Official Title: A Randomized Controlled Trial of Vitamin D Supplementation in Multiple Sclerosis

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Synopsis

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Rationale: Vitamin D insufficiency is a risk factor for multiple sclerosis (MS),[1] but it is uncertain if it also influences the prognosis of those with established disease. While vitamin D supplementation improves outcomes in an animal model of MS,[2, 3] human studies are limited. A vitamin D response element was recently identified in the promoter region of *HLA-DRB1*15*, a gene believed to be critical to MS risk, and 1, 25-dihydroxyvitamin D_3 increases its expression *in vitro*.[10] As such, vitamin D supplementation could even be harmful in established MS.[4, 5]

Our observational data show that vitamin D levels are inversely associated with subsequent relapse rate. However, other supplements that appeared to be helpful for other health conditions in observational studies had null or harmful effects in randomized trials.[12-14] We propose a randomized controlled trial of high- versus low-dose vitamin D_3 as an add-on to glatiramer acetate in patients with relapsing-remitting (RR) MS.

Study Design: Multicenter, randomized controlled, double-blind study of vitamin D_3 as add-on therapy to glatiramer acetate

Study Location: University of California, San Francisco; Johns Hopkins University; University of Pennsylvania; Washington University St. Louis; Oregon Health & Science University; Mt. Sinai School of Medicine; Cleveland Clinic; Yale University; Columbia University; Swedish Medical Center; University of Virginia; University of Rochester; Stanford University; Anne Arundel Medical Center; University of Massachusetts, Worcester and Dignity Health Medical Foundation.

Study Objectives:

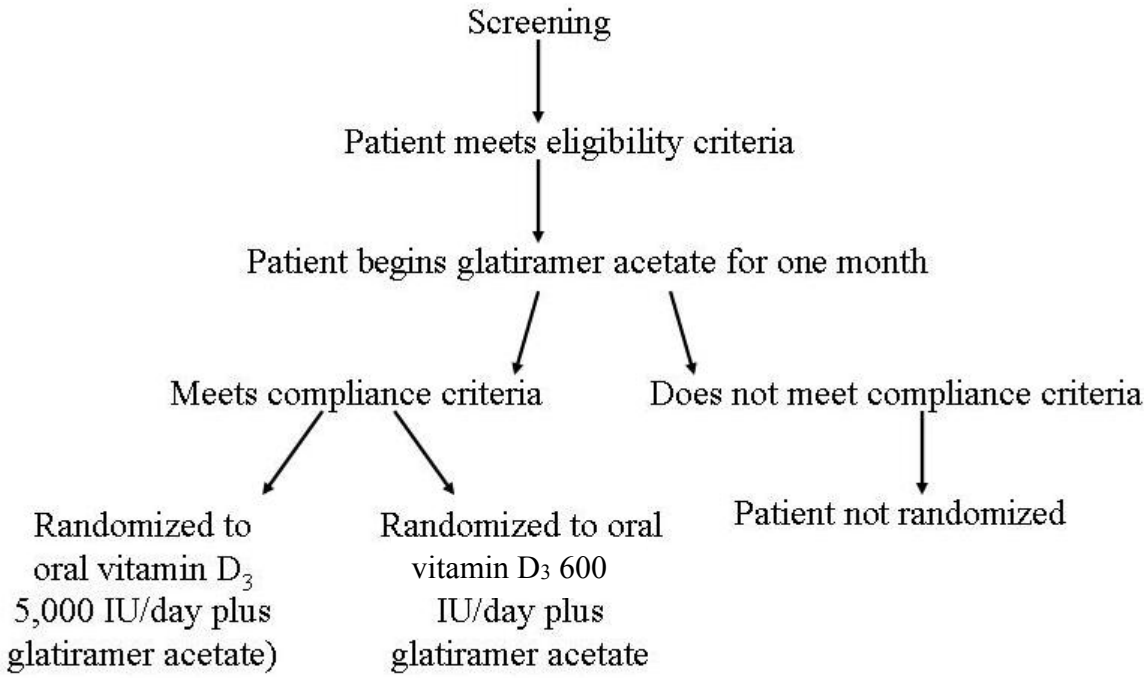
- To determine if high-dose versus low-dose vitamin D_3 supplementation as an add-on to first-line therapy for RRMS is associated with a decrease in the proportion of patients who experience a relapse.
- To determine if high-dose vitamin D_3 as an add-on to first-line therapy for MS is associated with a decrease in new T2 lesions in patients with RRMS.
- To determine if high-dose vitamin D_3 supplementation as an add-on to first-line therapy for MS is associated with a reduced rate of neurodegeneration.

Number of Planned Subjects: 172

Study Population: RRMS subjects (MAGNIMS criteria)[9], aged 18 to 50 years, with a baseline Expanded Disability Status score between 0.0 and 4.0 (and disease duration $\leq$ 10 years if MAGNIMS and McDonald RRMS, or $\leq$ 1 year if McDonald CIS meeting MAGNIMS RRMS criteria based on brain MRI). Patients who meet the McDonald RRMS criteria (rather than McDonald CIS that is now classified as MAGNIMS MS): must have had one clinical attack in

past two years AND at least one new silent T2 or gadolinium-enhancing lesion on brain MRI within the past year, or have had two clinical attacks in past two years, one of which occurred in the past year. Patients must have no prior history of treatment with rituximab, any chemotherapeutic agent, or total lymphoid irradiation. No treatment in the past six months with natalizumab, fingolimod, or fumarate. If patient has received glatiramer acetate, they have not been exposed to more than three months of treatment, and most recent treatment occurred greater than one month ago. Patients have received no treatment with other investigational therapies for MS and can't be receiving regular corticosteroids (e.g. scheduled pulse steroids or daily oral steroids). Patients must not have used interferon beta therapy or steroids for one month prior to screening and can't have used more than 1,000 IU supplemental vitamin D_3 (in addition to multivitamin) daily in the three months prior to screening. The 25-hydroxyvitamin D level must be $\geq$ 15 ng/mL within 30 days of screening; if not available, this will be added to the screening visit labs. Patients who have a level < 15 ng/mL may be referred back to their neurologist for appropriate repletion. Such patients may be rescreened for the vitamin D trial and enrolled successfully if all eligibility criteria are met at that time.

Treatment Groups: There will be a one-month run-in in which eligible subjects after screening will initiate subcutaneous glatiramer acetate 20 mg daily for one month. Those who are compliant ($\leq$ 3 missed doses in one month, assessed at the baseline visit) will be randomized (1:1) to high (5000 IU/day) or low (600 IU/day) dose oral vitamin D_3. Randomization will be stratified by center and schedule for each center will be based on randomly permuted blocks of varied size to ensure proper balance of treatment assignments within each center. Subjects will receive 1 tablet of vitamin D_3 daily and will also receive subcutaneous glatiramer acetate 20 mg daily:



Subjects who experience excessive activity (clinically, on MRI, or both) may be offered a switch from glatiramer acetate to another disease-modifying therapy for MS at the discretion of the treating physician but will remain in the study. This treatment will not be covered by the study.

Visit Schedule:

1st Year of Study

- Screening Visit
- Run-In Visit
- Baseline Visit
- Visits 1 through 4
- Premature Study Withdrawal Visit (if necessary)
- Unscheduled Relapse Visit (if necessary; within 5 days of onset of suspected relapse)

2nd Year of Study

- Visits 5 through 8
- Premature Study Withdrawal Visit (if necessary)
- Unscheduled Relapse Visit (if necessary; within 5 days of onset of suspected relapse)

Additional Subject Contact

Patients will be contacted by the study coordinator at months 1 and 2, six weeks after the month 3 visit and every three months thereafter (alternating with study visits) to assess for potential new symptoms and encourage compliance, using a standardized transcript.

Study Visit Window

All study visits will occur within 10 days of the scheduled visit. Visits that occur outside of the window will be recorded.

After we re-open from the COVID-19 pandemic, we will continuously assess whether there appear to be any impending COVID-19-related shutdowns or serious concerns that may prevent in-person study visits. During and after the COVID-19 pandemic, investigators will, at their discretion, be allowed to relax study windows as much as is needed in order to minimize risk to participants while also securing outcomes needed for the trial, such as end-of-study assessments. Questionnaires, although quite short, may be administered remotely at the discretion of the site PI.

In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in-person clinical trial visits or portions of clinical trial visits where determined to

be appropriate and where determined by the investigator not to increase the participant's risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

Efficacy Assessments: Clinical relapse assessments, EDSS, MSFC, MRI brain with and without gadolinium, low-contrast visual acuity (2.5% Sloan chart, measured binocularly), health-related quality of life (FAMS)

Safety Assessments: Patients will have screening laboratory tests (BUN, creatinine, calcium, AST, ALT, and urine pregnancy). Calcium and pregnancy tests will occur during the study. Suicidality assessment will occur every 3 months, per the FDA.

Assessment of last menstrual period and ordering of urine pregnancy tests for unexplained gaps will take the place of urine pregnancy tests during and after the COVID-19 pandemic.

Additional Assessments: 25-hydroxyvitamin D levels will be measured from samples at baseline and at months 3, 6, 12, and 24 (Heartland Laboratories, Ames, IA) as a batch at the end of the study to ensure the best possibility reproducibility. Serum will also be stored for use in immunologic studies as well as genetic studies.

Co-Consent for Genetics Study: All subjects will be asked at the screening visit to participate in a parallel study in which genotyping and gene expression analyses will be conducted using samples provided at study visits, as described in a related protocol. Participation in this study will be optional.

Statistical Analysis:

Primary Outcome: The proportion of subjects in each group who have had a relapse at two years

Secondary Outcomes

Clinical: annualized relapse rate; number of relapses requiring treatment with high-dose steroids; time to first on-study relapse; occurrence of sustained clinical disease progression on EDSS; change in MSFC score; change in low-contrast letter acuity; change in Functional Assessment in MS score

MRI: number of new or enlarging T2-weighted hyperintense MRI lesions; change in T2 lesion volume, percentage brain volume, normalized gray matter volume, and cortical thickness

Primary Predictor: Treatment group (high- versus low-dose vitamin D_3)

Secondary Predictor: Change in 25-hydroxyvitamin D level (baseline to month 24)

Study Activities:

	Screen	Run-In	Baseline	Months 3, 9, 15, 21	Months 6, 12, 18	Month 24	Relapse Visit
Informed consent	X						
Co-enrollment in genetics study	X						
Medical history and medication check	X	X	X	X	X	X	X
Clinical exam	X					X	X§§
Vital signs			X		X***	X	X§§
EDSS	X		X		X***	X	X
MSFC	X	X	X		X	X	
MRI			X		X***	X	
Suicidality assessment	X	X	X	X	X	X	X
Low-contrast acuity			X		X	X	
FAMS			X		X	X	
Sun exposure survey			X	X	X	X	
Skin tone card			X				
Fitzpatrick skin tone survey^			X			X	
Sodium screener					X***	X	
Screening labs	X						
Urine pregnancy test			X	X	X		
Calcium				X*	X	X	
Vitamin D level	X		X	X**	X	X	
Blood storage			X		X***	X	
Dispensing study drug			X	X	X		
Compliance check			X	X	X	X	
Relapse verification		X	X	X	X	X	X
Evaluation of blind						X	

*months 3 and 9; **month 3; ***month 12; §§ if clinically indicated; ^ slightly different version given at baseline and at final study visit; please consult the MOP

INTRODUCTION

Vitamin D deficiency is associated with a greater risk of MS. MS prevalence increases as distance from the equator increases,[10, 11] and those who migrate adopt the risk of the new area.[10, 12-14] Ultraviolet radiation (the main source of vitamin D) and skin cancer are inversely correlated with MS risk.[11, 16-20] In a nested case-control study, higher vitamin D levels conferred a lower subsequent risk of MS.[1] In experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, ultraviolet radiation or 1, 25-dihydroxyvitamin D_3 prevents the onset of the disease.[2, 3, 21]

Vitamin D is a potent immunomodulator. 1, 25-dihydroxyvitamin D_3 downregulates dendritic cells.[22, 23] It prevents the proliferation of and enhances apoptosis of activated B cells.[24] In mice, vitamin D receptor agonists reduce IL-17,[25] a modulator of EAE,[26] and inhibit proinflammatory T_H1 cells.[23] Vitamin D receptor agonists also promote CD4+CD25+T_{REG} cells.[27, 28] These data suggest that vitamin D plays key roles in innate and acquired immunity.[29] In EAE, giving vitamin D improves recovery [2, 3, 30-32] and reduces inflammation.[30, 33]

It is not known if vitamin D supplementation modifies the course of MS. Our preliminary data show a strong inverse association between vitamin D levels and subsequent relapse rates; these results were recently replicated in an adult MS cohort.[34, 35] Older pilot studies did not confirm the effect of vitamin D in MS, perhaps because they were small or non-randomized.[36-38] A recent pilot study randomized 49 MS patients to very high-dose versus discretionary vitamin D_3 (up to 4,000 IU/day) for one year and found a trend for better outcomes in the intervention group. However, subjects were not blinded or otherwise treated identically (limited follow-up of controls; actual control use of vitamin D not recorded).[39] Vitamin D increased the expression of *HLA-DRB1*15 in vitro*, presumably by acting on the concomitantly-reported vitamin D response element in the gene's promoter, suggesting that vitamin D could even worsen MS.[4] Moreover, other supplements that seemed beneficial for other diseases in observational studies had a null or negative impact in randomized trials.[6-8]

Vitamin D may be neuroprotective. As the duration of RRMS increases, many patients develop insidious disability progression, thought to be caused by neurodegeneration (the likely cause of volume loss, or atrophy, on brain MRI).[40, 41-48] Loss of normalized gray matter volume is associated with later disability, making it an attractive outcome measure for trials.[40, 49-54] Vitamin D and its receptor are widespread in the central nervous system.[55, 56] In cell culture excitotoxicity models, vitamin D was neuroprotective.[57-61] While there are no published data in MS, 1, 25 dihydroxyvitamin D_3 reduces activated T cell-mediated axonal damage in EAE.[62]

OBJECTIVES

Primary Objective

- To determine if high-dose versus low-dose vitamin D_3 supplementation as an add-on to first-line therapy for RRMS is associated with a decrease in the proportion of patients who experience a relapse.

Secondary Objectives

The secondary objectives of the study are to determine if high-dose vitamin D_3 as an add-on to first-line therapy for MS, as compared to low-dose vitamin D_3, is associated with:

- a decrease in new T2 lesions in patients with RRMS.
- a reduction in the loss of brain volume (whole brain; normalized gray matter; cortical thickness)
- a lower annualized relapse rate in the two-year study
- a reduction in the number of relapses requiring intravenous steroid therapy
- a prolongation of the time to the first relapse
- a reduction in the occurrence of sustained EDSS progression
- less worsening of disability as assessed by the MSFC
- less worsening of low-contrast letter acuity
- better health-related quality of life

We will also determine if high-dose vitamin D_3 as an add-on to first-line therapy for MS, as compared to low-dose vitamin D_3, is associated with the same effects as a function of time except for EDSS endpoint, as progression can only be confirmed after two years.

STUDY DESIGN

Overview

This is a prospective, double-blind, randomized multi-center trial of high- versus low-dose vitamin D_3 as add-on therapy to a standard therapy for RRMS, glatiramer acetate. Subjects will be randomized (1:1 ratio) to 600 IU or 5000 IU of oral vitamin D_3 daily for two years. The primary outcome will be the proportion of patients that experiences a clinical relapse during the study.

All study staff will be blinded to the treatment assignment; only in an emergency will unblinding occur. The study drug packages will be identical. Continental Vitamin Company (Los Angeles, CA) will manufacture capsules identical in size, color, odor, and taste. At the end of the study, subjects and coordinators will be asked to guess which group they were in.

Subjects who experience two or more relapses or who have excessive brain MRI activity at year 1 (or a combination; see "Study Treatment" section) may be offered a switch from glatiramer acetate to another disease-modifying therapy for MS at the discretion of the treating physician but will remain in the study. This treatment will not be covered by the study. For confirmed relapses, subjects may be offered a three-day course of high-dose intravenous methylprednisolone if deemed necessary by the treating physician. The cost of steroid treatment will not be covered by the study.

Overall Study Duration and Follow-Up

For the first year, the study will consist of the screening, run-in, and baseline visits, followed by visits at months 3, 6, 9, and 12 (visits 1-4). The second year will consist of visits at months 15, 18, 21, and 24 (visits 5-8). Visits 1, 3, 5, and 7 will be abbreviated visits at which the treating physician and examining physician do not need to perform any assessments. Premature withdrawal visits and unscheduled relapse visits will be conducted as needed. The unscheduled relapse visit may be merged with a scheduled visit if the two visits coincide and neither visit will fall out of window.

Efficacy Measures

Relapse: Relapses are new or recurring symptoms referable to the central nervous system lasting for at least 24 hours after a remission of 30 days or more since the prior event. Pseudo-exacerbations (worsening in the context of a fever/infection) will be excluded. Relapses must be accompanied by worsening of the EDSS (≥ 0.5 points) or in the Functional Systems (FS) scales (2 points on at least one FS scale or 1 point ≥ 2 FS scales), as determined by the *examining neurologist*.[63] Patients will be asked to report new symptoms within 48 hours of onset. Those with suspected relapses will be assessed within five days of onset. For confirmed relapses, subjects may be offered a three-day course of high-dose steroids if deemed necessary by the *treating physician*. The cost of steroid treatment will not be covered by the study. MRIs will be delayed for one month after the last date of steroid use.

We will also document probable relapses. These relapses include new or recurring symptoms referable to the central nervous system lasting for at least 24 hours after a remission of 30 days or more since the prior event. Pseudo-exacerbations (worsening in the context of a fever/infection) will be excluded. However, there will not be a requirement for an accompanying change on EDSS or FS scales. While relapse criteria are very stringent for many drug trials, relapses in practice may not involve a new examination finding (e.g. mild optic neuritis; sensory changes)

Disability Progression: A patient will be considered to have had sustained progression of disability if there is an increase in the EDSS score at month 12 by at least 1.0 point that is confirmed on the final examination, as performed by the *examining neurologist*, one year later.

MS Functional Composite (MSFC): The MSFC will be done by a *research coordinator* at the screening, run-in, and baseline visits and then every six months until month 24. The MSFC will also be performed at visits for relapses.[64]

Vision: Binocular low contrast letter acuity will be assessed by 2.5% Sloan charts.[65]

MRI: MRI scans will be performed at 3T and processed at baseline, month 12, and month 24. Since they are considered standard of care, the MRI scans will be read locally by the attending radiologist at each study site, and the local readings will be used for making decisions about changing therapy if disease activity is judged to be excessive, as detailed above, as well as for identifying and acting on incidental findings, as detailed in the IRB application. Prior to June 1, 2015, MRIs will be sent to Dr. Daniel Pelletier's laboratory at Yale University, which will perform MRI post-processing and analysis; laboratory members will be blinded to treatment group and disease course. In order to ensure the MRI scan quality is sufficient, each site will do a dry-run MRI scan on one individual. Since the MRI protocol being used is a standard clinical MS scan, the dry-run MRI may be conducted on a patient in the MS clinic who is already planning to have a follow-up scan as part of their routine clinical care. The scan will be loaded on a CD and sent to Yale University for approval. The dry-run MRI will be de-identified. For routine study participants, at Johns Hopkins, the study scans will be de-identified. If other sites do not wish to de-identify their scans, then the sites must provide proof to the study managers and to the Yale IRB that both their IRB-approved protocol and the IRB-approved patient consent form state that identifiers will remain on the CD and images. The discs will be stored in a secure facility and not used for any purpose other than this research study.

As of June 1, 2015, Dr. Pelletier's laboratory and all stored MRI study materials will move to University of Southern California (USC). Local IRBs will need to be updated that MRIs will be sent to USC for the remainder of the trial.

Brain lesions will be identified on simultaneously-viewed, high-resolution T1-weighted, T2-weighted, and proton density-weighted images. Regions of interest will be drawn based on a semi-automated threshold with manual editing utilizing in-house software, and T1- and T2-lesion masks will be created.[66] Intra- and inter-observer variability analyses will performed to ensure accuracy. T2-lesion volumes will be calculated by multiplying the area of the lesion by the slice thickness and the number of slices penetrated using in-house software. For atrophy, brain segmentation and normalization will be performed using SIENAX (Oxford, UK). Whole brain volume change will be calculated from high-resolution 3D T1-weighted images by SIENA.[67] Output will be converted into percentage brain volume change. Novel measures, such as cortical thickness, will also be assessed. The MRI endpoints will be evaluated as soon as the final subject completes the month 24 visit.

Health-related Quality of Life: The Functional Assessment in MS (FAMS) Version 4.0 will be administered at the beginning of each study visit at which it is scheduled.[68]

STUDY POPULATION

Inclusion Criteria:

1. Must meet MAGNIMS criteria for relapsing-remitting MS
2. Age 18 to 50 years
3. EDSS score ≤ 4.0
4. MS disease duration ≤ 10 years if McDonald RRMS; ≤ 1 year if meets MAGNIMS RRMS criteria but not McDonald RRMS criteria
5. If the patient meets the McDonald RRMS criteria (rather than McDonald CIS that is now classified as MAGNIMS MS):
    - Must have had one clinical attack in past two years AND at least one new silent T2 or gadolinium-enhancing lesion on brain MRI within the past year

    OR
    - Must have had two clinical attacks in past two years, one of which occurred in the past year
6. Females of child-bearing age must be willing to use at least one form of pregnancy prevention throughout the study.
7. Must have had a 25-hydroxyvitamin D level of ≥ 15 ng/mL within past 30 days
8. Must be willing to stop taking additional supplemental vitamin D, except as part of a multivitamin (can contain up to 600 IU), and must be willing to not take cod liver oil.

Exclusion Criteria:

1. Pregnant or nursing
2. Ongoing renal or liver disease
3. Known history of nephrolithiasis, hypercalcemia (non-spurious), sarcoidosis or other serious chronic illness including cancer (other than basal cell or squamous cell carcinoma of the skin), clinically relevant (non-benign) cardiac disease, or HIV.
4. Ongoing hyperthyroidism or active infection with *Mycobacterium* species
5. Known gastrointestinal disease (ulcerative colitis, Crohn's disease, celiac disease/gluten intolerance) or use of medications associated with malabsorption.
6. History of self-reported alcohol or substance abuse in past six months.
7. Prior history of treatment with rituximab, any chemotherapeutic agent, or total lymphoid irradiation. Treatment in the past six months with natalizumab, fingolimod, or fumarate is prohibited. If patient has received glatiramer acetate, exposure to more than three months of treatment is prohibited. No glatiramer acetate for one month prior to screening.Treatment with other unapproved therapies for MS is prohibited.
8. Use of interferon beta therapy for one month prior to screening
9. Use of more than 1,000 IU vitamin D daily (average) in the three months prior to screening
10. Condition that would limit the likelihood of completing the MRI procedures
11. Use of thiazide diuretics, digoxin, diltiazem, verapamil, cimetidine, heparin, low-molecular weight heparin, certain anticonvulsants (carbamazepine, phenytoin, or

phenobarbital), routine corticosteroids (eg scheduled monthly steroids, daily, etc), rifampin, or cholestyramine.

12. Steroids within a month of screening.
13. Recent active suicidal ideation in past 6 months (no "yes" answers to questions 2-5 on the screening Columbia Suicide Severity Rating Scale [C-SSRS]); no passive suicidal ideation in the past 2 months (answers "yes" to question 1 on the C-SSRS); suicidal attempts or preparatory attempts in the past 5 years (answers "yes" to any of the suicide attempt questions when referencing the past 5 years). Serum calcium.> 0.2 mg/dL above the upper limit of normal.

ENROLLMENT AND RANDOMIZATION

Patients who meet inclusion criteria and want to participate in the study will initiate therapy with glatiramer acetate for a one month run-in period. Those who successfully complete the run-in period and remain compliant with glatiramer acetate (compliance defined by no more than three missed doses in the run-in period) will then be randomized (stratified by site) in a 1:1 fashion to receive either 5,000 IU daily or 600 IU daily of oral vitamin D_3. The run-in was chosen so that it will reduce the risk of randomizing non-compliant individuals.

For subjects who have successfully completed the run-in, the study statistician will generate the randomization schedule (which will be stratified by site in order to account for differences in ultraviolet light in each location and will be based on randomly permuted blocks of varying sizes to ensure proper balance of treatment assignments within each center) in conjunction with the Investigational Drug Services (IDS) group at Johns Hopkins; the site coordinator (or study pharmacist, if necessitated by the site IRB) will distribute the study drug after confirming eligibility to the IDS. Each subject will be assigned a unique identification number, which will be used on all of the subject's Case Report Forms. In order to inform other providers and prevent unblinding, the subjects' primary care physician and regular neurologist will be informed that the subject is in the trial and will be asked not to check vitamin D levels during the trial in order to prevent unblinding.

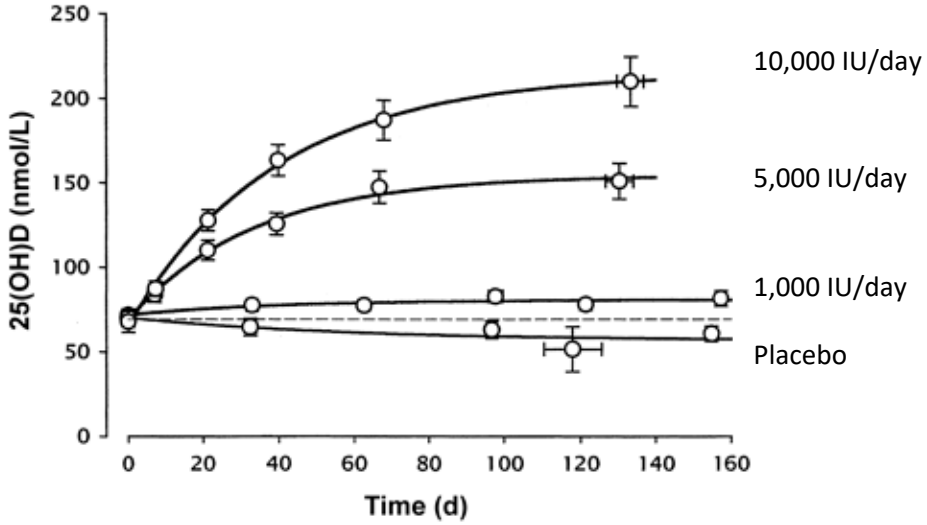
STUDY TREATMENT: DESCRIPTION AND ALLOCATION

Subjects will be randomized to oral vitamin D_3 5,000 IU or 600 IU once daily in a 1:1 fashion. All patients will be treated with subcutaneous glatiramer acetate 20 mg daily throughout the course of the study. Subjects who experience two or more relapses or excessive MRI activity (more than 3 new T2 lesions on the year 1 brain MRI) or one relapse plus more than 2 new T2 lesions on the year 1 MRI, may be offered a switch from glatiramer acetate to another disease-modifying therapy for MS at the discretion of the treating physician but will remain in the study. This treatment will not be covered by the study. The rules for discontinuing study treatment are described below. A subject may withdraw from the study if they choose to do so. The treating physician may also terminate a subject due to poor compliance, safety, or other issues.

Dose Rationale for Study Drug: The ideal serum level of 25-hydroxyvitamin D is still debated. Several experts have suggested that 30 ng/mL (75nmol/L; conversion factor from ng/mL to nmol/L is 2.496) is the minimum desired 25-hydroxyvitamin D level;[69] however, levels above 40 ng/mL were protective against MS.[1] Further, in our pediatric-onset MS study, there was no evidence of a threshold above which vitamin D was not important (up to 60 ng/mL, the highest level measured);[34] no threshold was determined in the Australian study either.[35] Many experts consider 10,000 IU the tolerable upper dose limit; in the most systematic pharmacokinetic study, this dose produced a final 25-hydroxyvitamin D level that approached 100 ng/mL (see Figure).[70, 71]

It thus seems likely that the appropriate target level is between 40 ng/mL and 100 ng/mL. In the Heaney vitamin D_3 pharmacokinetic study,[70] healthy subjects with a mean baseline level of 28 ng/mL were treated with vitamin D_3 5,000 IU daily for 20 weeks and had a final 25-hydroxyvitamin D level of 60 ng/mL. From our preliminary data, the mean 25-hydroxyvitamin D level in MS subjects was 22 ng/mL. We propose that 5,000 IU daily is a justifiable dose for the target 25-hydroxyvitamin D level of 60 ng/mL.

The control dose of 600 IU vitamin D_3, the current recommended daily allowance (RDA), was chosen for equipoise; however, this dose should produce little change in serum 25-hydroxyvitamin D levels (see Figure).[70]



Intervention Group: 86 subjects will receive glatiramer acetate 20 mg subcutaneously once a day, which will be given for one month prior to randomization, as described above. After randomization, subjects in this group will take 5,000 IU (125 mcg) of oral vitamin D_3 daily.

Control Group: 86 subjects will take glatiramer acetate 20 mg subcutaneously once daily. After one month, subjects randomized to this group will take 600 IU (15 mcg) of oral vitamin D_3 daily.

Methods for Maximizing Adherence and Compliance: The run-in period will allow investigators to assess compliance with glatiramer acetate. Patients who report missing more than three doses of glatiramer acetate during the run-in will not be eligible for randomization. Patients will be encouraged to take glatiramer acetate and the study drug together at the same time each day to minimize the chance that they will be forgotten. Patients will be instructed to take the medication within an hour of a meal, since oral vitamin D is fat-absorbed. A study coordinator will contact all subjects (texting or emailing, or phone call if preferred) to query about new symptoms and assess compliance. Subjects will be asked to complete an online or telephone survey if they are having new symptoms. Compliance will be assessed at each three-month visit by pill count.

Drug Accountability: The site principal investigator will maintain adequate records of the disposition of the study drug, including receipt, storage, dates, quantity of drug received, to whom dispensed, and accounts of any study drug accidentally or deliberately destroyed. A drug-dispensing log will be kept current for each participant and should contain the identification number of each participant and the date and quantity of drug dispensed.

Treatment Acquisition and Storage: All vitamin D will be stored in a secure location and will be dispensed by the study coordinator. The Johns Hopkins Investigational Drug Services will distribute the vitamin D capsules to each site to ensure study medication blinding. Vitamin D will be provided by Continental Vitamin Company; Teva Pharmaceuticals will provide glatiramer acetate.

SCHEDULE OF EVENTS

Study Personnel

At each site, the following personnel will participate in the study:

Treating physician: This person will be responsible for informed consent (a designee can consent e.g. the study coordinator), patient monitoring, adverse event assessment, physical examination and identifying possible relapses. The treating physician will remain blinded to treatment assignment.

Examining physician: This physician will perform the Expanded Disability Status Scale for scheduled and unscheduled visits for possible relapses. The examining physician will remain blinded to treatment assignment and to any details about a subject's course.

Pharmacist: If a site is unable to store and dispense the vitamin D and glatiramer acetate, a site pharmacy will store and distribute study medications.

Study coordinator: The coordinator will be responsible for administrative duties associated with study and will remain blinded to treatment assignment.

Injection training nurse: Injection training may be done by each site's nurse or referred to Teva, as determined by the site training physician.

Study technician: An examining technician will conduct the health-related quality of life, MSFC, and low-contrast letter acuity testing.

MRI technician: Each site technician will conduct the MRI protocol. This person will be blinded to treatment assignment and disease course.

Schedule of Events

Screening Visit

Treating physician

- Informed consent (can also be done by study coordinator)
- Consent for genetics study
- Eligibility criteria/verification
- Medical history
- Medication history
- Physical examination
- Suicidality assessment

Study coordinator

- Collection of blood for screening labs and vitamin D level (if not done within past month), and urine for pregnancy test (in women of childbearing potential)

Examining physician

- Expanded Disability Status Scale

Study technician

- MS Functional Composite

Run-in Visit

Study coordinator

- Suicidality assessment

- Instructions to start glatiramer acetate (training by nurse at each site/injection nurse)

Study technician

- MS Functional Composite

Baseline Visit (Day 0)

Treating physician

- Verification of compliance with glatiramer acetate injections
- Updating medical record (medical history, medications, contraceptive use)
- Adverse effect evaluation
- Documentation of relapses
- Suicidality assessment

Study coordinator

- Vital signs
- Collection of urine for pregnancy test
- Collection of blood for storage (for baseline 25-hydroxyvitamin D levels and future studies, including immunologic studies)
- Randomization
- Dispense study drug and glatiramer acetate
- Inform primary care doctor and neurologist of participation in study

Examining physician

- Expanded Disability Status Scale

Study technician

- MS Functional Composite
- Health-related quality of life (FAMS)
- Low-contrast visual acuity testing
- Sun Exposure Questionnaire
- Skin tone measurement (skin tone card, longer Fitzpatrick)

MRI technician

- Conducting the baseline brain MRI

Month 3, 9, 15, 21 Visits

Study coordinator

- Verification of compliance with glatiramer acetate injections and study drug
- Collection of blood for safety labs (months 3 and 9 only) and urine for pregnancy test
- Storage of blood for vitamin D levels (only at month 3)
- Updating medical record (medical history, medications, contraceptive use)
- Dispensing study drug and glatiramer acetate
- Suicidality assessment

Study technician

- Sun Exposure Questionnaire

Month 6, 12, 18 Visits

Treating physician

- Adverse event evaluation
- Suicidality assessment

Study coordinator

- Vital signs (month 12 only)
- Collection of blood for safety labs and urine for pregnancy test
- Collection of blood for storage for vitamin D levels
- Collection of blood for storage for future studies (month 12 only)
- Updating medical record (medical history, medications, contraceptive use)
- Verifying compliance with glatiramer acetate injections and study drug
- Dispensing study drug and glatiramer acetate

Examining physician

- Expanded Disability Status Scale (month 12 only)

Study technician

- MS Functional Composite
- Health-related quality of life (FAMS)
- Sodium Screener (month 12 only)

- Low-contrast visual acuity testing (binocular, 2.5%)
- Sun Exposure Questionnaire

MRI technician

- Conducting the brain MRI (month 12 only)

Month 24/End of Study Visit

Treating physician

- Physical examination including adverse event evaluation
- Suicidality assessment

Study coordinator

- Vital signs
- Collection of blood for safety labs (calcium only)
- Collection of blood for 25-hydroxyvitamin D levels
- Collection of blood for storage for future studies
- Updating medical record (medical history, medications, contraceptive use)
- Verifying compliance with glatiramer acetate injections and study drug
- Evaluation of the blind (patients, treating physician, examining physician, study technician)

Examining physician

- Expanded Disability Status Scale

Study technician

- MS Functional Composite
- Health-related quality of life (FAMS)
- Low-contrast visual acuity testing
- Sun Exposure Questionnaire
- Sodium screener
- Skin tone measurement (Fitzpatrick short version)

MRI technician

- Conducting the brain MRI

Unscheduled Relapse Visit

Treating physician

- Physical examination, if indicated, including adverse event evaluation
- Verification of relapse
- Determination of need for intravenous methylprednisolone
- Suicidality assessment

Study coordinator

- Vital signs, if indicated
- Urinalysis, urine culture and/or complete blood count (as needed, per the treating physician)
- Updating medication record

Examining physician

- Expanded Disability Status Scale

Additional Patient Contact

Between study visits, patients will be contacted by the study coordinator (months 1, 2, 4.5, 7.5, 10.5, 13.5, 16.5, 19.5, 22.5) to ask about new symptoms and encourage compliance, as well as to set up study visits.

Study Visit Windows

All study visits will occur within 10 days of the scheduled visit. Visits that occur outside of the window will be recorded.

During and after the COVID-19 pandemic, investigators will, at their discretion, be allowed to relax study windows as much as is needed in order to minimize risk to participants while also securing outcomes needed for the trial, such as end-of-study assessments.

SAFETY AND ADVERSE EVENT MONITORING

Screening will include blood (BUN, creatinine, AST, ALT, calcium), and urine pregnancy tests. Urine pregnancy testing will occur at baseline and every three months thereafter; serum calcium will be repeated at months 3, 6, 9, 12, 18, and 24. All safety data will be recorded; serious

adverse events will be reported on a serious adverse event form and in the chart. This algorithm will be used if hypercalcemia is detected during the study:

- Serum calcium level ≥1 mg/dL above the upper limit of normal: discontinue the study drug, and repeat labs in one week. If it normalizes, the drug will be restarted, and the level will be checked every other week for six weeks. If the level again is ≥1 mg/dL above the upper limit of normal, the patient will stop the drug for the rest of the study. If the repeat level is > 0.5 mg/dL above the upper limit of normal but is <1 mg/dL above the upper limit of normal, the patient will decrease the dose to one tablet every other day and will have serum calcium checked monthly for the rest of the study.
- Serum calcium is >0.5 mg/dL above the upper limit of normal but is <1 mg/dL above the upper limit of normal: labs repeated in one week; if the level continues to be in this range, patients will take only one tablet every other day for the remainder of the study and will have levels checked monthly until they normalize.
- Patients will be educated about symptoms of nephrolithiasis, will be instructed to call if they occur, and will be evaluated within 24 hours by the treating physician or by the Emergency Department. If nephrolithiasis is confirmed, the study drug will be stopped.

In compliance with the FDA regulations on assessing for suicidality in prospective trials (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM225130.pdf), the treating physician will administer the Columbia Classification Algorithm for Suicide Assessment at the screening visit, at baseline, and every three months as well as at unscheduled visits.

Adverse Event: An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom (or abnormal laboratory test), or disease temporally associated with the use of a medicinal product, whether or not it is considered related to the medicinal product. The investigator will record all study adverse events in the chart and will treat participants with adverse events appropriately, observing them until they resolve or stabilize. Adverse events will be collected from the start of the study until a participant terminates from the study; those that are unresolved at the time of termination will be followed until they resolve or up to 30 days.

Unexpected Adverse Event An adverse event is considered unexpected when its nature or severity is not consistent with the product information (e.g. package insert safety information, the investigational plan, the investigator's brochure, the protocol, or the informed consent form).

Serious Adverse Event: A serious adverse event (any adverse event that suggests a significant hazard, contraindication, side effect, or precaution) must be reported. This includes, but may not be limited to, death, a life-threatening event, inpatient hospitalization or prolongation of existing hospitalization, persistent or significant disability/ incapacity, congenital anomaly/ birth defect, other protocol-specified conditions, or an event that requires intervention to prevent permanent impairment or damage. Serious adverse events will be collected from informed consent signing until 30 days after study completion or until 30 days after a participant withdraws from the study.

Grading of Adverse Events
We will use the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 to report and grade all adverse events, whether or not they are related to disease progression or treatment. The relationship between an adverse event and the study drug will be determined by the site investigator and recorded on the appropriate form.

Serious Adverse Event Reporting The following process for reporting a serious adverse event will ensure compliance with the International Conference on Harmonisation guidelines:
1. The Institutional Review Board will be notified in one business day of a serious adverse event.
2. Standard reporting will occur if the event is serious, expected and drug-related, serious, expected and not drug-related, or serious, unexpected and not drug-related.
3. Expedited reporting is required if the event is serious, unexpected and drug-related. This type of event must be reported to the appropriate authorities within 15 days unless it is fatal or life-threatening; a fatal or life-threatening event must be reported within 7 days.

Pregnancy (Serious Adverse Event Reporting Requirements): Any pregnancy that occurs during a clinical study with an investigational drug will be reported as a serious adverse event, and pregnancies will be followed to their conclusion. Female participants should immediately inform the investigator of pregnancies and will stop taking the study medications. The investigator will report pregnancies to the Institutional Review board within one business day. The investigator will counsel the participant about the risks of continuing the pregnancy.

Criteria for Discontinuing Study Drug or Terminating the Study The study drug will be discontinued if the participant becomes pregnant or develops nephrolithiasis or persistently elevated serum calcium levels (as above), if it is in the participant's best interest (decided by the treating physician), if the participant experiences an adverse event grade 3 or higher that is at least possibly related to the study drug, or if the subject cannot tolerate the study drug or wants to discontinue treatment. Any death that is at least possibly related to the study will put the study on hold. The Data and Safety Monitoring Board will determine if it is safe to resume the study. Subjects who become suicidal during the study (codes 1-4, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM225130.pdf) will be referred to a psychiatrist or to a psychiatric emergency room for evaluation at the discretion of the site treating physician.

Data and Safety Monitoring Board (DSMB)

The independent DSMB consists of two neurologists (Amy Waldman, M.D., Children's Hospital of Philadelphia; Jill Conway, M.D., Carolinas Health Care) and one statistician (Ciprian Crainiceanu, Ph.D., Johns Hopkins University). The DSMB will meet, in person, or by teleconference, two times per year and will convene by teleconference or through e-mail any time a serious adverse event occurs.

For suicide, if there are more than three suicides or deaths in the study, the DSMB will be unblinded to treatment assignment. If the statistician deems this to be related to vitamin D dosage, the DSMB will be asked to make a recommendation about how to proceed. If more than 5 patients develop severe hypercalcemia (serum calcium ≥1 mg/dL above the upper limit of normal), a similar procedure will occur, with unblinding of the DSMB, the determination of whether this is likely related to vitamin D dose, and any recommendations for modification of the protocol. All serious adverse events will be reported to the DSMB members within one business day of the event's documentation. A recommendation from the DSMB when the thresholds above have been reached will be expected within three business days of the receipt of the report.

MRI and glatiramer acetate are part of the standard of care for MS patients, so adverse events will be reported to the DSMB, but the plan of care will be adjudicated and determined by the site PI, in consultation with the overall study PI, Dr. Mowry, as needed.

STATISTICAL ANALYSIS

Primary Outcome: The proportion of subjects in each group who have had a relapse at two years

Secondary Outcomes

Clinical: annualized relapse rate; number of relapses requiring treatment with high-dose steroids; time to first on-study relapse; proportion with probable relapse; annualized probable relapse rate; occurrence of sustained clinical disease progression on EDSS; change in MSFC score; change in low-contrast letter acuity over two years; change in Functional Assessment in MS score over two years

MRI: number of new or enlarging T2-weighted hyperintense MRI lesions; percentage of patients with a new T2 lesion; change in T2 lesion volume, percentage brain volume, normalized gray matter volume, and cortical thickness

Primary Predictor: Treatment group (high- versus low-dose vitamin D_3)

Secondary Predictor: Change in 25-hydroxyvitamin D level (baseline to month 24)

Statistical Analyses

Efficacy Outcomes: All statistical tests will be considered significant at a two-sided alpha of 0.05. Intention-to-treat will be used for all analyses.

The primary outcome is the proportion of individuals with a relapse during the two years of follow-up. Parameter estimates will be calculated using generalized estimating equations (GEE) with robust standard errors. A longitudinal logistic regression model with a saturated cell means model (including indicators for each visit and each visit by treatment interaction) and an unstructured covariance matrix will be used. The interaction term for the two-year visit

represents the comparison of the odds of relapse at two years for the two dose levels. Negative binomial regression will be used to compare the annual relapse rates and a Cox proportional hazards model will be used to compare the time to first relapse.

The occurrence of sustained EDSS progression will be assessed using the techniques described for the primary outcome. Longitudinal linear regression models will be used to evaluate changes in continuous outcomes (MS function composite score, T2 lesion volume, percentage brain volume, normalized gray matter volume, cortical thickness, low-contrast letter acuity, and Function Assessment in MS score). GEE with saturated mean models and unstructured covariance matrices will be applied. For all analyses, we will explore the potential effects of co-intervention (e.g. sun exposure;[72] use of multivitamin) and of baseline skin tone (using Skin Tone measurement card and Fitzpatrick scale) by adjusting for these variables in the models described above.

Adverse Event Outcomes: All participants who receive any vitamin D_3 will be included in the analysis of safety. Adverse events occurring before baseline, but not worsening or not related to study therapy during study, will not be considered as treatment-emergent adverse events.

Summary: The principal features of the design of this study and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principal features will require a protocol amendment that will then be subject to review by the study sponsor.

Role of Johns Hopkins as Coordinating Center

Dr. Mowry, the coordinating center PI, has the contact information for all centers. The study managers, Sandra Cassard and Susan Emrich, will assist Dr. Mowry in the oversight of the trial conduct. Dr. Mowry and/or the study managers will assist the participating centers in the submission of their IRB approval documents and consent forms and in drafting any response letters to the IRBs. All approved IRB applications and consent forms will be submitted to the coordinating center prior to starting the study at each site for review by Dr. Mowry/overall study managers to ensure modifications will not threaten the integrity of the study or the safety of the patients.

When modifications to the protocol are initiated by the coordinating center, the study managers will disseminate those modifications to each participating site. The managers will follow up within 10 days to ensure that the modifications have been submitted by each site to their IRB and will check in every three weeks after submission until written confirmation acknowledging the protocol modifications are provided from each site's IRB. The study managers will also be responsible for training study coordinators at participating sites in data entry. REDCap, a web-based database, will be used for data collection. The study managers will do spot checks of all data, using faxed data entry sheets (coded to maintain confidentiality) to assess for data entry

errors. The study managers will monitor data entry on an ongoing basis to ensure data are entered efficiently and within an expected window of subjects' study visits.

Protocol events and deviations will be reported directly to the study PI (Dr. Mowry) and to the study managers within 5 business days; serious adverse events must be reported immediately by e-mail and phone to Dr. Mowry and the study managers.

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