not.

To date, the use of MD1003 in MS patients appears safe. However, preclinical findings identified a putative teratogenicity potential of MD1003. As a consequence, we recommend that women with progressive MS of childbearing age use effective contraception while taking MD1003. If not using effective contraception, MD1003 treated men should use condoms with female partner who are either of childbearing potential or are pregnant.

Overall, the results of one pilot study and one randomized trial with MD1003 in progressive MS have opened new possibilities for treatment and care of this disease. Whether these encouraging results will be confirmed by other and larger studies, necessarily in less disabled patients, constitute the major remaining issue. If the answer is “yes”, we can expect the development of a new therapeutic class targeting biological pathways involved in axonal/neural cell energy or myelin production for the treatment of progressive MS.

Declaration of Interest

D Bernard is an employee of MedDay Pharmaceuticals; author DM Mock has served as a consultant for MedDay Pharmaceuticals; GL Defer has received personal compensation for scientific advisory board activities from BiogenIdec, Novartis, Sanofi Aventis, Genzyme and
Teva pharmaceutical Industries Ltd and has received funding for travel and/or speaker honoraria from Merck Serono, BiogenIdec, Guerbet, Sanofi-Aventis, Novartis, Genzyme and Teva pharmaceutical Industries Ltd. GL Defer’s institution received grants supporting research in his department from Merck Serono, BiogenIdec, Sanofi-Aventis and Novartis. GL Defers is an investigator in the MS-SPI and MS-ON studies sponsored by MedDay Pharmaceuticals. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
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Papers of special note have been highlighted as:
* of interest
** of considerable interest


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77. * Phase III MS-SPI study of MD1003 in patients with progressive MS - Primary endpoint

* Phase III MS-SPI study of MD1003 in patients with progressive MS - Secondary endpoints


* Phase III MS-SPI study of MD1003 in patients with chronic visual loss due to MS


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Figure 1. **Metabolism of biotin: main reactions.** The main metabolites of biotin in human plasma and in urine are 1) bisnorbiotin, produced by one round of beta-oxidation on biotin, and 2) biotin sulfoxide, produced by sulfur oxidation of biotin. Holocarboxylase synthetase covalently attaches biotin to a lysyl residue of the apocarboxylase to form the functional holocarboxylase. Biocytin (biotinyllysine) is a product of proteolysis of biotinylated proteins. Biocytin is further hydrolyzed by biotinidase to release free biotin and lysine. Figure adapted with permission from [28].
Figure 2. Pathways involving biotin-dependent carboxylases. Biotin-dependent carboxylases are represented as black bars: pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase, and propionyl-CoA carboxylase generate intermediates for the tricarboxylic acid (TCA) cycle at three different entry points: oxaloacetate, succinate and acetyl-CoA; and acetyl-CoA carboxylase catalyzes the rate limiting, committed step in fatty acid biosynthesis: the cytosolic synthesis of malonyl-CoA from acetyl-CoA. Figure adapted with permission from [27].
Figure 3. Overview of putative mechanism of action of high-dose biotin in multiple sclerosis. Targets of biotin are: (a) ACC: acetyl-CoA carboxylase (b) PC: pyruvate carboxylase, (c) PCC: propionyl-CoA carboxylase, (d) MCC: methylcrotonyl-CoA carboxylase. Activation of ACC may increase myelin synthesis in oligodendrocytes and remyelination whereas activation of PC, PCC, and MCC may lead to increase ATP production in neurons (and astrocytes).
Figure 4. Mean plasma biotin levels (±SEM) after single oral administration of 100, 200, and 300 mg MD1003 in fasted condition (n=8 healthy adults). Single doses of MD1003 (biotin 100-300 mg) in capsules were rapidly absorbed and distributed in healthy adults (n=4 men and 4 women). The elimination half-life varied between 7.8 and 18.8 hours. For doses ranging between 100 mg and 300 mg, both mean plasmatic peak concentration ($C_{max}$) and area under the plasma drug concentration-time curve (AUC) increased less than proportionally to the dose. There was a slight food effect leading to increased exposure when MD1003 was administered with food.
Table 1. Summary of results from phase III clinical trials in progressive MS

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>n</th>
<th>Intervention</th>
<th>Trial duration (years)</th>
<th>Pre-trial progression (months)</th>
<th>Participants</th>
<th>EDSS change</th>
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<tbody>
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<td>Azathioprine (2.5 mg/kg/day)</td>
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<td>The MS Study Group [98]</td>
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<td>Cyclosporin (adjusted to reach plasma levels of 300–500 ng/ml)</td>
<td>2</td>
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<td>Canadian Cooperative MS Study Group [99]</td>
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<td>C: Cyclophosphamide (1g/alternate day IV until white cell count ≤4.5 × 10⁹/L) and oral prednisone or P: plasma exchange (40 mL/kg weekly for 20 weeks), oral cyclophosphamide (1.5–2.0 mg/kg/day), and oral prednisone</td>
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<td>≥12</td>
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<td>Noseworthy et al. [100]</td>
<td>1998</td>
<td>199</td>
<td>Sulfasalazine (500 mg/day weekly increased by 500 mg/day up to 2 g/day)</td>
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<td>Interferon-beta-1b (8M IU alternate days)</td>
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P value

NS: not significant
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<td>Roquinimex (1.0, 2.5, and 7.5 mg/day)</td>
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<td>Rice et al. [103]</td>
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<td>50†</td>
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<tr>
<td>Pohlau et al. [110]</td>
<td>2007</td>
<td>231</td>
<td>Immunoglobulin (0.4 g/kg per month)</td>
<td>2</td>
<td>≥12</td>
<td>-</td>
<td>-</td>
<td>48</td>
</tr>
<tr>
<td>Wolinsky et al.</td>
<td>2007</td>
<td>943</td>
<td>Glatiramer acetate (20 mg SC per day)</td>
<td>3</td>
<td>≥6</td>
<td>11</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Hawker et al.</td>
<td>2009</td>
<td>439</td>
<td>Rituximab (1000 mg infusions twice in four courses)</td>
<td>2</td>
<td>≥12</td>
<td>9</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Freedman et al.</td>
<td>2011</td>
<td>612</td>
<td>Myelin basic protein 8298 (500 mg IV 6 monthly)</td>
<td>Recent</td>
<td>9</td>
<td>-</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Zajicek et al.</td>
<td>2013</td>
<td>498</td>
<td>Dronabinol (max 28 mg per day)</td>
<td>3</td>
<td>≥12</td>
<td>-</td>
<td>52</td>
<td>61</td>
</tr>
<tr>
<td>INFORMS [17]</td>
<td>2015</td>
<td>969</td>
<td>Fingolimod (0.5 mg per day)</td>
<td>3</td>
<td>≥24</td>
<td>5.8</td>
<td>NR</td>
<td>0</td>
</tr>
<tr>
<td>MS-SPI [75]</td>
<td>2015</td>
<td>154</td>
<td>Biotin (300 mg/day)</td>
<td>3</td>
<td>≥24</td>
<td>15.7</td>
<td>51</td>
<td>64</td>
</tr>
<tr>
<td>ORATORIO [19]</td>
<td>2015</td>
<td>732</td>
<td>Ocrelizumab (600 mg infusions every 24 weeks for 120 weeks)</td>
<td>4.5</td>
<td>-</td>
<td>6.5</td>
<td>44</td>
<td>0</td>
</tr>
</tbody>
</table>

Data taken from [96]. MS = multiple sclerosis; SPMS = secondary progressive multiple sclerosis; PPMS = primary progressive multiple sclerosis; EDSS = Expanded Disability Status Scale; IV = intravenous; SC = subcutaneous; NR = not reported; * Positive score represents worsening, negative score represents improvements; † Remainder had relapsing-remitting multiple sclerosis; ‡ Not separated; § Data taken from original publication; ¶ Trial terminated early, exploratory outcomes taken from patients attending final follow-up; †† 12 mg/m² dose; †‡ Active/placebo ratio of 2:1 (all other studies are 1:1, 1:1:1, or 1:1:1:1).
<table>
<thead>
<tr>
<th>Study name</th>
<th>Primary objective</th>
<th>Primary efficacy endpoint</th>
<th>Study design/population</th>
<th>Status</th>
<th>Anticipated completion date</th>
<th>EudraCT No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-SPI</td>
<td>To demonstrate the superiority of MD1003 at 300 mg/day over placebo in clinical improvement of patients with spinal progressive MS</td>
<td>Proportion of patients with decreased EDSS* or ≥20% improvement in TW25 at month 9 (confirmed at month 12) compared with best baseline values</td>
<td>12 months’ treatment followed by 12-month open-label extension phase (154 patients randomized 2:1)</td>
<td>Ongoing (double-blind phase completed, analysis completed)</td>
<td>Jan 2016</td>
<td>2013-002113-35</td>
</tr>
<tr>
<td>MS-ON</td>
<td>To demonstrate the superiority of MD1003 at 300 mg/day over placebo in the visual improvement of patients suffering from chronic visual loss after optic neuritis related to MS</td>
<td>Mean change in best corrected visual acuity (logMAR) at 100% contrast between baseline and month 6 of the diseased eye**</td>
<td>6 months’ treatment followed by 6-month open-label extension phase (93 patients randomized 2:1)</td>
<td>Ongoing (double-blind phase completed, analysis ongoing)</td>
<td>Jan 2016</td>
<td>2013-002112-27</td>
</tr>
<tr>
<td>MD1003-</td>
<td>To demonstrate the</td>
<td>Mean change of 2MWT time</td>
<td>12 months’ treatment</td>
<td>Ongoing</td>
<td>2016</td>
<td>2014-000698-</td>
</tr>
<tr>
<td>AMN</td>
<td>superiority of MD1003 at 300 mg/day over placebo in the clinical improvement of patients with AMN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>between month 12 and baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>followed by 12-month open-label extension phase (67 patients randomized 2:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(unblinded)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2MWT, 2 minutes walking test; AMN, adrenomyeloneuropathy; EDSS, Expanded Disability Status Scale; EudraCT, European Clinical Trials Database; MS, multiple sclerosis; TW25, Timed 25-Foot Walk

* Defined as a decrease of ≥0.5 point if initial EDSS from 6 to 7 and a decrease of ≥1 point if initial EDSS from 4.5 to 5.5

** Defined as the eye with the worst visual acuity (<5/10) at baseline and with evidence of worsening during the past three years
Table 3. Reported safety from clinical use of high-dose biotin

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number of patients</th>
<th>Dose and route of administration</th>
<th>Treatment duration</th>
<th>Adverse effects reported</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-month-old infant with metabolic acidosis and ketosis</td>
<td>1</td>
<td>10 mg/day orally</td>
<td>3 weeks</td>
<td>No adverse effects, other than diarrhea</td>
<td>Informatics Inc., 1974 [58]</td>
</tr>
<tr>
<td>Infants with Leiner’s disease or other forms of dermatitis</td>
<td>28</td>
<td>20 mg orally or 0.1 mg/day i.m.</td>
<td>3–5 weeks</td>
<td>No adverse effects</td>
<td>Informatics Inc., 1974 [58]</td>
</tr>
<tr>
<td>Infants with dermatitis seborrhoides</td>
<td>30</td>
<td>5 mg/day i.m. for 6–10 days, then orally until day 10 or 15</td>
<td>2 weeks</td>
<td>No adverse effects</td>
<td>Informatics Inc., 1974 [58]</td>
</tr>
<tr>
<td>Infants with generalized seborrheic dermatitis</td>
<td>25</td>
<td>5 mg i.v.</td>
<td>1 injection</td>
<td>No adverse effects</td>
<td>Messaritakis et al., 1975 [83]</td>
</tr>
<tr>
<td>2-year-old with propionic acidemia and secondary ketotic hyperglycemia</td>
<td>1</td>
<td>5 mg twice daily orally</td>
<td>5 days</td>
<td>No adverse effects</td>
<td>Informatics Inc., 1974 [58]</td>
</tr>
<tr>
<td>211 patients with erythroderma and 192 with seborrheic dermatitis</td>
<td>403</td>
<td>4 tablets orally or 1-2 ampules i.m.- containing 5 mg (daily doses of up to 100 mg)</td>
<td>1–3 weeks</td>
<td>No adverse effects</td>
<td>Informatics Inc., 1974 [58]</td>
</tr>
<tr>
<td>Patients with onychodystrophy</td>
<td>5</td>
<td>120 mg/day orally</td>
<td>40 days</td>
<td>No adverse effects</td>
<td>Informatics Inc., 1974 [58]</td>
</tr>
<tr>
<td>Infants with seborrheic dermatitis and infants with Leiner’s disease</td>
<td>11</td>
<td>Total dosage ranged from 3 mg over 2 weeks to 10 mg injected over 4 days</td>
<td>2 weeks</td>
<td>No adverse effects</td>
<td>Informatics Inc., 1974 [58]</td>
</tr>
<tr>
<td>Subject</td>
<td>Number of patients</td>
<td>Dose and route of administration</td>
<td>Treatment duration</td>
<td>Adverse effects reported</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------------</td>
<td>----------------------------------</td>
<td>--------------------</td>
<td>--------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Holocarboxylase Synthetase Deficiency</td>
<td>11</td>
<td>5-80 mg day orally</td>
<td>Not specified</td>
<td>No adverse effects</td>
<td>Michalski et al., 1989 [84]</td>
</tr>
<tr>
<td>Hemodialysis patients</td>
<td>11</td>
<td>50 mg i.v.</td>
<td>2 months</td>
<td>No adverse effects</td>
<td>Koutsikos et al., 1996 [85]</td>
</tr>
<tr>
<td>Protein deficient children</td>
<td>22</td>
<td>10 mg/day orally</td>
<td>15 days</td>
<td>No adverse effects</td>
<td>Expert Group in Vitamins and Minerals, 2003 [65]</td>
</tr>
<tr>
<td>Patient with holocarboxylase synthetase deficiency</td>
<td>1</td>
<td>200 mg/day orally</td>
<td>Not specified</td>
<td>No adverse effects</td>
<td>Santer et al., 2003 [86]</td>
</tr>
<tr>
<td>Patients with biotin-responsive basal ganglia disease</td>
<td>8</td>
<td>5 mg/kg/day orally</td>
<td>Months to years months</td>
<td>No adverse effects</td>
<td>Ozand et al., 1998 [35]</td>
</tr>
<tr>
<td>Patient with biotin-responsive basal ganglia disease</td>
<td>1</td>
<td>5 mg/kg/day orally</td>
<td>Several months</td>
<td>No adverse effects</td>
<td>Adhisivam et al., 2007 [87]</td>
</tr>
<tr>
<td>Patients with biotin-responsive chronic progressive encephalopathies</td>
<td>10</td>
<td>50 to 200 mg/day</td>
<td>Several months</td>
<td>No adverse effects</td>
<td>Dabbagh et al., 1994 [88]</td>
</tr>
<tr>
<td>Patients with biotin-responsive basal ganglia disease</td>
<td>3</td>
<td>100 mg/day</td>
<td>Several months</td>
<td>No adverse effects</td>
<td>Straussberg et al., 2002 [89]</td>
</tr>
<tr>
<td>Patients with biotin-responsive basal ganglia disease</td>
<td>1</td>
<td>5 mg/kg/day</td>
<td>For one year</td>
<td>No adverse effects</td>
<td>Yamada et al., 2010 [90]</td>
</tr>
<tr>
<td>Patients with biotin-responsive basal ganglia disease</td>
<td>1</td>
<td>5 mg/day, increased to 15 mg/day and 30 mg/day</td>
<td>Several months</td>
<td>No adverse effects</td>
<td>El-Hajj et al., 2008 [91]</td>
</tr>
<tr>
<td>Subject</td>
<td>Number of patients</td>
<td>Dose and route of administration</td>
<td>Treatment duration</td>
<td>Adverse effects reported</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>--------------------</td>
<td>----------------------------------</td>
<td>--------------------</td>
<td>------------------------------------------------------------------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>Patients with biotin-responsive basal ganglia disease and SCL19A3 mutations</td>
<td>2</td>
<td>100 to 600 mg/day</td>
<td>4 years</td>
<td>No adverse effects</td>
<td>Debs et al., 2010 [38]</td>
</tr>
<tr>
<td>Patients with progressive MS</td>
<td>23</td>
<td>100 to 600 mg/day</td>
<td>2 to 84 months</td>
<td>One death from heart failure not considered to be related to the treatment</td>
<td>Sedel et al., 2015 [74]</td>
</tr>
</tbody>
</table>

*i.m.: intramuscular route, i.v.: intravenous route; MS: multiple sclerosis*