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High-Dose Vitamin D in Clinically Isolated Syndrome Typical of Multiple Sclerosis

The D-Lay MS Randomized Clinical Trial

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IMPORTANCE Vitamin D deficiency is a risk factor for multiple sclerosis (MS) and is associated with the risk of disease activity, but data on the benefits of supplementation are conflicting.

OBJECTIVE To evaluate the efficacy of high-dose cholecalciferol as monotherapy in reducing disease activity in patients with clinically isolated syndrome (CIS) typical for MS.

DESIGN, SETTING, AND PARTICIPANTS The D-Lay MS trial was a parallel, double-blind, randomized placebo-controlled clinical trial in 36 MS centers in France. Patients were enrolled from July 2013 to December 2020 (final follow-up on January 18, 2023). Untreated patients with CIS aged 18 to 55 years with CIS duration less than 90 days, serum vitamin D concentration less than 100 nmol/L, and diagnostic magnetic resonance imaging (MRI) meeting 2010 criteria for dissemination in space or 2 or more lesions and presence of oligoclonal bands were recruited.

INTERVENTION Patients were randomized 1:1 to receive oral cholecalciferol 100 000 IU (n = 163) or placebo (n = 153) every 2 weeks for 24 months.

MAIN OUTCOMES AND MEASURES The primary outcome measure was disease activity, defined as occurrence of a relapse and/or MRI activity (new and/or contrast-enhancing lesions) over 24 months of follow-up, also analyzed as separate secondary outcomes.

RESULTS Of the 316 participants enrolled and randomized (median [IQR] age, 34 [28-42] years; 70% women), the primary analysis included 303 patients (95.9%) who took at least 1 dose of the study drug and 288 (91.1%) ultimately completed the 24-month trial. Disease activity was observed in 94 patients (60.3%) in the vitamin D group and 109 patients (74.1%) in the placebo group (hazard ratio [HR], 0.66 [95% CI, 0.50-0.87]; $P = .004$), and median time to disease activity was longer in the vitamin D group (432 vs 224 days; log-rank $P = .003$). All 3 secondary MRI outcomes reported significant differences favoring the vitamin D group vs the placebo group: MRI activity (89 patients [57.1%] vs 96 patients [65.3%]; HR, 0.71 [95% CI, 0.53-0.95]; $P = .02$), new lesions (72 patients [46.2%] vs 87 patients [59.2%]; HR, 0.61 [95% CI, 0.44-0.84]; $P = .003$), and contrast-enhancing lesions (29 patients [18.6%] vs 50 patients [34.0%]; HR, 0.47 [95% CI, 0.30-0.75]; $P = .001$). All 10 secondary clinical outcomes showed no significant difference, including relapse, which occurred in 28 patients (17.9%) in the vitamin D group vs 32 (21.8%) in the placebo group (HR, 0.69 [95% CI, 0.42-1.16]; $P = .16$). Results were similar in a subset of 247 patients meeting updated 2017 diagnostic criteria for relapsing-remitting MS at treatment initiation. Severe adverse events occurred in 17 patients in the vitamin D group and 13 in the placebo group, none of which were related to cholecalciferol.

CONCLUSIONS AND RELEVANCE Oral cholecalciferol 100 000 IU every 2 weeks significantly reduced disease activity in CIS and early relapsing-remitting MS. These results warrant further investigation, including the potential role of pulse high-dose vitamin D as add-on therapy.

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Visual Abstract

Supplemental content

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Group Information: A complete list of the D-Lay MS Investigators appears in Supplement 3.

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Female sex, obesity, smoking, Epstein-Barr virus infection, and vitamin D deficiency are risk factors for multiple sclerosis (MS).[1] MS typically starts with an acute episode involving the central nervous system, such as optic neuritis (inflammation of the optic nerve), transverse myelitis (inflammation of the spinal cord), or brainstem syndromes, termed a *clinically isolated syndrome* (*CIS*), although CIS does not always convert to MS. The risk factors for relapse, defining the conversion to clinically definite MS, include the presence of cerebrospinal fluid (CSF) oligoclonal bands, high number of T2-weighted fluid-attenuated inversion recovery (T2/FLAIR) lesions on brain index MRI, and younger age, while a role of lower vitamin D levels is suspected.[2,3] In patients with MS, lower vitamin D levels are associated with relapse risk, new MRI T2/FLAIR lesions, and disability.[4-7] Vitamin D induces pleiotropic immune regulations, decreasing differentiation of effector T and B cells, promoting regulatory subsets, modulating innate immune cells, and reducing immune cell trafficking at the blood-brain barrier level and microglial and astrocytic activation, thus supporting vitamin D therapy to reduce MS activity and progression.[8]

Given their potential synergistic immunomodulatory effects, vitamin D supplementation trials have been performed as add-on therapy with interferon beta in patients with MS.[9,10] Although neither study found significant differences in their respective primary end points (no evidence of disease activity at 48 weeks[9] or change in the relapse rate at 96 weeks[10]), they suggested other potential benefits: the SOLAR trial found fewer new or active lesions,[9] while a post hoc analysis of patients who completed follow-up in the CHOLINE study showed reduced relapse rate, fewer new hypointense T1-weighted lesions, and less disability progression.[10] A pilot vitamin D monotherapy study including 30 patients with untreated acute optic neuritis with vitamin D levels less than 75 nmol/L revealed a significant benefit of 50 000 IU per week of vitamin D supplementation on relapse and MRI activity at 48 weeks.[11] In contrast, a placebo-controlled study including 204 patients with a CIS testing cholecalciferol at 3 different daily doses (1000 IU, 5000 IU, and 10 000 IU) failed to show radiological or clinical benefit at 48 weeks.[12] Meta-analyses revealed no effect of vitamin D on relapses or disability,[13] but a trend toward reduction of new T2/FLAIR lesions, highlighting the need for large randomized clinical trials.[14-16]

This study aimed to evaluate the adverse events and efficacy of vitamin D as monotherapy in patients with recent CIS suggestive of MS (MRI showing dissemination in space according to 2010 McDonald criteria or ≥2 lesions and presence of oligoclonal bands) to reduce disease activity.

Key Points

Question Does vitamin D reduce disease activity in patients with clinically isolated syndrome (CIS) typical for multiple sclerosis when given at a high dose as monotherapy?

Findings In this randomized clinical trial including 303 patients treated with oral high-dose cholecalciferol or placebo, the primary outcome measure of disease activity, defined as occurrence of a relapse and/or magnetic resonance imaging activity (new and/or contrast-enhancing lesions) over 24 months of follow-up, was observed in 94 patients (60.3%) in the vitamin D group and 109 patients (74.1%) in the placebo group (hazard ratio, 0.66). This was a statistically significant difference.

Meaning Oral high-dose cholecalciferol reduced disease activity in clinically isolated syndrome and in early relapsing-remitting multiple sclerosis.

Methods

Trial Design

The D-Lay MS study was a multicenter, double-blind, parallel, 1:1 randomized, phase 3 placebo-controlled clinical trial conducted in patients with CIS over 24 months, enrolling from July 16, 2013, to December 23, 2020, with the last patient visit on January 18, 2023, in 36 MS centers in France. This study complied with French law, the Declaration of Helsinki, and Good Clinical Practice. The trial protocol (Supplement 1) was approved by the ethics committee Sud Méditerranée III (#2013.02.08 ter) and prospectively registered on ClinicalTrials.gov (NCT01817166). This report conforms with Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines. All participants provided written informed consent.

Patients

The study population included patients aged 18 to 55 years with CIS with diagnostic brain MRI including T2/FLAIR and T1-weighted gadolinium imaging, with or without spinal cord MRI, showing dissemination in space according to the 2010 revised McDonald criteria or the presence of at least 2 MRI lesions consistent with MS and positive cerebrospinal fluid, defined by the presence of at least 2 unique oligoclonal bands, in the past 90 days.[17,18] Patients already receiving a disease-modifying therapy were excluded. Patients with serum vitamin D levels greater than 100 nmol/L and history of hypercalcemia, sarcoidosis, or tuberculosis were excluded to avoid potential overdose. Full eligibility criteria are available in Supplement 2.

Patients were also classified according to the CIS phenotype (optic neuritis vs other symptoms), high-dose intravenous methylprednisolone pulse therapy, overweight (body mass index [BMI] >25), and severe vitamin D insufficiency (serum vitamin D <30 nmol/L) at baseline. Because the McDonald criteria were updated during the study period, allowing the diagnosis of relapsing-remitting MS (RRMS) accurately and more rapidly in patients with CIS (when presence of active lesions or CSF oligoclonal bands), a subgroup of patients fulfilling the 2017 criteria for MS before treatment initiation was also created for supplementary analysis.[19] Patient characteristics were compared between the periods before vs after the update of these diagnosis criteria.

Trial Procedure

Participants were randomly assigned using computer-generated random numbers in a 1:1 ratio to receive either oral

100 000 IU cholecalciferol or placebo vial every 2 weeks for 24 months or until the occurrence of disease activity. Randomization was performed by an independent statistician in blocks of 4 and stratified according to center and presence of contrast-enhancing lesions on diagnostic MRI. The active and placebo treatments were produced by Nexpharma (see eMethods in Supplement 2). The site investigators, patients, and outcome assessors were blinded to treatment assignments.

Medical visits occurred at baseline; after 3, 12, and 24 months; and at relapse. At each visit, patients underwent brain and spinal cord MRI, neurologists assessed patients' disability using Expanded Disability Status Scale (EDSS) and Paced-Auditory Serial Addition Test 3 seconds (PASAT), and patients completed the EuroQoL 5-Dimension 5-Level (EQ-5D-5L) and 36-Item Short Form Survey (SF-36) health questionnaires, Fatigue Scale for Motor and Cognitive Functions (FSMC), and the Hospital Anxiety and Depression Scale (HADS) (see eMethods in Supplement 2 for scoring of scales, tests, and questionnaires). Blood and spot urine samples were collected at each visit to assess serum and urine calcium and creatinine levels. All data were collected on paper case report forms and reported on an electronic case report form on OpenClinica web software. Experimental treatment commenced after the baseline brain and spinal cord MRI, performed within 14 days after the baseline visit. Patients who agreed received a text message reminder every 2 weeks. When a patient reached the primary outcome, a dedicated visit was performed and treatment was discontinued. Protocol follow-up visits were cancelled except the study end visit.

MRI included brain 3D-FLAIR, unenhanced and gadolinium-enhanced T1-weighted sequences, and spinal cord T2- and gadolinium-enhanced T1-weighted sequences. Interpretation of MRI scans and assessment of MRI activity was performed locally by the neuroradiologist and reviewed by the treating neurologist who detailed the localization of the lesions in the electronic case report form. Data monitoring was performed in each center in person and in compliance with Good Clinical Practice guidelines.

An independent oversight committee was established to ensure safety. Adverse events were continuously reported and graded according to Common Terminology Criteria for Adverse Events version 3.

Outcomes

The primary outcome was the presence of disease activity, defined by the first occurrence of relapse or MRI activity (ie, new or unequivocally enlarging brain FLAIR or spinal cord T2 lesions or contrast-enhancing T1 lesions on follow-up MRI).[20] This outcome corresponds to the risk of conversion to RRMS according to the 2005 McDonald criteria in use at the time of protocol conception,[17] and is best adapted to the current criteria and definition of MS activity.[19] Time to disease activity was defined as the time between treatment start and the first evidence of disease activity. Secondary outcomes included disease activity description, with the occurrence of relapse, MRI activity, and presence and number of new/enlarging or contrast-enhancing lesions at the time of disease activity. Other secondary outcomes included EDSS, PASAT, EQ-5D-5L, SF-36, FSMC, and HADS scores until disease activity or at the end of follow-up, as well as kidney function and serum and urinary calcium levels at each visit (see eMethods in Supplement 2). Additional secondary outcomes could not be analyzed in the study due to insufficient transfer of MRI scans for centralized reading and the need for additional funds to measure serum concentrations of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 at each visit. Adverse events were counted according to severity and Common Terminology Criteria for Adverse Events grade.

Sample Size Calculation

To have 90% power to detect a 16% difference in the rate of disease activity between the 2 groups (85% with placebo vs 69% with vitamin D)[21] with an α risk of 5%, an expected rate of unusable data of 10%, and a 10% dropout rate, 316 patients were necessary.

Statistical Analysis

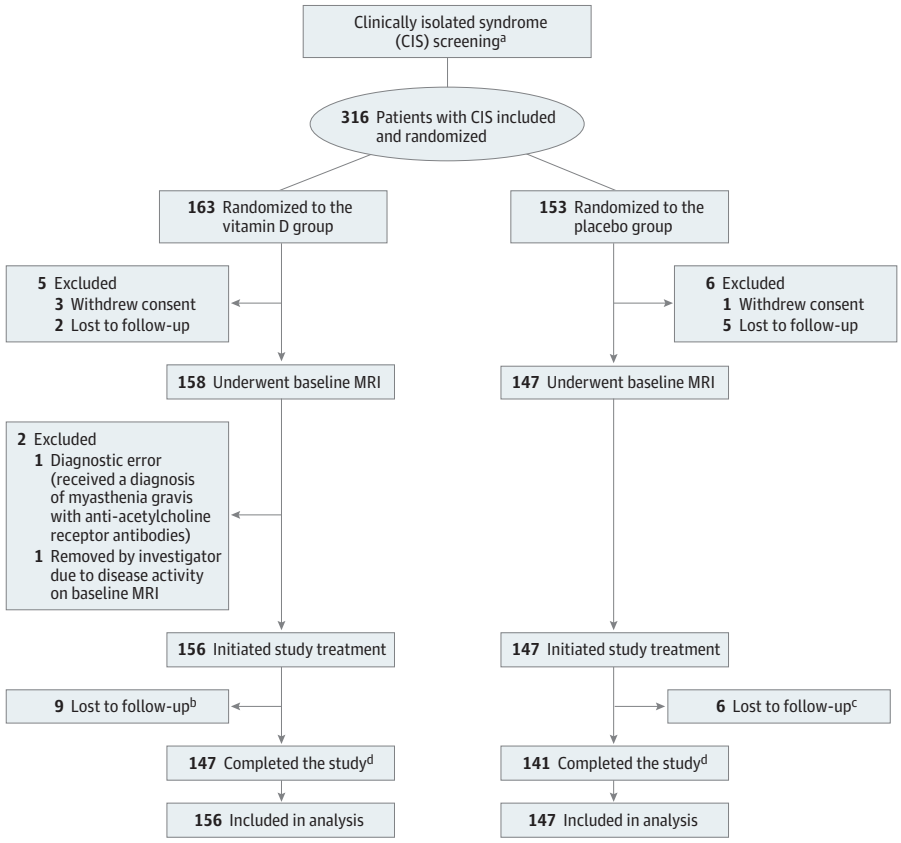
Participants who took at least 1 dose of vitamin D or placebo were included in the full analysis set and analyzed according to their randomization group (**Figure 1**), including any patient with conversion at baseline MRI who inappropriately started the medication. Patient characteristics were described with percentages for categorical variables and with median and IQR values for quantitative variables. The time between treatment start and disease activity is represented using the Kaplan-Meier method, and the time between the groups was compared using the log-rank test. The effect of vitamin D on disease activity occurrence was analyzed using a random-effects Cox model, considering the treatment group as the factor of interest adjusting on randomization stratification factors (ie, center and contrast-enhancing lesions on diagnostic MRI), with center considered as random effect. A fully adjusted model was performed adjusting for supplemental predefined prognostic factors (age, sex, number of contrast-enhancing lesions on diagnostic MRI, baseline EDSS score, and the time between CIS and first treatment administration), as well as 2 additional prognostic factors (serum vitamin D levels and high-dose intravenous methylprednisolone pulse therapy after CIS), which were added prior to data review and unblinding. Results are presented as hazard ratios (HRs) with 95% CIs.

The patient number needed to treat was calculated as the inverse of the absolute risk reduction with the 95% CI.

Quantitative secondary end points were analyzed using linear mixed models with treatment group, time, and treatment group × time interaction adjusted as above as fixed effects. The models included a participant-specific random intercept to account for data repetition. For secondary outcomes, when the primary outcome was met during follow-up, the subsequent measures were censored. The analysis of secondary end points and subgroup analyses were not adjusted for multiple testing and should be interpreted as exploratory.

A modifying effect of different variables on the effect of treatment on the primary end point (ie, disease activity) was analyzed by applying the same partially adjusted model but adding an interaction term between the treatment group and

Figure 1. Flow of Participants in the D-Lay MS Clinical Trial



MRI indicates magnetic resonance imaging.

[a]Enrollment information was not collected.

[b]One patient before month 3, 3 before month 6, 1 before month 12, and 4 after month 12.

[c]One patient before month 6, 2 before month 12, and 3 after month 12.

[d]No disease activity with follow-up until month 24 or disease activity.

the variables with a potential modifying effect. For each variable, groups were defined post hoc according to the literature in early MS: age (<36 y, ≥36 y),[22] sex (male, female),[2] number of T2 lesions (<9, ≥9),[2] number of contrast-enhancing lesions (0, 1, ≥2),[22] baseline EDSS score (0, >0),[23] presence of T2 spinal cord lesions (0, >0, missing),[22] serum vitamin D levels (<30 nmol/L, ≥30 nmol/L),[7] BMI (<25, ≥25),[24] optic neuritis (yes, no),[2] high-dose intravenous methylprednisolone pulse therapy (yes, no),[25] and presence of CSF oligoclonal bands (yes, no).[2] These results are represented using a subgroup analysis plot.

A post hoc sensitivity analysis was also performed excluding participants who developed disease activity at the time of baseline MRI prior to starting the study drug. Because the McDonald criteria were updated during the study period, allowing the diagnosis of RRMS accurately and more rapidly after CIS, we also performed an ancillary analysis of the outcomes in the subgroup of patients fulfilling the 2017 criteria for MS before treatment initiation.[19]

Patients lost to follow-up after treatment initiation were censored at the last contact date. In the different models, multiple imputation was performed to handle missing data for adjustment variables, except for the interval between CIS onset and treatment initiation, in which missing intervals were replaced by the observed time between CIS and MRI plus the median time between MRI and treatment initiation observed in other patients. For each model, sensitivity analyses were performed without imputation to verify the robustness of the results.

The frequency of serious adverse events in both groups was compared with a χ^2 test. Statistical analyses were conducted using R version 4.3.3 (R Foundation for Statistical Computing) using the 2-tailed α level of .05.

Results

Patient Characteristics

A total of 316 patients with CIS were enrolled and randomized (Figure 1). Four patients withdrew consent and 9 did not start experimental treatment for different reasons; these individuals were excluded from analyses (7 in the vitamin D group and 6 in the placebo group). The analysis was conducted on 303 patients (156 in the vitamin D group and 147 in the placebo group; Figure 1), including 19 patients with conversion at baseline MRI who inappropriately started the medication (9 in the vitamin D group and 10 in the placebo group).

Groups showed similar baseline demographic, clinical, and MRI characteristics (**Table 1**). The median (IQR) age was 34 (28-42) years, 70% of participants were women, 33% had optic neuritis, and the median (IQR) baseline EDSS score was 1.0 (0-2.0). MS 2010 criteria for dissemination in space were fulfilled in 274 patients (92%) at CIS diagnosis, and 257 (85%) received high-dose intravenous methylprednisolone pulse therapy. Oligoclonal bands in the CSF were present in 203 of 240 patients (85%) tested. Among the 279 patients with data allowing

assessment of the 2017 McDonald criteria, 247 patients (89%) fulfilled the requirements for RRMS diagnosis. The median (IQR) vitamin D level at diagnosis was 45 (31-66) nmol/L, with severe vitamin D deficiency (<30 nmol/L) present in 68 patients (22.4%). The median time from CIS onset to randomization was 54 days and from CIS onset to initiation of the investigational treatment was 60 days.

Outcomes

During the 24-month follow-up, disease activity was observed less frequently in the vitamin D group (94 patients [60.3%]) compared with the placebo group (109 patients [74.1%]), and median time to disease activity was 432 (95% CI, 360-727) days in the vitamin D group vs 224 (95% CI, 104-360) days in the placebo group (log-rank test $P = .003$; **Figure 2**). The number needed to treat to prevent 1 case of disease activity over the 24-month study period was 7.2 (95% CI, 4.1-29.0).

The HR for disease activity in the vitamin D group, compared with the placebo group, after partial adjustment was 0.66 (95% CI, 0.50-0.87; $P = .004$; **Table 2**). Results were similar in a post hoc analysis without imputation (eTable 1 in Supplement 2) and after excluding the 19 patients who experienced disease activity prior to starting the study treatment: 85 of 147 (57.8%) in the vitamin D group experienced disease activity vs 99 of 137 (72.3%) in the placebo group (HR, 0.64 [95% CI, 0.48-0.86]; $P = .003$). During the 24-month follow-up, in the vitamin D group vs the placebo group, MRI activity was observed less frequently (89 patients [57.1%] vs 96 patients [65.3%]; HR, 0.71 [95% CI, 0.53-0.95]; $P = .02$), new enhancing lesions were observed less frequently (72 patients [46.2%] vs 87 patients [59.2%]; HR, 0.61 [95% CI, 0.44-0.84]; $P = .003$), and contrast-enhancing lesions were observed less frequently (29 patients [18.6%] vs 50 patients [34.0%]; HR, 0.47 [95% CI, 0.30-0.75]; $P = .001$) (Table 2).

No significant difference was observed for relapse (28 patients [17.9%] in the vitamin D group vs 32 [21.8%] in the placebo group; HR, 0.69 [95% CI, 0.42-1.16]; $P = .16$). Vitamin D had no significant impact on disability (EDSS, PASAT), fatigue (FSMC), quality of life (EQ-5D-5L, SF-36), or depression and anxiety symptoms (HADS) during follow-up (Table 2).

All results remained unchanged after full adjustment (Table 2).

Table 1. Baseline Patient Characteristics

Characteristic	No. (%)	
	Vitamin D (n = 156)	Placebo (n = 147)
Age, median (IQR), y	35 (28-42)	34 (27-40)
Female	103 (66)	108 (73)
Male	53 (34)	39 (27)
Body mass index, median (IQR)	24.1 (21.2-27.5) [n = 153]	23.5 (21.6-27.7) [n = 144]
History of infectious mononucleosis	16 (10)	12 (8.2)
Current smoking	57 (37)	55 (37)
Vitamin D level, median (IQR), nmol/L	49.5 (34.0-67.0)	42.5 (29.0-63.0)
Severe vitamin D deficiency (<30 nmol/L)	29/155 (19)	39/145 (27)
Optic neuritis	55 (35)	43 (31)
High-dose intravenous methylprednisolone pulse therapy	129 (83)	128 (87)
EDSS score, median (IQR)[a]	1.0 (0-2.0)	1.0 (0-2.0)
<9 FLAIR brain lesions	75/151 (50)	61/143 (43)
Contrast-enhancing lesions		
0	74 (47)	77 (52)
1	67 (43)	55 (37)
≥2	15 (10)	15 (10)
T2 spinal cord lesions	n = 151	n = 139
0	68 (46)	66 (48)
1	44 (29)	34 (24)
≥2	39 (25)	39 (28)
Met 2010 MRI DIS criteria[b]	141/153 (92)	133/144 (92)
Presence of CSF oligoclonal bands	97/121 (62)	106/119 (72)
Met 2017 MS diagnostic criteria[c]	126/144 (88)	121/135 (90)
Time between CIS onset and randomization, median (IQR), d	54 (39-74)	54 (37-76)
Time between CIS onset and study drug initiation, median (IQR), d	61 (48-80) [n = 149]	60 (46-83) [n = 142]

Abbreviations: CIS, clinically isolated syndrome; CSF, cerebrospinal fluid; DIS, dissemination in space; EDSS, Expanded Disability Status Scale; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging.

[a] See Supplement 2 for additional information about scoring.

[b] Indicates patients with DIS according to 2010 McDonald criteria.

[c] Indicates patients with multiple sclerosis (MS) at baseline according to 2017 McDonald criteria (see Methods in Supplement 2).

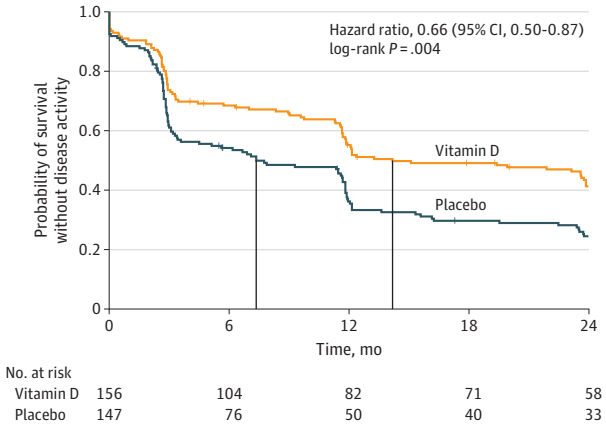
Ancillary Analysis

Subgroup analysis of the primary outcome in the 247 patients fulfilling the 2017 McDonald criteria for RRMS at baseline showed an HR for disease activity in the vitamin D group, compared with placebo, of 0.66 (95% CI, 0.49-0.89; $P = .007$) after partial adjustment (eTable 2 in Supplement 2). MRI activity (HR, 0.71 [95% CI, 0.51-0.97]; $P = .03$), occurrence of new/enlarging lesions (HR, 0.59 [95% CI, 0.42-0.83]; $P = .003$), and occurrence of contrast-enhancing lesions (HR, 0.49 [95% CI, 0.30-0.81]; $P = .006$) on follow-up MRI scans were also significantly reduced in the vitamin D group compared with the placebo group (eTable 2 in Supplement 2). No significant difference was observed for relapse (HR, 0.70 [95% CI, 0.40-1.21]; $P = .20$). The revision of MS diagnostic criteria had a minor impact on inclusion rate over time (eFigure 1 in Supplement 2). Patient characteristics were similar before and after the update, with the only significant differences being lower EDSS score (1.25 before 2017 update vs 1.0 after 2017 update; $P = .03$) and more contrast-enhancing lesions ($P = .04$) (eTable 3 in Supplement 2).

Factors Influencing Outcomes

Interaction between the treatment group and prognostic factors revealed that patients who benefited most from vitamin D were those without spinal cord lesions at diagnosis (HR, 0.23 vs 0.85; P value for interaction = .01), with severe vitamin D deficiency (HR, 0.33 vs 0.78; P value for interaction = .03), and with normal BMI at baseline (HR, 0.53 vs 0.95; P value for interaction = .048) (**Figure 3**). Age, sex, CIS phenotype, high-dose intravenous methylprednisolone pulse therapy, number

Figure 2. Disease Activity in the Treatment Groups During the 2 Years of Follow-Up



Vertical solid lines represent median time to disease activity for each group (14.2 [95% CI, 11.8-24.1] months for the vitamin D group and 7.3 [95% CI, 3.4-11.8] months for the placebo group). Median (IQR) observation time was 23.7 (23.4-24.2) months for the vitamin D group and 23.8 (23.6-24.0) months for the placebo group.

Table 2. Efficacy Measures

Outcome[a]	No. of events		Difference in incidence rates, patient-years	Partial adjustment[b]		Full adjustment[c]	
	Vitamin D (n = 156)	Placebo (n = 147)		HR (95% CI)	*P* value	HR (95% CI)	*P* value
Primary outcome							
Disease activity	94	109	−0.33	0.66 (0.50 to 0.87)	.004	0.65 (0.49 to 0.87)	.004
Secondary outcomes[d]							
Relapse	28	32	−0.10	0.69 (0.42 to 1.16)	.16	0.68 (0.40 to 1.16)	.15
MRI activity	89	96	−0.26	0.71 (0.53 to 0.95)	.02	0.72 (0.53 to 0.97)	.03
Contrast-enhancing lesions	29	50	−0.23	0.47 (0.30 to 0.75)	.002	0.50 (0.31 to 0.80)	.005
New or enlarging lesions	72	87	−0.28	0.61 (0.44 to 0.84)	.003	0.62 (0.44 to 0.86)	.004
	Annual change (SE)	Annual change (SE)		Δ of annual change (95% CI)		Δ of annual change (95% CI)	
EDSS	−0.17 (0.05)	−0.14 (0.06)		−0.03 (−0.18 to 0.12)	.67	−0.02 (−0.17 to 0.13)	.75
PASAT	3.29 (0.49)	3.15 (0.62)		0.14 (−1.41 to 1.69)	.86	0.09 (−1.47 to 1.64)	.91
EQ-5D-5L	0.03 (0.01)	0.02 (0.01)		0.01 (−0.02 to 0.05)	.40	0.01 (−0.02 to 0.05)	.50
SF-36 physical	1.41 (0.41)	1.10 (0.52)		0.31 (−0.99 to 1.61)	.64	0.27 (−1.02 to 1.55)	.68
SF-36 mental	2.38 (0.59)	2.97 (0.75)		−0.59 (−2.46 to 1.29)	.54	−0.67 (−2.56 to 1.22)	.49
FSMC	−0.77 (0.82)	0.97 (1.03)		−1.74 (−4.32 to 0.84)	.19	−1.45 (−4.00 to 1.11)	.27
HADS total	−0.54 (0.30)	−0.62 (0.39)		0.08 (−0.87 to 1.04)	.86	0.16 (−0.80 to 1.12)	.74
HADS anxiety	−0.52 (0.18)	−0.40 (0.24)		−0.12 (−0.70 to 0.46)	.68	−0.09 (−0.67 to 0.50)	.78
HADS depression	0.00 (0.17)	−0.25 (0.21)		0.25 (−0.28 to 0.79)	.35	0.29 (−0.24 to 0.83)	.29

Abbreviations: CIS, clinically isolated syndrome; EDSS, Expanded Disability Status Scale; EQ-5D-5L, EuroQoL 5-Dimension 5-Level; FSMC, Fatigue Scale for Motor and Cognitive Functions; HADS, Hospital Anxiety and Depression Scale; HR, hazard ratio; PASAT, Paced-Auditory Serial Addition Test 3 seconds; SF-36, 36-Item Short Form Survey.

[a] See Supplement 2 for information on scoring of scales, tests, and questionnaires.

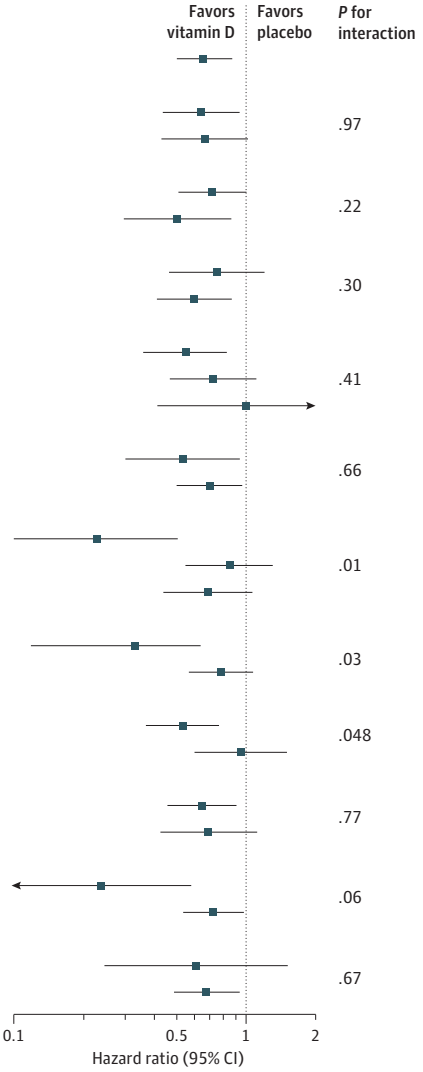
[b] The partial adjustment was performed on the center and the presence of contrast-enhancing lesions on the baseline magnetic resonance imaging (MRI) scan.

[c] The full adjustment was performed on additional baseline characteristics (age, sex, number of T2-weighted fluid-attenuated inversion recovery (T2/FLAIR) lesions, EDSS score, plasma vitamin D levels, high-dose intravenous methylprednisolone pulse therapy, and time from CIS onset). There were no missing data for the partially adjusted model. Multiple imputation was performed to manage missing data for adjustment variables in the fully adjusted model: number of T2/FLAIR lesions (3.0%): 5 in the vitamin D group and 4 in the placebo group; EDSS score (0.3%): 1 in the placebo group; serum vitamin D level (1.0%): 1 in the vitamin D group and 2 in the placebo group; time between CIS and treatment onset (4.0%): 7 in the vitamin D group and 5 in the placebo group.

[d] Missing data for quantitative secondary end points during the follow-up (percentage of missing value among all values during the follow-up visits at 0, 3, 12 or 24 months: EDSS: 2% in the vitamin D group and 3% in the placebo group; PASAT: 11% in the vitamin D group and 7% in the placebo group; EQ-5D-5L: 3% in the vitamin D group and 2% in the placebo group; SF-36 physical: 4% in the vitamin D group and 3% in the placebo group; SF-36 mental: 4% in the vitamin D group and 3% in the placebo group; FSMC: 8% in the vitamin D group and 7% in the placebo group; HADS total: 7% in the vitamin D group and 7% in the placebo group; HADS anxiety: 5% in the vitamin D group and 5% in the placebo group; HADS depression: 6% in the vitamin D group and 4% in the placebo group.

Figure 3. Interaction Between Covariates and Vitamin D

Variable	No. of participants with event/total No.		Hazard ratio (95% CI)	P for interaction
	Vitamin D	Placebo		
Overall (no stratification)	94/156	109/147	0.66 (0.50-0.87)	
Age, y				
<36	51/80	66/84	0.64 (0.44-0.93)	.97
≥36	43/76	43/63	0.66 (0.43-1.02)	
Sex				
Female	64/103	78/108	0.71 (0.51-1.00)	.22
Male	30/53	31/39	0.50 (0.29-0.86)	
No. of brain FLAIR lesions				
<9	40/75	36/61	0.75 (0.46-1.20)	.30
≥9	52/76	71/82	0.59 (0.41-0.86)	
No. of contrast-enhancing lesions				
0	38/74	55/77	0.55 (0.36-0.84)	
1	43/67	40/55	0.72 (0.46-1.11)	.41
≥2	13/15	14/15	0.99 (0.41-2.39)	
EDSS score				
0	23/46	34/45	0.53 (0.30-0.93)	.66
>0	71/110	75/101	0.69 (0.50-0.97)	
Presence of T2 spinal cord lesions				
No	10/31	22/30	0.23 (0.10-0.51)	
Yes	43/60	40/53	0.85 (0.54-1.32)	.01
Missing	41/65	47/64	0.68 (0.44-1.07)	
Serum vitamin D level, nmol/L				
<30	15/29	33/39	0.33 (0.17-0.63)	.03
≥30	78/126	74/106	0.78 (0.56-1.07)	
Body mass index				
<25	51/88	70/90	0.53 (0.37-0.76)	.048
≥25	43/65	37/54	0.95 (0.60-1.50)	
Optic neuritis				
No	60/101	78/102	0.64 (0.45-0.91)	.77
Yes	34/55	31/45	0.69 (0.42-1.12)	
IVMP				
No	10/27	15/19	0.23 (0.09-0.58)	.06
Yes	84/129	94/128	0.72 (0.53-0.97)	
Presence of CSF oligoclonal bands				
No	11/24	8/13	0.61 (0.24-1.51)	.67
Yes	62/97	79/106	0.67 (0.48-0.94)	



Squares represent hazard ratios (HRs) after adjustment on randomization strata (contrast-enhancing lesions) and centers. Bars represent 95% CIs. Missing data per group: fluid-attenuated inversion recovery (FLAIR): 4 in the vitamin D group and 5 in the placebo group; Expanded Disability Status Scale (EDSS) score: 1 in the placebo group; serum vitamin D level: 1 in the vitamin D group and 2 in the placebo group; body mass index: 3 in the vitamin D group and 3 in the placebo group; cerebrospinal fluid (CSF) oligoclonal bands: 35 in the vitamin D group and 28 in the placebo group. See Supplement 2 for details of the EDSS, which evaluates neurological disability associated with multiple sclerosis. IVMP indicates high-dose intravenous methylprednisolone pulse therapy.

of brain T2/FLAIR lesions, presence of contrast-enhancing lesions, and EDSS score at baseline did not significantly influence the effects of vitamin D on the primary outcome (Figure 3).

Adverse Events

Thirty-three serious adverse events were reported in 30 patients during the study (17 patients [10.9%] in the vitamin D group and 13 patients [8.8%] in the placebo group; χ^2 test $P = .55$; eTable 4 in Supplement 2), none of which were suggestive of hypercalcemia or related to the study drug. Mild hypercalcemia (calcium level of 2.6-2.88 mmol/L) was observed in only 2 patients at 3 and 12 months in the placebo group (eTable 5 in Supplement 2), with similar urinary calcium/creatinine ratios on spot sample results during follow-up (eTable 6 in Supplement 2). Neither kidney failure (eTable 7 in Supplement 2) nor moderate or severe hypercalcemia (calcium level >2.88 mmol/L) were reported during follow-up (eTable 5 in Supplement 2).

Discussion

This randomized clinical trial showed that high-dose cholecalciferol monotherapy initiated within 90 days after diagnosis of CIS strongly suggestive of MS reduced disease activity significantly compared with placebo.

These results aligned with a pilot study testing the efficacy of vitamin D monotherapy for 48 weeks in 30 patients with untreated optic neuritis.[11] In the current study, the efficacy of vitamin D was similar between patients with CIS with or without optic neuritis, extending its potential target population to all CIS phenotypes.

The efficacy of vitamin D observed in this study contrasts with the recent PrevANZ study testing vitamin D in untreated patients with CIS.[12] This might be related to the lack of power of the PrevANZ study due to the 3 vitamin D doses tested, lower patient numbers, shorter treatment duration, and higher dropout rates. Moreover, the PrevANZ study included older patients with CIS with fewer T2 lesions and fewer people who smoke, resulting in fewer patients showing disease activity. Furthermore, the patients in the current study had lower baseline vitamin D levels and lower BMI, both of which influenced vitamin D efficacy in the study. One hypothesis is that the daily oral vitamin D supplementation used in the PrevANZ study might have had different immune effects compared with the high-dose pulse therapy used in this study, as explored in transcriptomic analyses of samples from patients included in both studies, although comparative analyses are lacking.[26,27] When compared with placebo-controlled phase 3 trials in CIS, the patients in the current study had comparable baseline characteristics in terms of optic neuritis frequency, EDSS score, contrast-enhancing lesions, and high-dose intravenous methylprednisolone pulse therapy, and the HR for disease activity with cholecalciferol observed in our study (0.66 [95% CI, 0.50-0.87]) was similar to that of teriflunomide (0.65 [95% CI, 0.51-0.82]) in the TOPIC trial),[28] and slightly more than interferon beta-1a and interferon beta-1b treatment (0.49 [95% CI, 0.38-0.64] in the REFLEX trial,[29] and 0.54 [95% CI, 0.43-0.67] in the BENEFIT trial, respectively).[21] Interestingly, the early separation of Kaplan-Meier curves suggest an early effect of Vitamin D on disease activity that is sensitively detected by MRI, while the parallel shape after 3 to 6 months could indicate the maintenance of the treatment effect over 24 months, as observed in previous CIS therapeutic trials (eg, the BENEFIT and TOPIC studies).[21,28] Altogether, this suggests that cholecalciferol could represent an inexpensive therapeutic alternative, with low risk of adverse events, after a CIS, especially in populations with limited access to disease-modifying therapies.

Because a subgroup analysis also showed that cholecalciferol significantly reduced disease activity in patients with RRMS according to the most recent McDonald diagnostic criteria,[19] vitamin D supplementation might also benefit all patients with early-stage RRMS, especially after careful exclusion of patients at risk for hypercalcemia, including those with sarcoidosis and tuberculosis. This is supported by the low frequency and severity of adverse events in the high-dose vitamin D group in the study.

Patients who benefited most from vitamin D were those with severe vitamin D deficiency (<30 nmol/L). This is in line with the 2024 report showing that low vitamin D levels before initiation of disease-modifying therapy increase the risk of disease activity in MS, with a causality relationship,[30] and strongly encourages further studies to examine the benefit of vitamin D add-on therapy in patients with vitamin D deficiency when starting a disease-modifying therapy.

Combination therapy, whereby 2 individually suboptimal agents are given simultaneously, could be imagined to have a beneficial therapeutic effect as seen in infections, cancers, and rheumatoid inflammatory diseases, but results in MS are so far disappointing.[31] The low risk of adverse events and promising efficacy profile of high-dose vitamin D observed in the D-Lay MS trial makes vitamin D an ideal candidate for future studies as an add-on therapy to improve the risk-benefit ratio of existing therapies.

In line with results of the current study, add-on therapy studies with vitamin D, CHOLINE and SOLAR, suggested benefits on MRI activity in combination with interferon beta-1a, although they were negative for the primary end point (reduction of MS relapse).[9,10] Thus, a larger-scale study with 24 months of follow-up is needed to assess efficacy of high-dose vitamin D supplementation as add-on therapy to reduce evidence of disease activity in treated patients with MS.

The study design prioritized patient safety, with brain and spinal cord MRI at 3, 12, and 24 months, allowing the early switch to disease-modifying therapy on detection of disease activity through MRI activity. Hence, the relapse rate was very low, which may have limited the ability to detect a significant difference in relapse risk reduction after vitamin D therapy. However, MRI activity is considered a more sensitive and objective measure of disease activity. In accordance with the low relapse rate, vitamin D had no impact on EDSS score progression and other clinical outcomes in this study.

Interestingly, patients without spinal cord lesions also benefited the most from vitamin D, while other good prognostic factors (optic neuritis, low EDSS score, absence of contrast-enhancing lesions, and no high-dose intravenous methylprednisolone pulse therapy at baseline MRI) did not influence vitamin D efficacy, suggesting that the impact of vitamin D is not limited to MS with good prognosis.

Limitations

This study has several limitations. First, the detection of events by imaging was only possible at the fixed times of the scheduled MRI scans, which did not necessarily correspond to the precise moment when the lesion developed. This phenomenon, which affected both groups, is unlikely to compromise the validity of the results, but could potentially lead to a lower precision of the CIs obtained. It also means that the median times reported in the groups represent fixed visit times rather than actual times at which the MRI findings developed, and should therefore be interpreted with caution. Second, because EDSS score was not measured frequently enough to assess 3- or 6-month confirmed disability accumulation, it was not possible to estimate the percentage of patients reaching no evidence of disease activity-3 at 24 months, a composite measure used to assess the effectiveness of treatments in clinical trials combining no clinical relapses, no radiological activity, and no disability progression (measured by EDSS score). Accordingly, relapse-associated worsening and progression independent of relapse activity could not be estimated

here.[32] These measures help elucidate the nature of disease progression, capturing the stepwise progression induced by incomplete recovery from relapses (relapse-associated worsening) or gradual disease progression (progression independent of relapse activity). Third, relapses and MRI activity were not diagnosed by central adjudication. However, patients were followed up in MS centers with solid clinical trial experience and expert neuroradiologist interpretation was reviewed by the treating neurologist who detailed the localization of the lesions in a standardized case report form. Fourth, the multiple comparisons of the secondary end points and subgroup analyses raise the potential for type I errors, meaning that these findings should be interpreted cautiously. Fifth, despite the long enrollment period and the revision of MS diagnostic criteria in 2017, there was only a slightly decreased enrollment rate over time, and few differences in patient characteristics after 2017 (lower EDSS score, but more contrast-enhancing lesions; see eFigure 1 and eTable 3 in Supplement 2). Sixth, whether the patient population was a diverse and/or representative sample could not be addressed because French law precludes collecting race and ethnicity data in this setting.

Conclusions

This randomized clinical trial showed the efficacy and low risk of adverse events of oral cholecalciferol 100 000 IU monotherapy every 2 weeks to reduce disease activity in patients with CIS and early RRMS. These results make high-dose vitamin D an interesting candidate for further studies evaluating add-on therapy in the therapeutic strategy for managing MS.

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REFERENCES

1. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA*. 2006;296(23):2832-2838. doi:10.1001/jama.296.23.2832

2. Tintore M, Rovira À, Río J, et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. *Brain*. 2015;138(Pt 7):1863-1874. doi:10.1093/brain/awv105

3. Kuhle J, Disanto G, Dobson R, et al. Conversion from clinically isolated syndrome to multiple sclerosis: a large multicentre study. *Mult Scler*. 2015;21(8):1013-1024. doi:10.1177/1352458514568827

4. Simpson S Jr, Taylor B, Blizzard L, et al. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. *Ann Neurol*. 2010;68(2):193-203. doi:10.1002/ana.22043

5. Mowry EM, Waubant E, McCulloch CE, et al. Vitamin D status predicts new brain magnetic resonance imaging activity in multiple sclerosis. *Ann Neurol*. 2012;72(2):234-240. doi:10.1002/ana.23591

6. Thouvenot E, Orsini M, Daures JP, Camu W. Vitamin D is associated with degree of disability in patients with fully ambulatory relapsing-remitting multiple sclerosis. *Eur J Neurol*. 2015;22(3):564-569. doi:10.1111/ene.12617

7. Ascherio A, Munger KL, White R, et al. Vitamin D as an early predictor of multiple sclerosis activity and progression. *JAMA Neurol*. 2014;71(3):306-314. doi:10.1001/jamaneurol.2013.5993

8. Galoppin M, Kari S, Soldati S, et al. Full spectrum of vitamin D immunomodulation in multiple sclerosis: mechanisms and therapeutic implications. *Brain Commun*. 2022;4(4):fcac171. doi:10.1093/braincomms/fcac171

9. Hupperts R, Smolders J, Vieth R, et al; SOLAR Study Group. Randomized trial of daily high-dose vitamin D_3 in patients with RRMS receiving subcutaneous interferon β-1a. *Neurology*. 2019;93(20):e1906-e1916. doi:10.1212/WNL.0000000000008445

10. Camu W, Lehert P, Pierrot-Deseilligny C, et al. Cholecalciferol in relapsing-remitting MS: a randomized clinical trial (CHOLINE). *Neurol Neuroimmunol Neuroinflamm*. 2019;6(5):e597. doi:10.1212/NXI.0000000000000597

11. Derakhshandi H, Etemadifar M, Feizi A, et al. Preventive effect of vitamin D3 supplementation on conversion of optic neuritis to clinically definite multiple sclerosis: a double blind, randomized, placebo-controlled pilot clinical trial. *Acta Neurol Belg*. 2013;113(3):257-263. doi:10.1007/s13760-012-0166-2

12. Butzkueven H, Ponsonby AL, Stein MS, et al. Vitamin D did not reduce multiple sclerosis disease activity after a clinically isolated syndrome. *Brain*. 2024;147(4):1206-1215. doi:10.1093/brain/awad409

13. Yuan X, Guo L, Jiang C, Yang X, Huang J. The effect of different administration time and dosage of vitamin D supplementation in patients with multiple sclerosis: a meta-analysis of randomized controlled trials. *Neuroimmunomodulation*. 2021;28(3):118-128. doi:10.1159/000515131

14. McLaughlin L, Clarke L, Khalilidehkordi E, Butzkueven H, Taylor B, Broadley SA. Vitamin D for the treatment of multiple sclerosis: a meta-analysis. *J Neurol*. 2018;265(12):2893-2905. doi:10.1007/s00415-018-9074-6

15. Zheng C, He L, Liu L, Zhu J, Jin T. The efficacy of vitamin D in multiple sclerosis: a meta-analysis. *Mult Scler Relat Disord*. 2018;23:56-61. doi:10.1016/j.msard.2018.05.008

16. Mahler JV, Solti M, Apóstolos-Pereira SL, Adoni T, Silva GD, Callegaro D. Vitamin D_3 as an add-on treatment for multiple sclerosis: a systematic review and meta-analysis of randomized controlled trials. *Mult Scler Relat Disord*. 2024;82:105433. doi:10.1016/j.msard.2024.105433

17. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald criteria". *Ann Neurol*. 2005;58(6):840-846. doi:10.1002/ana.20703

18. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol*. 2011;69(2):292-302. doi:10.1002/ana.22366

19. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018;17(2):162-173. doi:10.1016/S1474-4422(17)30470-2

20. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology*. 2014;83(3):278-286. doi:10.1212/WNL.0000000000000560

21. Kappos L, Polman CH, Freedman MS, et al. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. *Neurology*. 2006;67(7):1242-1249. doi:10.1212/01.wnl.0000237641.33768.8d

22. Lebrun-Frénay C, Rollot F, Mondot L, et al; RISC, SFSEP, and OFSEP Investigators. Risk factors and time to clinical symptoms of multiple sclerosis among patients with radiologically isolated syndrome. *JAMA Netw Open*. 2021;4(10):e2128271. doi:10.1001/jamanetworkopen.2021.28271

23. Zhang Y, Cofield S, Cutter G, Krieger S, Wolinsky JS, Lublin F. Predictors of disease activity and worsening in relapsing-remitting multiple sclerosis. *Neurol Clin Pract*. 2022;12(4):e58-e65. doi:10.1212/CPJ.0000000000001177

24. Munger KL, Bentzen J, Laursen B, et al. Childhood body mass index and multiple sclerosis risk: a long-term cohort study. *Mult Scler*. 2013;19(10):1323-1329. doi:10.1177/1352458513483889

25. Milligan NM, Newcombe R, Compston DA. A double-blind controlled trial of high dose methylprednisolone in patients with multiple sclerosis: 1. clinical effects. *J Neurol Neurosurg Psychiatry*. 1987;50(5):511-516. doi:10.1136/jnnp.50.5.511

26. Yeh WZ, Gresle M, Lea R, et al. The immune cell transcriptome is modulated by vitamin D_3 supplementation in people with a first demyelinating event participating in a randomized placebo-controlled trial. *Clin Immunol*. 2024;262:110183. doi:10.1016/j.clim.2024.110183

27. Galoppin M, Rival M, Louis A, et al. CITE-seq reveals inhibition of NF-κB pathway in B cells from vitamin D-treated multiple sclerosis patients. *bioRxiv*. Preprint posted online September 28, 2023. doi:10.1101/2023.09.25.559400

28. Miller AE, Wolinsky JS, Kappos L, et al; TOPIC Study Group. Oral teriflunomide for patients with a first clinical episode suggestive of multiple sclerosis (TOPIC): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Neurol*. 2014;13(10):977-986. doi:10.1016/S1474-4422(14)70191-7

29. Comi G, De Stefano N, Freedman MS, et al. Comparison of two dosing frequencies of subcutaneous interferon beta-1a in patients with a first clinical demyelinating event suggestive of multiple sclerosis (REFLEX): a phase 3 randomised controlled trial. *Lancet Neurol*. 2012;11(1):33-41. doi:10.1016/S1474-4422(11)70262-9

30. Giordano A, Clarelli F, Pignolet B, et al. Vitamin D affects the risk of disease activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2025;96(2):170-176. doi:10.1136/jnnp-2024-334062

31. Sorensen PS, Magyari M, Sellebjerg F. An update on combination therapies for multiple sclerosis: where are we now? *Expert Rev Neurother*. 2023;23(12):1173-1187. doi:10.1080/14737175.2023.2289572

32. Lublin FD, Häring DA, Ganjgahi H, et al. How patients with multiple sclerosis acquire disability. *Brain*. 2022;145(9):3147-3161. doi:10.1093/brain/awac016