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Efficacy of vitamin D_3 as add-on therapy in patients with relapsing–remitting multiple sclerosis receiving subcutaneous interferon beta-1a: A Phase II, multicenter, double-blind, randomized, placebo-controlled trial

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ABSTRACT

Recent studies have demonstrated the immunomodulatory properties of vitamin D, and vitamin D deficiency may be a risk factor for the development of MS. The risk of developing MS has, in fact, been associated with rising latitudes, past exposure to sun and serum vitamin D status. Serum 25-hydroxyvitamin D [25(OH)D] levels have also been associated with relapses and disability progression. The identification of risk factors, such as vitamin D deficiency, in MS may provide an opportunity to improve current treatment strategies, through combination therapy with established MS treatments. Accordingly, vitamin D may play a role in MS therapy. Small clinical studies of vitamin D supplementation in patients with MS have reported positive immunomodulatory effects, reduced relapse rates and a reduction in the number of gadolinium-enhancing lesions. However, large randomized clinical trials of vitamin D supplementation in patients with MS are lacking.

SOLAR (***S***upplementation of Vigant**OL**® oil versus placebo as ***A***dd-on in patients with relapsing–remitting multiple sclerosis receiving **R**ebif® treatment) is a 96-week, three-arm, multicenter, double-blind, randomized, placebo-controlled, Phase II trial (NCT01285401). SOLAR will evaluate the efficacy of vitamin D_3 as add-on therapy to subcutaneous interferon beta-1a in patients with RRMS. Recruitment began in February 2011 and is aimed to take place over 1 calendar year due to the potential influence of seasonal differences in 25(OH)D levels.

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1. Introduction

Multiple sclerosis (MS) is a chronic, inflammatory and degenerative disease of the central nervous system, believed to be triggered by environmental factors in patients with complex genetic-risk profiles [1]. Current disease-modifying drugs can reduce relapses and may delay disability progression in patients with relapsing–remitting MS (RRMS) [2–5], the most common disease course at onset [6].

Vitamin D deficiency is a putative risk factor for MS [7,8]. Identifying such risk factors as potential targets of therapy may provide an opportunity to improve current MS treatment strategies. The risk of developing MS has been correlated to rising latitudes, past exposure to sun and serum 25-hydroxyvitamin D [25(OH)D] level [9]. Levels of vitamin D are determined by dietary intake, sun exposure and genetic make-up [10]. Vitamin D_3 (cholecalciferol), the naturally occurring form

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of vitamin D, is predominantly found in two metabolite forms in the circulation: the prehormone, 25-hydroxyvitamin D; and the thousand-times less abundant, biologically active metabolite, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. Circulating concentrations of 1,25$(OH)_2$D are mainly the result of a 25-hydroxylation step in the kidney, which is regulated according to the needs of calcium homeostasis [11]. This activation step into 1,25$(OH)_2$D can also occur within other tissues, including immune-modulating cells, where the activation is not affected by calcium homeostasis; in this case 1,25$(OH)_2$D is not thought to enter the circulation, but rather its role is to moderate immune function by autocrine and paracrine mechanisms, suggesting that vitamin D may have a role in human physiology beyond skeletal and calcium homeostasis [12].

In several studies, a potential causal association between serum 25(OH)D concentrations and MS disease activity has been observed. A high chance of remaining relapse-free has been associated with higher serum 25(OH)D levels [8], with each 10 nmol/L increase in 25(OH)D resulting in a reduction in the risk of relapse of up to 12% [13]. In addition, in pediatric-onset MS and clinically isolated syndrome, every 10 ng/mL increase in adjusted 25(OH)D level was associated with a 34% decrease in the rate of subsequent relapses [14] (equating to an approximate 14% decrease in the rate of subsequent relapses per 10 nmol/L increase). Furthermore, serum 25(OH)D and 1,25$(OH)_2$D levels have been reported to be lower during MS relapses than during remission [15,16]. An inverse relation between Expanded Disability Status Scale (EDSS) progression and vitamin D levels has also been reported [8].

Functional interaction of vitamin D with the main MS-linked HLA-DRB1*1501 allele [17], or selective immune system regulation [18] are possible mechanisms underlying the modulating effect of vitamin D on MS pathobiology. The biologically active metabolite of vitamin D has been shown to induce suppression of interleukin (IL)-2, IL-1 and lymphocyte proliferation [19,20]. High levels of serum 25(OH)D have been associated with an improved regulatory T cell function and with skewing of the Th1/Th2 balance towards a Th2 type response, suggesting that vitamin D may be an important regulator of peripheral T cell function in MS [21]. A reduced proliferative response of CD4+ T cells to myelin peptides was observed in patients who received high-dose vitamin D supplementation (up to 40000 IU daily), when compared with that in patients without supplementation and when compared with that prior to treatment [22]. Strong immunomodulatory properties of molecules derived from vitamin D_3 have also been demonstrated in murine experimental autoimmune encephalomyelitis (EAE), a model of MS. TX527, an analog of 1,25$(OH)_2D_3$, can attenuate EAE and results in significant disease protection in combination with interferon (IFN)-beta, superior to the effects of the individual treatments [23].

Small preliminary studies of vitamin D_3 supplementation, with or without calcium, have reported positive immunomodulatory effects [22], reduced relapse rates [24] and a reduction in the number of gadolinium (Gd)-enhancing lesions [25] in patients with MS. Furthermore, vitamin D supplementation in patients with MS raised serum transforming growth factor beta-1 levels [26], which may indicate a neuroprotective effect.

In a recent pilot study, patients with RRMS receiving IFN-beta were supplemented with high-dose (20000 IU daily) vitamin D_3, the same compound that will be used in the SOLAR study, for 12 weeks [27]. A shift towards an anti-inflammatory cytokine profile, with an increased proportion of IL-10+ CD4+ T cells and a decrease in the ratio between IFN-γ+ and IL-4+ CD4+ T cells was observed, thus suggesting that combining IFN-beta with vitamin D_3, to further modulate the immune system of patients with MS, may be beneficial [27].

Although there is no direct evidence demonstrating the clinical benefit of combining vitamin D_3 with IFN-beta therapy in MS, the majority of patients in studies examining vitamin D in MS were also receiving IFN-beta. To date, these studies have been limited to assessing the association between the risk of relapse and vitamin D level [8,13,14], and the safety of high-dose vitamin D [22,25,27] in MS. The recent pilot study of high-dose vitamin D_3 treatment [27], and the potential synergism for IFN-beta and vitamin D in a mouse model of MS [23], suggest that a double-blind placebo-controlled trial is necessary to determine the potential add-on effects of vitamin D_3 to IFN-beta treatment.

The SOLAR (***S***upplementation of Vigant***OL***® oil versus placebo as ***A***dd-on in patients with relapsing–remitting multiple sclerosis receiving ***R***ebif® treatment) study is designed to assess the efficacy of vitamin D_3 as add-on therapy to subcutaneous (sc) IFN beta-1a in patients with RRMS (NCT01285401).

2. Methods

2.1. SOLAR trial design, objectives and rationale

SOLAR is a 96-week, three-arm, multicenter, double-blind, randomized, placebo-controlled, Phase II trial (Fig. 1). The primary objective of the SOLAR study is to assess the efficacy of vitamin D_3 (cholecalciferol, Vigantol® oil, Merck KGaA, Darmstadt, Germany) versus placebo as add-on therapy in patients with RRMS receiving sc IFN beta-1a (Rebif®, Merck Serono S.A. – Geneva, Switzerland), 44 μg three times weekly (tiw).

The secondary objectives are to: assess changes in clinical and magnetic resonance imaging (MRI) parameters; investigate the safety profile of study treatment over 96 weeks; and explore pharmacogenetics, gene expression and circulating biomarkers, and potential correlations with vitamin D_3 treatment outcomes.

A total of 348 patients with RRMS and with serum 25(OH)D levels <150 nmol/L will be randomized (1:1) to receive vitamin D_3 or placebo as add-on therapy to IFN beta-1a, 44 μg sc tiw. Patients randomized to vitamin D_3 will receive 7000 IU (175 μg) daily for 4 weeks. If treatment with vitamin D is tolerated (as described below), patients will receive 14000 IU (350 μg) daily for a further 92 weeks; otherwise, patients will continue to receive 7000 IU daily. In case of further intolerance, vitamin D_3 supplementation will be withdrawn; patients will be encouraged to maintain the assessment schedule until the end of the treatment period during which vitamin D_3 withdrawal occurred. The stability of the vitamin D_3 compound is guaranteed for the entire shelf life of the product. An unspecified number of patients (estimated to be 10) with supraphysiological

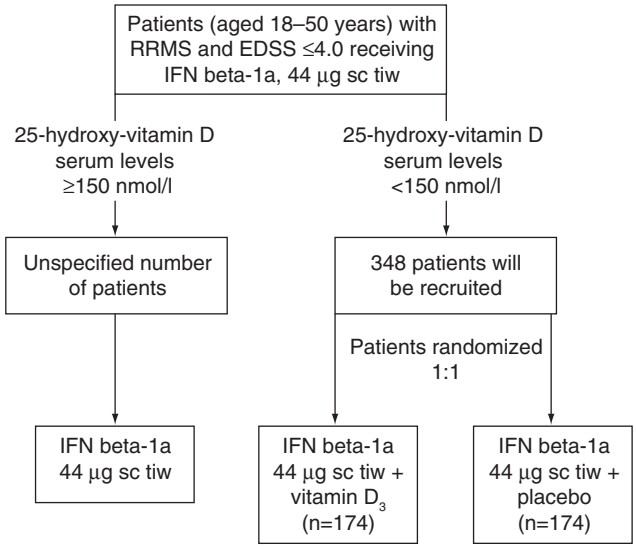

Fig. 1. Patient recruitment and randomization. EDSS, Expanded Disability Status Scale; IFN, interferon; RRMS, relapsing–remitting multiple sclerosis; sc, subcutaneous; tiw, three times weekly.

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serum 25(OH)D levels ≥150 nmol/L will receive IFN beta-1a, 44 μg sc tiw, only. For all patients, if treatment with IFN beta-1a, 44 μg sc tiw, is not well tolerated, patients will receive IFN beta-1a, 22 μg sc tiw, based on the physician's decision.

Vitamin D_3 will be administered as an oral solution (500 μg/mL) each morning during breakfast, as the absorption of a fat-soluble vitamin benefits from the contemporaneous intake of fat (e.g. dropping the daily dose on a piece of buttered bread). sc IFN beta-1a will be administered before going to bed. The exception to this schedule will be during visits to the trial site where vitamin D_3, or placebo, will be administered only after the collection of blood samples.

Serum calcium (<2.6 mmol/L) levels, as well as the urinary ratio of calcium to creatinine (<1.0), will be determined before the start of treatment, and at week 4, week 12 and every 12 weeks thereafter. If the urinary ratio of calcium to creatinine is elevated, this measurement will be repeated as soon as possible to confirm the result before any action is taken, as urine calcium levels are highly variable. If any parameter is above the mentioned upper limit of normal for patients receiving 14000 IU daily, the dose of vitamin D_3 may be decreased by 50% (i.e. from 14000 IU daily to 7000 IU daily) or omitted for 1 month with a re-administration of 50% of the dose thereafter. All patients will continue to receive their dose of IFN beta-1a for the duration of the study.

2.2. Rationale for high-dose vitamin D_3 dosing in combination with IFN-beta

To be able to differentiate treatment effects between the vitamin D_3 and placebo-treated arms, patients will be supplemented with high doses of vitamin D_3, as a lower dose of vitamin D_3 within a controlled trial setting may not reveal potential benefits [22]. In addition, evidence for high-dose vitamin D_3 effectiveness in the context of an immunomodulatory treatment is supported by results from two recent studies: where patients with RRMS, receiving IFN-beta, were supplemented with 20000 IU daily vitamin D_3 [27]; and where patients with MS received escalating vitamin D doses up to 40000 IU daily over 28 weeks, followed by 10000 IU daily over 12 weeks, and further downtitrated to 0 IU daily [22].

Studies investigating 25(OH)D levels have suggested that a concentration exceeding 100 nmol/L is optimal in healthy individuals [28], and may reduce the risk of MS [29]. In addition, studies assessing vitamin D adequacy in fracture prevention and preservation of bone mineral density suggest that concentrations of 25(OH)D should exceed 75 nmol/L [30]. Moreover several cohort studies among patients with MS have shown that patients with RRMS are environmentally exposed to physiological 25(OH)D levels up to 150 nmol/L [8,13,14].

A study designed to test the tolerability of specific 25(OH)D concentrations, reported a reduction in the number of Gd-enhancing lesions in patients with MS receiving 1200 mg elemental Ca/day alongside increasing doses of vitamin D_3, from 28000 to 280000 IU weekly [25]. Furthermore, evidence of immunomodulatory effects was observed in patients with MS treated with high-dose oral vitamin D, which resulted in a mean serum 25(OH)D peak of 413 nmol/L [22]. Therefore, we selected a dose of 7000 IU daily for 4 weeks, followed by 14000 IU daily for a further 92 weeks.

2.3. Dose safety and potential side effects

Baseline serum 25(OH)D levels of 150 nmol/L in patients treated with 14000 IU vitamin D_3 per day, corresponding to 350 μg, is expected to increase the 25(OH)D serum level by 245 nmol/L, based on a 0.70 nmol increase for each additional 1 μg vitamin D_3 input [31]. Therefore, the expected resulting serum 25(OH)D level of 395 nmol/L in patients receiving vitamin D_3 will be below the critical threshold of 500 nmol/L that may lead to hypercalcaemia [32]. In a previous study, the serum 25(OH)D levels of patients with MS receiving high doses of vitamin D_3 (increasing doses of vitamin D_3 from 28000 to 280000 IU weekly, for 28 weeks) was initially 78 nmol/L, rising to 386 nmol/L after treatment, meaning that patients reached twice the top of the physiologic range without eliciting hypercalcaemia or hypercalciuria [25]. No significant adverse advents (AEs) were observed in patients with MS treated with high doses of vitamin D_3 (up to 40000 IU daily over 28 weeks, followed by 10000 IU daily for 12 weeks, and further down-titrated to 0 IU daily), leading to the conclusion that approximately 10000 IU daily was a safe regimen [22]. Vitamin D_3 was well tolerated and resulted in desirable vitamin D_3 levels in a study investigating long-term (1 year) and short-term (8 weeks) safety in healthy adolescents at doses of 14000 IU weekly, equivalent to 2000 IU daily [33]. In addition, 15 patients with RRMS receiving IFN-beta were recently supplemented with 20000 IU daily of vitamin D_3 for 12 weeks without a dose-escalation scheme. The resulting median serum 25(OH)D level of 348 nmol/L did not induce AEs such as hypercalcaemia or hypercalciuria [27].

2.4. Rationale for the three-arm study design

There is currently no consensus on the optimal physiological range of vitamin D_3 in the general population, nor a recognized threshold below which individuals should be considered as vitamin D_3-deficient. Therefore, to avoid potential low vitamin D_3 level selection bias, a serum 25(OH)D level of <150 nmol/L has been defined as a cut-off value. In addition to stratifying randomized patients by serum 25(OH)D levels at baseline, patients will also be stratified (in order of importance) by body mass index (due to the fat-soluble nature of vitamin D_3), sex and number of relapses in the past 2 years, in order to assess the impact of these variables on clinical outcomes and to assure equal distribution among the treatment arms. Avoiding potential vitamin D_3 level selection bias and stratifying patients by variables is considered to be the most ethically acceptable study design, considering the potential benefits of vitamin D_3 supplementation in patients with RRMS. In addition, this approach will allow the potential synergistic mode of action (MoA) of IFN-beta with vitamin D_3 in MS to be explored, with respect to IFN-beta with placebo.

Samples from patients in the third arm, with supraphysiological serum 25(OH)D levels (≥150 nmol/L), will be used in exploratory analyses. Pharmacogenetic analyses will investigate whether common genetic polymorphisms distinguish these patients from those with serum 25(OH)D levels <150 nmol/L. In addition, it will also be of interest to determine whether these patients have a more benign course of disease than those with serum 25(OH)D levels <150 nmol/L, suggesting natural protection.

2.5. Patient criteria

Patients who meet the following criteria will be eligible for the study: aged 18–50 years; have a diagnosis of RRMS (McDonald 2005 criteria [34]); have a brain and/or spinal MRI with findings typical of MS; have a first clinical event occurring within 5 years prior to screening; have had at least relapse, or one or more Gd-enhancing or new T2 MRI lesions within the 12 months prior to screening; have an EDSS score ≤4.0 at screening; are currently and for the first time receiving sc IFN beta-1a tiw for a minimum of 90 days and for not longer than 12 months prior to the baseline visit. All patients must be willing and able to comply with the protocol for the duration of the trial and provide written informed consent.

The main exclusion criteria are: pregnancy or lactation; any disease other than MS that could better explain signs and symptoms; complete transverse myelitis or bilateral optic neuritis; a relapse within 30 days prior to study day 1 (SD1); use of corticosteroids or adrenocorticotrophic hormone within 30 days prior to SD1; abnormalities of vitamin D-related hormonal system other than due to low dietary intake or decreased sun exposure; urine calcium/creatinine (mmol/mmol) ratio >1.0 or hypercalcaemia (11 mg/100 cc [5.5 mEq/L]); inadequate liver function (defined by alanine aminotransferase [ALT] >3 times upper

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limit of normal [ULN]; aspartate aminotransferase >3 times ULN; alkaline phosphatase >2.5 times ULN; or bilirubin >1.5 times ULN, if associated with any elevation of ALT or alkaline phosphatase); concomitant medications that influence vitamin D metabolism other than corticosteroids (e.g., phenytoin, barbiturates, thiazide diuretics and cardiac glycosides); currently taking >400 IU (>10 μg) of vitamin D supplement daily; or conditions with increased susceptibility to hypercalcaemia (e.g., known arrhythmia or heart disease, treatment with digitalis or hydrochlorothiazide, nephrolithiasis).

2.6. Randomization

Patients with serum 25(OH)D levels <150 nmol/L will be randomized 1:1 at SD1, by means of an interactive voice-response system, to vitamin D_3 or placebo, and will be double-blinded for all assessments. Patients with serum 25(OH)D levels ≥150 nmol/L will be automatically assigned to the sc IFN beta-1a treatment group and undergo all trial assessments without receiving either vitamin D_3 or placebo.

2.7. Primary endpoints

The primary endpoint will be a composite endpoint of MRI and clinical variables. The primary MRI endpoint will be the mean number of combined unique active (CUA) lesions at week 48. The primary clinical endpoint will be the proportion of relapse-free patients at week 96, chosen as previous studies have demonstrated correlations between vitamin D supplementation and relapse-free status [8,13,14,22]. The composite endpoint will be assessed in a hierarchical manner, with the primary MRI endpoint measured at week 48 and the primary clinical endpoint measured at week 96.

2.8. Secondary endpoints

Secondary clinical endpoints will include relapses and chronic disease (i.e. disability) progression. Relapse-related endpoints will be measured by the: proportion of relapse-free patients at week 48; time to first documented relapse; annualized relapse rate at weeks 48 and 96; total number of reported relapses at all time points; and requirement for glucocorticoid treatment during the trial. Secondary disability-related endpoints will be measured by the: proportion of patients free from confirmed EDSS progression at weeks 24, 48, 72 and 96; time to 24 weeks' sustained disability progression on EDSS; and proportion of patients with 24 weeks' sustained disability progression on EDSS at weeks 48 and 96.

The secondary MRI endpoints (Table 1) will assess the anti-inflammatory effect (lesion activity, disease activity and burden of disease) and a potential neuroprotective effect (development of black holes and brain atrophy) of vitamin D supplementation. Additional secondary efficacy endpoints will measure the proportion of patients free from disease activity and the change in cognitive function from baseline at weeks 48 and 96, measured by the Symbol Digit Modalities Test (SDMT).

2.9. Safety and immunogenicity endpoints

Safety endpoints will be evaluated up to week 96 and will include safety data from all completed trial visits, including: the occurrence of AEs, serious AEs and laboratory abnormalities; adherence to treatment; change from baseline in bone mass density at week 96 (test optional); reasons for trial termination; serum calcium level; urine calcium/creatinine ratios; and serum 25(OH)D_3 and serum 1,25$(OH)_2D_3$ levels. Immunogenicity endpoints will include the proportion of patients, measured from baseline, who are positive for binding antibodies (BAbs) and neutralizing antibodies (NAbs) to IFN beta.

Table 1
Additional MRI-based secondary endpoints.

Scanning sequence	Endpoint
T1 (Gd-enhancing)	Mean number of new T1 Gd-enhancing lesions per patient per scan at weeks 48 and 96 Cumulative number of T1 Gd-enhancing lesions at weeks 48 and 96 Proportion of patients free from T1 Gd-enhancing lesions at weeks 48 and 96
T1 (Gd-enhancing) and T2	Cumulative number of new CUA lesions at weeks 48 and 96 Mean number of CUA lesions per patient per scan at week 96
T2	Mean change from baseline in the total volume of T2 lesions at weeks 48 and 96 (T2 BOD; mm^3)
T1 non-enhancing	Proportion of patients free from new T1 hypointense lesions (black holes) at weeks 48 and 96 Percentage of new T1-hypointense lesions (black holes) at weeks 48 and 96 within the subgroup of new or enlarging non-enhancing T2 lesions Mean change from baseline in total volume of T1 hypointense lesions at weeks 48 and 96 (mm^3)
Other	Percent brain volume change at weeks 48 and 96 from baseline, and at week 96 from week 48 Proportion of T1 Gd-enhancing lesions at SD1 that transform into black holes at weeks 48 and 96

BOD, burden of disease; CUA, combined unique active; Gd, gadolinium, MRI, magnetic resonance imaging; SD1, study day 1.

2.10. Assessments

Patients will undergo 12 visits during the trial period (Fig. 2). MRI assessments will be performed at SD1 and weeks 48 and 96. Neurological function will be assessed at screening, SD1 and every 12 weeks thereafter. Cognitive function (SDMT) will be assessed at SD1 and weeks 24, 48, 72 and 96. Relapses will be evaluated at SD1, week 4, week 12 and every 12 weeks thereafter up to week 96. BAbs and NAbs to IFN beta-1a will be measured at SD1, week 12 and every 12 weeks thereafter up to week 96. Serum 25(OH)D levels will be measured at screening, SD1 and weeks 48 and 96. Gene expression profiling will be performed at SD1 and weeks 24, 48, 72 and 96, and pharmacogenetic biomarkers investigated in samples taken at SD1.

2.11. Biomarkers and pharmacogenetics

Circulating biomarkers, gene expression, and pharmacogenetic analyses will be performed on blood and plasma samples to identify potential surrogate biomarkers. Biomarker analysis will investigate potential correlations between vitamin D_3 add-on therapy and clinical- and MRI-based outcomes. In addition, biomarker analysis will explore the pharmacological response to treatment and the MoA of vitamin D_3. Clinical and MRI outcomes will be evaluated in relation to single nucleotide polymorphisms of the vitamin D binding protein, vitamin D receptor, CYP27B1, CYP24A1, CYP2R1, and in MS-related CD25, CD127 and HLA-DRB1*1501. Circulating protein and gene expression profiling will be measured at relapse. It is also important to note that different or additional biomarkers may be analyzed to reflect the latest advances in biomarker research.

2.12. Sample size and statistical analysis

The study is 80% powered to detect an increase from 53% (placebo group) to 69% (vitamin D_3 group) in the proportion of relapse-free patients at week 96. The study is also powered to detect futility based upon the CUA lesion count per patient at week 48 at the interim analysis. Assuming that the number of CUA lesions per patient follows an approximate negative binomial distribution, with a mean (SD) for treatment group 1 (patients receiving vitamin D_3 and IFN beta-1a, 44 μg sc tiw) of 1.03 (1.61) and treatment group 2 (patients receiving

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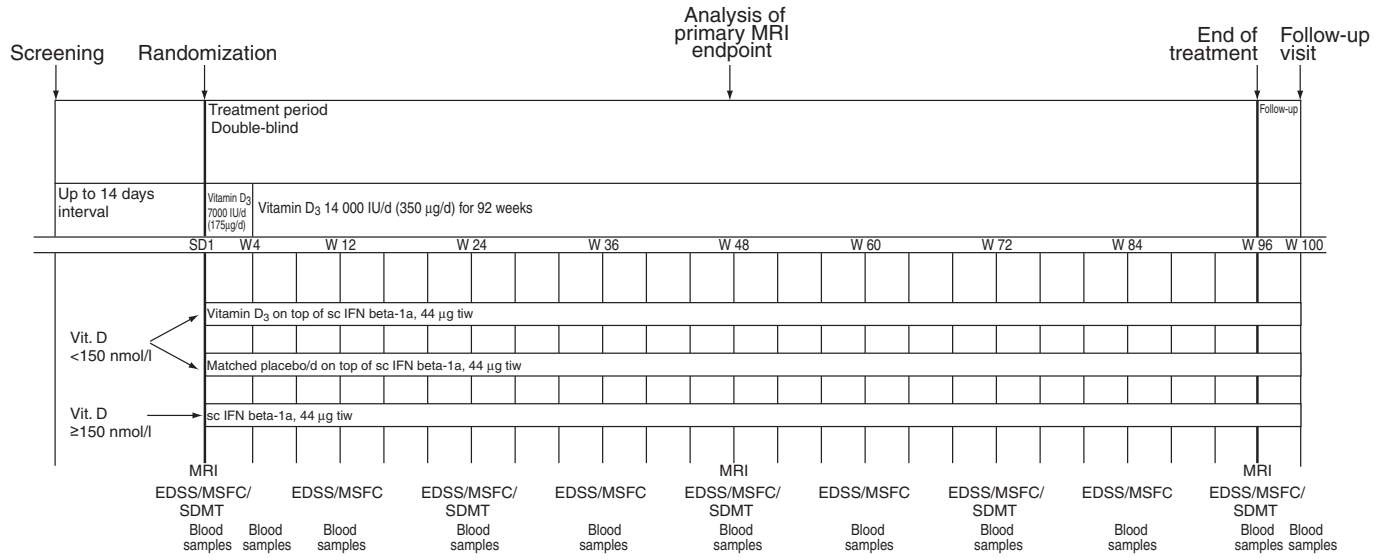

Fig. 2. Study design. EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; MSFC, multiple sclerosis functional composite; sc, subcutaneous; SD1, study day 1; tiw, three times weekly; SDMT, Symbol Digit Modalities Test.

placebo and IFN beta-1a, 44 µg sc tiw) of 0.48 (1.11), then 200 patients (100 per arm) provides 80% power to detect this increase in CUA lesion count over treatment group 3 (IFN beta-1a, 44 µg sc tiw only) with $p<0.05$. The study is designed to detect a decrease in the mean number of CUA lesions at week 48.

The planned sample size of 348 patients with 25(OH)D serum levels <150 nmol/L in the two randomized arms is based upon the primary clinical endpoint.

2.13. Patient recruitment

The SOLAR study will be conducted in Europe, involving approximately 53 sites across Belgium, Denmark, Estonia, Finland, Germany, Hungary, Latvia, Lithuania, Norway, Switzerland, The Netherlands and Italy. Recruitment of patients from northern and southern Europe will allow for an investigation of the effects of latitude (sun exposure) on treatment outcomes. Recruitment is aimed to take place over 1 calendar year due to the potential influence of seasonal differences in vitamin D levels. Recruitment began in half of the countries in February/March and will begin in April/May in the other half, which should result in a close to linear recruitment curve, with an accrual rate estimate of 0.5 patients per-month per-site. The site initiation sequence is performed only in relation to the approval of the study documents by the site IRB/EC and contract finalization, and is not dictated by any other factors (e.g. latitude of the site).

2.14. Sub-studies

The SOLAR(IUM) trial will be a parallel, immunological sub-study in The Netherlands. The trial will assess whether, in patients supplemented with vitamin D_3 for 1 year, the CD4+ T cell compartment displays a more pronounced anti-inflammatory state when compared with placebo-supplemented patients. The primary endpoint will be the difference in distribution of cytokine profile of peripheral CD4+ T lymphocytes by flow cytometry at week 48 between the two randomized treatment groups.

A second sub-study in Switzerland will aim to investigate the effect of vitamin D treatment on antigen-specific T cells in patients with MS. The secondary objective will be to assess the immunomodulatory effect of sc IFN beta-1a with or without vitamin D_3 supplementation compared with no immunomodulatory treatment, using the two randomized treatment groups. For this analysis, a fourth group of patients will be enrolled: patients with RRMS without any treatment for the past 3 months, matched with the randomized patients in terms of EDSS score, age and sex.

3. Conclusions

Although the mechanisms underlying the modulating effect of vitamin D on MS pathobiology are not currently fully understood, current literature suggests that vitamin D is a likely environmental factor contributing to the risk of developing MS. In addition, initial studies indicate that the dose of vitamin D_3 we plan to use in this study may be clinically beneficial to patients with MS, without inducing AEs. SOLAR will be the first large, placebo-controlled international study to assess vitamin D_3 as add-on therapy to sc IFN beta-1a in patients with RRMS, using clinical and MRI outcomes. The study will also consider confounding factors, serum 25(OH)D levels reached, sunlight exposure and latitude. Further, analyses of specific genetic sequences already described will be performed to evaluate correlations between specific genetic preconditions and treatment response. In addition, the SOLAR study will provide important information that will assist with the design of future trials of vitamin D supplementation in MS treatment, by identifying how different populations of patients with MS respond to vitamin D_3.

Role of funding

The study is sponsored by Merck Serono S.A. – Geneva, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany), who developed and approved the study design, and also supported the preparation of this manuscript.

Conflict of interest

J Smolders has received a consultancy fee from Merck Serono and traveling fees from Biogen Idec. *U Hostalek* is an employee of Merck KGaA, Darmstadt, Germany. *M Beelke* is an employee of Merck Serono S.A. – Geneva, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany). *L Ghazi-Visser* is an employee of Merck B.V. – Schiphol-Rijk, The Netherlands (an affiliate of Merck KGaA, Darmstadt, Germany). *F Barkhof* has received consultancy fees from Bayer-Schering Pharma, Sanofi-Aventis, Biogen Idec, UCB, Merck Serono,

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