Clinical trial results:
Dose-related effects of vitamin D on immune responses in patients with clinically isolated syndrome or early multiple sclerosis and healthy control participants. An exploratory randomised double blind placebo controlled study

Summary

EudraCT number	2012-000635-68
Trial protocol	IE
Global end of trial date	09 June 2015

Results information

Result version number	v1 (current)
This version publication date	04 December 2018
First version publication date	04 December 2018
Summary attachment (see zip file)	Effects of vitamin D3 in clinically isolated syndrome and healthy control participants: A double-blind randomised controlled trial (O'Connell et al. - 2017 - Effects of vitamin D3 in clinically isolated syndr.pdf) Dose-related effects of vitamin D on immune responses in patients with clinically isolated syndrome and healthy control participants: study protocol for an exploratory randomized double- blind placebo (O'Connell et al. - 2013 - Dose-related effects of vitamin D on immune respon.pdf)

Trial information

Trial identification

Sponsor protocol code	2012CIS/VD/SVUH

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT01728922
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	University College Dublin
Sponsor organisation address	Belfield, Dublin, Ireland,
Public contact	Neurology Department, St Vincent's University Hospital, 00353 12214179, n.neuadcc@st-vincents.ie
Scientific contact	Neurology Department, St Vincent's University Hospital, 00353 12214179, n.neuadcc@st-vincents.ie

Notes:

Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	05 June 2015
Is this the analysis of the primary completion data?	Yes
Primary completion date	05 June 2015
Global end of trial reached?	Yes
Global end of trial date	09 June 2015
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

To determine the effects of vitamin D supplementation at two doses a) 4,667 IU daily b) 9,333 IU daily compared to c) placebo over a four and six-month period on the frequency of CD4 T cell subsets and cytokine responses by PBMC in 1) patients with the clinically isolated syndrome or early MS not treated with disease modifying therapies 2) healthy control participants

Protection of trial subjects:

All patients provided written informed consent to participate in the study prior to being screened.
Patients were free to withdraw from the study at any time without giving a reason. Patients were advised that if they requested to withdraw from the study, at any time during the trial, then this would have no negative consequences.
The investigator could also withdraw patients from the trial if they deemed it appropriate for safety or ethical reasons or if it was considered to be to be detrimental to the well-being of the patient.
Safety monitoring:
Blood biochemistry including LFTs, U&E and serum calcium were measured at all study visits. In addition serum parathyroid hormone was assessed at screening. Serum or urine pregnancy testing in women of child-bearing potential was performed at screening and baseline visits.

Background therapy: -

Evidence for comparator:

There is thus accumulating evidence that vitamin D deficiency increases susceptibility to MS and that vitamin D supplementation reduces disease activity by immunomodulatory mechanisms. It seems probable that serum vitamin D levels of greater than 100nmol/L are required to produce the immunological effects of vitamin D. Recent studies have suggested that despite supplementation it is difficult to achieve adequate levels of vitamin D in people with MS and showed different responses to supplementation raising the possibility of a different vitamin D pharmacokinetics in people with MS compared to controls. Thus, in MS patients with vitamin D deficiency, doses of 5000 to 10,000 IU/day are likely to be needed to achieve the desired immunological response. Most patients with RRMS, once diagnosed, commence treatment with first line disease modifying therapies (DMTs). Several RCTs are underway examining the effects vitamin D supplementation in RRMS patients already receiving first-line DMTs on clinical and MRI outcomes. In the UK and Ireland patients with the clinically isolated syndrome (CIS) are not usually commenced on DMTs until there is clinical or MRI evidence of dissemination in time. One may recruit these CIS patients, at risk of developing RRMS, into trials of potentially therapeutic agents in order to examine the efficacy of a therapy in preventing the development of clinically definite multiple sclerosis. We have therefore designed this RCT in order to examine, over a 24-week treatment period, in CIS patients and healthy control participants, randomized to either of two doses of vitamin D (5000 or 10,000 IU) or placebo, the effects on immunological measures as a primary outcome. In addition, in the CIS patients clinical and MRI efficacy measures will be secondary outcomes.

Actual start date of recruitment	02 April 2012
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	Ireland: 67
Worldwide total number of subjects	67
EEA total number of subjects	67

Notes:

Subjects enrolled per age group

In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	67
From 65 to 84 years	0
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

Participants were screened against inclusion and exclusion criteria following discussion of the trial and provision of signed, fully informed consent.

Pre-assignment

Screening details:

39 healthy participants and 32 participants with clinical isolated syndrome were randomised into three trial arms each: placebo, 5,000 IU vitamin D and 10,000 IU vitamin D.

Period 1

Period 1 title	Baseline Visit
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Assessor

Blinding implementation details:

Patients, healthy control participants, and study staff, with the exception of the hospital pharmacist and internal monitor were blinded until the study database was locked at the end of the trial. The IMP and the placebo were identical in appearance, as was the labeling and packaging.

Arms

Are arms mutually exclusive?	Yes
Arm title	Healthy control - placebo

Arm description:

Healthy participants randomised to be receiving placebo

Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One placebo dose daily

Arm title	CIS - placebo

Arm description:

Participants with clinical isolated syndrome randomised to be receiving placebo

Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One placebo dose daily

Arm title	Healthy control - 5,000 IU vitamin D

Arm description:

Healthy participants randomised to be receiving 5,000 IU vitamin D

Arm type	Experimental

Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:
One 10,000 IU vitamin D3 dose daily

Arm title	CIS - 5,000 IU vitamin D

Arm description:
Participants with clinical isolated syndrome randomised to be receiving 5,000 IU vitamin D

Arm type	Experimental
Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:
One 10,000 IU vitamin D3 dose daily

Arm title	Healthy control - 10,000 IU vitamin D

Arm description:
Healthy participants randomised to be receiving 10,000 IU vitamin D

Arm type	Experimental
Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:
One 10,000 IU vitamin D3 dose daily

Arm title	CIS - 10,000 IU vitamin D

Arm description:
Participants with clinical isolated syndrome randomised to be receiving 10,000 IU vitamin D

Arm type	Experimental
Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:
One 10,000 IU vitamin D3 dose daily

Number of subjects in period 1	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D
Started	12	7	13
Completed	11	7	13
Not completed	1	0	0
Consent withdrawn by subject	1	-	-

Number of subjects in period 1	CIS - 5,000 IU vitamin D	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D
Started	10	13	12
Completed	10	13	12
Not completed	0	0	0
Consent withdrawn by subject	-	-	-

Period 2

Period 2 title	Study Period
Is this the baseline period?	No
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Assessor

Blinding implementation details:

Patients, healthy control participants, and study staff, with the exception of the hospital pharmacist and internal monitor were blinded until the study database was locked at the end of the trial. The IMP and the placebo were identical in appearance, as was the labeling and packaging.

Arms

Are arms mutually exclusive?	Yes
Arm title	Healthy control - placebo

Arm description:

Healthy participants receiving placebo

Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One placebo dose daily

Arm title	CIS - placebo

Arm description:

Participants with clinical isolated syndrome receiving placebo

Arm type	Placebo
Investigational medicinal product name	Placebo
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One placebo dose daily

Arm title	Healthy control - 5,000 IU vitamin D

Arm description:

Healthy participants receiving 5,000 IU vitamin D

Arm type	Experimental

Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One 10,000 IU vitamin D3 dose daily

Arm title	CIS - 5,000 IU vitamin D

Arm description:

Participants with clinical isolated syndrome receiving 5,000 IU vitamin D

Arm type	Experimental
Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One 10,000 IU vitamin D3 dose daily

Arm title	Healthy control - 10,000 IU vitamin D

Arm description:

Healthy participants receiving 10,000 IU vitamin D

Arm type	Experimental
Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One 10,000 IU vitamin D3 dose daily

Arm title	CIS - 10,000 IU vitamin D

Arm description:

Participants with clinical isolated syndrome receiving 10,000 IU vitamin D

Arm type	Experimental
Investigational medicinal product name	Vitamin D3
Investigational medicinal product code	
Other name	Vigantol, Cholecalciferol
Pharmaceutical forms	Oral drops
Routes of administration	Enteral use

Dosage and administration details:

One 10,000 IU vitamin D3 dose daily

Number of subjects in period 2	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D
Started	11	7	13
Completed	11	7	13
Not completed	0	0	0
Consent withdrawn by subject	-	-	-

Protocol deviation	-	-	-

Number of subjects in period 2	CIS - 5,000 IU vitamin D	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D
Started	10	13	12
Completed	10	11	12
Not completed	0	2	0
Consent withdrawn by subject	-	1	-
Protocol deviation	-	1	-

Baseline characteristics

Reporting groups	
Reporting group title	Healthy control - placebo
Reporting group description:	
Healthy participants randomised to be receiving placebo	
Reporting group title	CIS - placebo
Reporting group description:	
Participants with clinical isolated syndrome randomised to be receiving placebo	
Reporting group title	Healthy control - 5,000 IU vitamin D
Reporting group description:	
Healthy participants randomised to be receiving 5,000 IU vitamin D	
Reporting group title	CIS - 5,000 IU vitamin D
Reporting group description:	
Participants with clinical isolated syndrome randomised to be receiving 5,000 IU vitamin D	
Reporting group title	Healthy control - 10,000 IU vitamin D
Reporting group description:	
Healthy participants randomised to be receiving 10,000 IU vitamin D	
Reporting group title	CIS - 10,000 IU vitamin D
Reporting group description:	
Participants with clinical isolated syndrome randomised to be receiving 10,000 IU vitamin D	

Reporting group values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D
Number of subjects	12	7	13
Age categorical Units: Subjects			
In utero			
Preterm newborn infants (gestational age < 37 wks)			
Newborns (0-27 days)			
Infants and toddlers (28 days-23 months)			
Children (2-11 years)			
Adolescents (12-17 years)			
Adults (18-64 years)			
From 65-84 years			
85 years and over			
Age continuous Units: years			
arithmetic mean	29.1	34.3	30.3
standard deviation	± 4.7	± 10.6	± 3.7
Gender categorical Units: Subjects			
Female	10	6	10
Male	2	1	3
Ever smoked			
Has the study subject ever smoked?			
Units: Subjects			
Yes	4	3	3

No	8	4	10

Body Mass Index Units: kg/m2			
arithmetic mean	27.6	26.7	23.5
standard deviation	± 4.3	± 4.2	± 3.3
IFN-γ+ CD4+ proliferating T cells Units: percent			
median	4.9	4.7	8.5
inter-quartile range (Q1-Q3)	2.5 to 7.5	4.3 to 5.6	5 to 10.9
Il-17+ CD4+ proliferating T-cells Units: percent			
median	0.8	0.3	1
inter-quartile range (Q1-Q3)	0.3 to 1.5	0.2 to 0.9	0.5 to 1.6
IFN-γ from stimulated PBMCs Units: pg/ml			
median	5852	183	6954
inter-quartile range (Q1-Q3)	746 to 14895	60 to 420	1671 to 10836
IL-17 from stimulated PBMCs Units: pg/ml			
median	512	1090	169
inter-quartile range (Q1-Q3)	80 to 1420	621 to 1776	33 to 1006
IL-10 from stimulated PBMCs Units: pg/ml			
median	1528	1192	1571
inter-quartile range (Q1-Q3)	407 to 7274	562 to 1438	321 to 3657
Seasonally adjusted serum 25(OH)D concentrations Units: nnmol/L			
arithmetic mean	49.5	53.7	57.2
standard deviation	± 17	± 15.8	± 20.7
Plasma parathyroid hormone concentration Units: pmol/L			
arithmetic mean	5.0	3.3	4.2
standard deviation	± 2.0	± 1.0	± 1.1
Serum calcium concentrations Units: mmol/L			
arithmetic mean	2.3	2.4	2.3
standard deviation	± 0.1	± 0.1	± 0.1

Reporting group values	CIS - 5,000 IU vitamin D	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D
Number of subjects	10	13	12
Age categorical Units: Subjects			
In utero			
Preterm newborn infants (gestational age < 37 wks)			
Newborns (0-27 days)			
Infants and toddlers (28 days-23 months)			

Children (2-11 years)			
Adolescents (12-17 years)			
Adults (18-64 years)			
From 65-84 years			
85 years and over			
Age continuous			
Units: years			
arithmetic mean	32.7	30.5	37.2
standard deviation	± 4.6	± 5.1	± 8.7
Gender categorical			
Units: Subjects			
Female	5	6	8
Male	5	7	4
Ever smoked			
Has the study subject ever smoked?			
Units: Subjects			
Yes	5	2	7
No	5	11	5
Body Mass Index			
Units: kg/m2			
arithmetic mean	26.3	25.4	30.6
standard deviation	± 3.4	± 3.2	± 5.6
IFN-γ+ CD4+ proliferating T cells			
Units: percent			
median	5.8	9.3	7.3
inter-quartile range (Q1-Q3)	3.3 to 6.8	7 to 12.6	1.2 to 10.3
Il-17+ CD4+ proliferating T-cells			
Units: percent			
median	1.1	0.6	0.8
inter-quartile range (Q1-Q3)	0.6 to 2.9	0.4 to 0.9	0.1 to 2
IFN-γ from stimulated PBMCs			
Units: pg/ml			
median	514	8735	341
inter-quartile range (Q1-Q3)	259 to 1618	2307 to 22084	124 to 1230
IL-17 from stimulated PBMCs			
Units: pg/ml			
median	317	153	1208
inter-quartile range (Q1-Q3)	208 to 989	72 to 1487	595 to 1587
IL-10 from stimulated PBMCs			
Units: pg/ml			
median	1596	3093	1271
inter-quartile range (Q1-Q3)	618 to 2174	466 to 6130	361 to 1599
Seasonally adjusted serum 25(OH)D concentrations			
Units: nnmol/L			
arithmetic mean	51.5	45.6	55.2
standard deviation	± 16.9	± 8.8	± 16.8
Plasma parathyroid hormone concentration			
Units: pmol/L			
arithmetic mean	2.6	4.8	3.2
standard deviation	± 0.6	± 1.9	± 1.7

Serum calcium concentrations Units: mmol/L			
arithmetic mean	2.4	2.3	2.4
standard deviation	± 0.1	± 0.1	± 0.1

Reporting group values	Total		
Number of subjects	67		
Age categorical Units: Subjects			
In utero	0		
Preterm newborn infants (gestational age < 37 wks)	0		
Newborns (0-27 days)	0		
Infants and toddlers (28 days-23 months)	0		
Children (2-11 years)	0		
Adolescents (12-17 years)	0		
Adults (18-64 years)	0		
From 65-84 years	0		
85 years and over	0		
Age continuous Units: years			
arithmetic mean			
standard deviation	-		
Gender categorical Units: Subjects			
Female	45		
Male	22		
Ever smoked			
Has the study subject ever smoked?			
Units: Subjects			
Yes	24		
No	43		
Body Mass Index Units: kg/m2			
arithmetic mean			
standard deviation	-		
IFN-γ+ CD4+ proliferating T cells Units: percent			
median			
inter-quartile range (Q1-Q3)	-		
Il-17+ CD4+ proliferating T-cells Units: percent			
median			
inter-quartile range (Q1-Q3)	-		
IFN-γ from stimulated PBMCs Units: pg/ml			
median			
inter-quartile range (Q1-Q3)	-		
IL-17 from stimulated PBMCs Units: pg/ml			
median			

inter-quartile range (Q1-Q3)	-		
IL-10 from stimulated PBMCs Units: pg/ml			
median			
inter-quartile range (Q1-Q3)	-		
Seasonally adjusted serum 25(OH)D concentrations Units: nnmol/L			
arithmetic mean			
standard deviation	-		
Plasma parathyroid hormone concentration Units: pmol/L			
arithmetic mean			
standard deviation	-		
Serum calcium concentrations Units: mmol/L			
arithmetic mean			
standard deviation	-		

End points

End points reporting groups

Reporting group title	Healthy control - placebo

Reporting group description:

Healthy participants randomised to be receiving placebo

Reporting group title	CIS - placebo

Reporting group description:

Participants with clinical isolated syndrome randomised to be receiving placebo

Reporting group title	Healthy control - 5,000 IU vitamin D

Reporting group description:

Healthy participants randomised to be receiving 5,000 IU vitamin D

Reporting group title	CIS - 5,000 IU vitamin D

Reporting group description:

Participants with clinical isolated syndrome randomised to be receiving 5,000 IU vitamin D

Reporting group title	Healthy control - 10,000 IU vitamin D

Reporting group description:

Healthy participants randomised to be receiving 10,000 IU vitamin D

Reporting group title	CIS - 10,000 IU vitamin D

Reporting group description:

Participants with clinical isolated syndrome randomised to be receiving 10,000 IU vitamin D

Reporting group title	Healthy control - placebo

Reporting group description:

Healthy participants receiving placebo

Reporting group title	CIS - placebo

Reporting group description:

Participants with clinical isolated syndrome receiving placebo

Reporting group title	Healthy control - 5,000 IU vitamin D

Reporting group description:

Healthy participants receiving 5,000 IU vitamin D

Reporting group title	CIS - 5,000 IU vitamin D

Reporting group description:

Participants with clinical isolated syndrome receiving 5,000 IU vitamin D

Reporting group title	Healthy control - 10,000 IU vitamin D

Reporting group description:

Healthy participants receiving 10,000 IU vitamin D

Reporting group title	CIS - 10,000 IU vitamin D

Reporting group description:

Participants with clinical isolated syndrome receiving 10,000 IU vitamin D

Primary: The Effects of Oral Vitamin D Compared to Placebo on the Percentage IFN-γ+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline.

End point title	The Effects of Oral Vitamin D Compared to Placebo on the Percentage IFN-γ+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline.[1]

End point description:

This outcome measure will be assessed at 16 weeks and compared to baseline.

End point type	Primary

End point timeframe:

assessed at 16 weeks

Notes:

[1] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: percentage				
median (inter-quartile range (Q1-Q3))	5.9 (1.4 to 12.6)	7 (4.3 to 9)	6.5 (3.1 to 11.5)	4.9 (3.6 to 10.2)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: percentage				
median (inter-quartile range (Q1-Q3))	11.3 (3.3 to 13.1)	7 (2.3 to 8.6)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on the Percentage IFN-γ+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.

End point title	The Effects of Oral Vitamin D Compared to Placebo on the Percentage IFN-γ+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.[2]

End point description:

End point type	Primary

End point timeframe:

Assessed at 24 weeks

Notes:

[2] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: percentage				
median (inter-quartile range (Q1-Q3))	6.2 (3.8 to 12.6)	6 (1.1 to 7.3)	8.2 (4.8 to 12.3)	6.4 (4.7 to 8.6)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: percentage				
median (inter-quartile range (Q1-Q3))	8.9 (4.5 to 12.3)	5.1 (2.5 to 7.6)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on the Percentage IL-17+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline.

End point title	The Effects of Oral Vitamin D Compared to Placebo on the Percentage IL-17+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline.[3]

End point description:

End point type	Primary

End point timeframe:

Assessed at 16 weeks

Notes:

[3] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: percentage				
median (inter-quartile range (Q1-Q3))	0.8 (0.3 to 1.9)	0.6 (0.2 to 1.9)	0.6 (0.3 to 0.9)	1.4 (0.8 to 2.9)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: percentage				
median (inter-quartile range (Q1-Q3))	0.5 (0.3 to 1.2)	1.3 (0.6 to 3.2)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on the Percentage IL-17+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.

End point title	The Effects of Oral Vitamin D Compared to Placebo on the Percentage IL-17+ CD4+ Proliferating T-cells as a Fraction of Total T-cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.[4]

End point description:

End point type	Primary

End point timeframe:

Assessed at 24 weeks

Notes:

[4] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: percentage				
median (inter-quartile range (Q1-Q3))	1.3 (0.4 to 2.8)	0.8 (0 to 13)	0.5 (0.3 to 1.7)	1.7 (0.5 to 2.4)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: percentage				
median (inter-quartile range (Q1-Q3))	0.7 (0.5 to 1.6)	0.7 (0.3 to 2.9)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on IFN-γ Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline.

End point title	The Effects of Oral Vitamin D Compared to Placebo on IFN-γ Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline.[5]

End point description:

End point type	Primary

End point timeframe:

Assessed at 16 weeks

Notes:

[5] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	6129 (1179 to 16005)	103 (0 to 842)	2160 (1780 to 8896)	1303 (414 to 1910)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	6918 (2664 to 20006)	757 (191 to 1739)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on IFN-γ Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.

End point title	The Effects of Oral Vitamin D Compared to Placebo on IFN-γ Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.[6]

End point description:

End point type	Primary

End point timeframe:

Assessed at 24 weeks

Notes:

[6] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	7620 (2969 to 17173)	450 (13 to 810)	8253 (3860 to 16915)	698 (299 to 1957)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	8948 (2905 to 21193)	484 (160 to 2161)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on IL-17 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline

End point title	The Effects of Oral Vitamin D Compared to Placebo on IL-17 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline[7]

End point description:	
End point type	Primary
End point timeframe:	
Assessed at 16 weeks	

Notes:

[7] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	90 (34 to 1795)	1606 (762 to 3160)	190 (77 to 1265)	669 (183 to 1066)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	230 (51 to 1487)	1098 (781 to 2303)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on IL-17 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline

End point title	The Effects of Oral Vitamin D Compared to Placebo on IL-17 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline[8]
End point description:	
End point type	Primary
End point timeframe:	
Assessed at 24 weeks	

Notes:

[8] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not

supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	473 (119 to 2081)	1399 (665 to 1850)	267 (152 to 1012)	805 (211 to 2151)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	416 (149 to 1525)	1676 (628 to 2078)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on IL-10 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline

End point title	The Effects of Oral Vitamin D Compared to Placebo on IL-10 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 16 Weeks Compared to Baseline[9]

End point description:

End point type	Primary

End point timeframe:

Assessed at 16 weeks

Notes:

[9] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	1793 (153 to 2556)	1213 (658 to 2176)	958 (250 to 2356)	1146 (667 to 1682)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	1201 (489 to 3365)	1183 (84 to 2052)		

Statistical analyses

No statistical analyses for this end point

Primary: The Effects of Oral Vitamin D Compared to Placebo on IL-10 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.

End point title	The Effects of Oral Vitamin D Compared to Placebo on IL-10 Concentrations in Peripheral Blood Mononuclear Cells in Patients With CIS and Healthy Control Participants (HCP) at 24 Weeks Compared to Baseline.[10]

End point description:

End point type	Primary

End point timeframe:

Assessed at 24 weeks

Notes:

[10] - No statistical analyses have been specified for this primary end point. It is expected there is at least one statistical analysis for each primary end point.
Justification: The statistical analysis performed was within-group baseline to endpoint testing. This type of analysis is statistically invalid in the context of randomised controlled trials and its input is not supported by EudraCT. Please refer to the results paper for review of the results of the statistical analysis.

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	1452 (615 to 3838)	881 (302 to 2528)	1616 (416 to 2865)	1001 (735 to 2003)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	13	12		
Units: pg/ml				
median (inter-quartile range (Q1-Q3))	3251 (1023 to 5908)	1140 (140 to 1855)		

Statistical analyses

No statistical analyses for this end point

Secondary: The Number of New T2 Lesions Compared to Baseline Among the Study Group.

End point title	The Number of New T2 Lesions Compared to Baseline Among the Study Group.
End point description:	
End point type	Secondary
End point timeframe:	
Assessed at 24 weeks	

End point values	CIS - placebo	CIS - 5,000 IU vitamin D	CIS - 10,000 IU vitamin D	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	7	9	10	
Units: lesions per participant				
arithmetic mean (standard deviation)	0.6 (± 0.8)	1.4 (± 1.7)	0.4 (± 0.5)	

Statistical analyses

No statistical analyses for this end point

Secondary: The Number of New Gadolinium Enhancing Lesions Compared to Baseline Among the Study Group.

End point title	The Number of New Gadolinium Enhancing Lesions Compared to Baseline Among the Study Group.
End point description:	
End point type	Secondary

End point timeframe:	
Assessed at 24 weeks	

End point values	CIS - placebo	CIS - 5,000 IU vitamin D	CIS - 10,000 IU vitamin D	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	7	9	10	
Units: lesions per participant				
arithmetic mean (standard deviation)	0.1 (± 0.4)	0.1 (± 0.3)	0 (± 0)	

Statistical analyses

No statistical analyses for this end point

Secondary: Number of Patients With New Disease Activity by MRI

End point title	Number of Patients With New Disease Activity by MRI
End point description:	
End point type	Secondary
End point timeframe:	
Assessed at 24 weeks	

End point values	CIS - placebo	CIS - 5,000 IU vitamin D	CIS - 10,000 IU vitamin D	
Subject group type	Reporting group	Reporting group	Reporting group	
Number of subjects analysed	7	9	10	
Units: participants	3	5	5	

Statistical analyses

No statistical analyses for this end point

Other pre-specified: Change in Seasonally-adjusted Serum 25(OH)D Levels at 16 Weeks

End point title	Change in Seasonally-adjusted Serum 25(OH)D Levels at 16 Weeks
End point description:	
End point type	Other pre-specified
End point timeframe:	
Assessed at 16 weeks	

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: nmol/L				
arithmetic mean (standard deviation)	4 (± 12)	7 (± 26)	83 (± 27)	81 (± 63)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	11	12		
Units: nmol/L				
arithmetic mean (standard deviation)	152 (± 71)	121 (± 35)		

Statistical analyses

Statistical analysis title	Intergroup comparison at 16 weeks
Statistical analysis description: Comparison of 5,000 IU vitamin D arm and 10,000 IU vitamin D arm to placebo arm.	
Comparison groups	Healthy control - placebo v Healthy control - 5,000 IU vitamin D v Healthy control - 10,000 IU vitamin D
Number of subjects included in analysis	35
Analysis specification	Pre-specified
Analysis type	superiority
P-value	< 0.001
Method	t-test, 2-sided

Statistical analysis title	Intergroup comparison at 16 weeks
Statistical analysis description: Comparison of 5,000 IU vitamin D arm and 10,000 IU vitamin D arm to placebo arm.	
Comparison groups	CIS - placebo v CIS - 5,000 IU vitamin D v CIS - 10,000 IU vitamin D
Number of subjects included in analysis	29
Analysis specification	Pre-specified
Analysis type	superiority
P-value	< 0.001
Method	t-test, 2-sided

Statistical analysis title	Intergroup comparison at 16 weeks
Statistical analysis description:	
Comparison between 5,000 IU vitamin D arm and 10,000 IU vitamin D arm	
Comparison groups	CIS - 5,000 IU vitamin D v CIS - 10,000 IU vitamin D
Number of subjects included in analysis	22
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.08
Method	t-test, 2-sided

Other pre-specified: Change in Seasonally-adjusted Serum 25(OH)D Levels at 24 Weeks

End point title	Change in Seasonally-adjusted Serum 25(OH)D Levels at 24 Weeks
End point description:	
End point type	Other pre-specified
End point timeframe:	
Assessed at 24 weeks	

End point values	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D	CIS - 5,000 IU vitamin D
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	11	7	13	10
Units: nmol/L				
arithmetic mean (standard deviation)	2 (± 14)	18 (± 34)	92 (± 35)	76 (± 57)

End point values	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	11	12		
Units: nmol/L				
arithmetic mean (standard deviation)	136 (± 71)	115 (± 51)		

Statistical analyses

Statistical analysis title	Intergroup comparison at 24 weeks
Statistical analysis description:	
Comparison of 5,000 IU vitamin D arm and 10,000 IU vitamin D arm to placebo arm.	
Comparison groups	Healthy control - 10,000 IU vitamin D v Healthy control - 5,000 IU vitamin D v Healthy control - placebo

Number of subjects included in analysis	35
Analysis specification	Pre-specified
Analysis type	superiority
P-value	< 0.001
Method	t-test, 2-sided

Statistical analysis title	Intergroup comparison at 24 weeks
Statistical analysis description: Comparison between 5,000 IU vitamin D arm and 10,000 IU vitamin D arm	
Comparison groups	Healthy control - 5,000 IU vitamin D v Healthy control - 10,000 IU vitamin D
Number of subjects included in analysis	24
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.06
Method	t-test, 2-sided

Statistical analysis title	Intergroup comparison at 24 weeks
Statistical analysis description: Comparison of 5,000 IU vitamin D arm and 10,000 IU vitamin D arm to placebo arm.	
Comparison groups	CIS - placebo v CIS - 5,000 IU vitamin D v CIS - 10,000 IU vitamin D
Number of subjects included in analysis	29
Analysis specification	Pre-specified
Analysis type	superiority
P-value	< 0.001
Method	t-test, 2-sided

Statistical analysis title	Intergroup comparison at 24 weeks
Statistical analysis description: Comparison between 5,000 IU vitamin D arm and 10,000 IU vitamin D arm	
Comparison groups	CIS - 5,000 IU vitamin D v CIS - 10,000 IU vitamin D
Number of subjects included in analysis	22
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.02
Method	t-test, 2-sided

Adverse events

Adverse events information

Timeframe for reporting adverse events:	
Adverse events were assessed at each visit (9 visits) during the trial over the entire study period (30 weeks)	
Assessment type	Systematic

Dictionary used

Dictionary name	NA
Dictionary version	NA

Reporting groups

Reporting group title	Healthy control - placebo
Reporting group description:	
Healthy participants randomised to be receiving placebo	
Reporting group title	CIS - placebo
Reporting group description:	
Participants with clinical isolated syndrome randomised to be receiving placebo	
Reporting group title	Healthy control - 5,000 IU vitamin D
Reporting group description:	
Healthy participants randomised to be receiving 5,000 IU vitamin D	
Reporting group title	CIS - 5,000 IU vitamin D
Reporting group description:	
Participants with clinical isolated syndrome randomised to be receiving 5,000 IU vitamin D	
Reporting group title	Healthy control - 10,000 IU vitamin D
Reporting group description:	
Healthy participants randomised to be receiving 10,000 IU vitamin D	
Reporting group title	CIS - 10,000 IU vitamin D
Reporting group description:	
Participants with clinical isolated syndrome randomised to be receiving 10,000 IU vitamin D	

Serious adverse events	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D
Total subjects affected by serious adverse events			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
number of deaths (all causes)	0	0	0
number of deaths resulting from adverse events	0	0	0
Surgical and medical procedures			
Lumbar discectomy			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences causally related to treatment / all	0 / 0	0 / 1	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0

Serious adverse events	CIS - 5,000 IU vitamin D	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D
Total subjects affected by serious			

adverse events			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
number of deaths (all causes)	0	0	0
number of deaths resulting from adverse events	0	0	0
Surgical and medical procedures			
Lumbar discectomy			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences causally related to treatment / all	0 / 0	0 / 0	0 / 0
deaths causally related to treatment / all	0 / 0	0 / 0	0 / 0

Frequency threshold for reporting non-serious adverse events: 0 %

Non-serious adverse events	Healthy control - placebo	CIS - placebo	Healthy control - 5,000 IU vitamin D
Total subjects affected by non-serious adverse events			
subjects affected / exposed	9 / 13 (69.23%)	8 / 9 (88.89%)	6 / 13 (46.15%)
Surgical and medical procedures			
Dental procedure			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Banding haemorrhoids			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
General disorders and administration site conditions			
Temperature			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Weight loss			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	1	0	0
Lightheadedness			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Fatigue			
subjects affected / exposed	0 / 13 (0.00%)	2 / 9 (22.22%)	0 / 13 (0.00%)
occurrences (all)	0	2	0
Shivering			

subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Missed oral contraceptive pill			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Immune system disorders			
Hay fever			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Reproductive system and breast disorders			
Vaginal thrush			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Delayed menstrual cycle			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	1	0	0
Psychiatric disorders			
Anxiety			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Depression			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Investigations			
Colonoscopy			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Nervous system disorders			
Headache			
subjects affected / exposed	1 / 13 (7.69%)	3 / 9 (33.33%)	0 / 13 (0.00%)
occurrences (all)	1	6	0
Tingling/sensory symptoms			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Reduced visual acuity			

subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Neuropathic pain			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Relapse			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Blood and lymphatic system disorders			
Anaemia			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Low iron			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Ear and labyrinth disorders			
Fullness of left ear			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Eye disorders			
Eye strain			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Eye pain			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Gastrointestinal disorders			
Heartburn			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	1 / 13 (7.69%)
occurrences (all)	0	1	1
Diarrhoea			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	2	0	1
Vomiting			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	1	0	0
Nausea			

subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Rectal bleeding			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Abdominal pain			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	1	0	0
Skin and subcutaneous tissue disorders			
Acne			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Rash			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Swelling			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Sebaceous cyst			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Flushing			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Renal and urinary disorders			
Haematuria			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Elevated urea			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Musculoskeletal and connective tissue disorders			
Knee injury			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	1	0	0
Muscular pain			

subjects affected / exposed	0 / 13 (0.00%)	2 / 9 (22.22%)	0 / 13 (0.00%)
occurrences (all)	0	2	0
Bursitis			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Joint injury			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Infections and infestations			
Respiratory tract infection			
subjects affected / exposed	3 / 13 (23.08%)	1 / 9 (11.11%)	1 / 13 (7.69%)
occurrences (all)	4	1	1
Conjunctivitis			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Varicella zoster virus infection			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	0	0	0
Herpes simplex virus infection			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	0 / 13 (0.00%)
occurrences (all)	0	1	0
Urinary tract infection			
subjects affected / exposed	0 / 13 (0.00%)	1 / 9 (11.11%)	1 / 13 (7.69%)
occurrences (all)	0	1	1
Tooth abscess			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Tonsillitis			
subjects affected / exposed	0 / 13 (0.00%)	0 / 9 (0.00%)	1 / 13 (7.69%)
occurrences (all)	0	0	1
Athlete's foot			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	1	0	0
Norovirus infection			
subjects affected / exposed	1 / 13 (7.69%)	0 / 9 (0.00%)	0 / 13 (0.00%)
occurrences (all)	1	0	0

Non-serious adverse events	CIS - 5,000 IU vitamin D	Healthy control - 10,000 IU vitamin D	CIS - 10,000 IU vitamin D
Total subjects affected by non-serious adverse events			
subjects affected / exposed	8 / 11 (72.73%)	10 / 13 (76.92%)	10 / 12 (83.33%)
Surgical and medical procedures			
Dental procedure			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	0	1	0
Banding haemorrhoids			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
General disorders and administration site conditions			
Temperature			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	1	0	0
Weight loss			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Lightheadedness			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Fatigue			
subjects affected / exposed	3 / 11 (27.27%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	3	0	1
Shivering			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	1	0	0
Missed oral contraceptive pill			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Immune system disorders			
Hay fever			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	0	1	0
Reproductive system and breast disorders			

Vaginal thrush			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Delayed menstrual cycle			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Psychiatric disorders			
Anxiety			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	3 / 12 (25.00%)
occurrences (all)	0	1	3
Depression			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	1	0	0
Investigations			
Colonoscopy			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Nervous system disorders			
Headache			
subjects affected / exposed	4 / 11 (36.36%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	5	1	0
Tingling/sensory symptoms			
subjects affected / exposed	2 / 11 (18.18%)	0 / 13 (0.00%)	4 / 12 (33.33%)
occurrences (all)	3	0	5
Reduced visual acuity			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	2 / 12 (16.67%)
occurrences (all)	0	0	2
Neuropathic pain			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	2
Relapse			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	1	0	0
Blood and lymphatic system disorders			
Anaemia			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	0	1	0

Low iron			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Ear and labyrinth disorders			
Fullness of left ear			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Eye disorders			
Eye strain			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Eye pain			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	1	0	0
Gastrointestinal disorders			
Heartburn			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	0	1	0
Diarrhoea			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Vomiting			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Nausea			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Rectal bleeding			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Abdominal pain			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Skin and subcutaneous tissue disorders			
Acne			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	0	1	0

Rash			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Swelling			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Sebaceous cyst			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Flushing			
subjects affected / exposed	2 / 11 (18.18%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	2	0	0
Renal and urinary disorders			
Haematuria			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Elevated urea			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	2	0	0
Musculoskeletal and connective tissue disorders			
Knee injury			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Muscular pain			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	3 / 12 (25.00%)
occurrences (all)	1	0	4
Bursitis			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Joint injury			
subjects affected / exposed	1 / 11 (9.09%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	1	0	0
Infections and infestations			
Respiratory tract infection			
subjects affected / exposed	2 / 11 (18.18%)	5 / 13 (38.46%)	4 / 12 (33.33%)
occurrences (all)	7	5	4

Conjunctivitis			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	0	1	0
Varicella zoster virus infection			
subjects affected / exposed	0 / 11 (0.00%)	1 / 13 (7.69%)	0 / 12 (0.00%)
occurrences (all)	0	1	0
Herpes simplex virus infection			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Urinary tract infection			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Tooth abscess			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	1 / 12 (8.33%)
occurrences (all)	0	0	1
Tonsillitis			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Athlete's foot			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0
Norovirus infection			
subjects affected / exposed	0 / 11 (0.00%)	0 / 13 (0.00%)	0 / 12 (0.00%)
occurrences (all)	0	0	0

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
28 July 2014	Inclusion/Exclusion criteria: Previous treatment with beta-interferons or glatiramer acetate or steroids in the last three months. Participants already taking supplemental vitamin D. In discussion with the study team we felt these changes will improve recruitment without impacting on the scientific integrity of the trial or the safety of the participants. The main reason for the change is that steroids are a mainstay of treatment of acute episodes (relapses) and a 3 month steroid washout was initially proposed to prevent any steroid hangover effect but on review this is too long and unnecessary. We also noted that a number of participants were taking very low doses of vitamin D having read about Clinically Isolated Syndrome on the internet or in multivitamin preparations. Given that there is no way of controlling for dietary vitamin D intake as a number of foods are now fortified it was felt that doses less than 1000IU per day would be noted but should not exclude participation in the trial. The changes are reflected below: Previous treatment with beta-interferons or glatiramer acetate in the last 3 months or steroids in the last 4 weeks. Participants already taking supplemental vitamin D at greater than 1000IU per day. We wish to clarify a typographical error with reference to the age range for controls. On both the synopsis and section 10.3.3 (version 8.0: 14/3/2012) screening procedures the age range is listed as 20-40 years, however under section 10.2.2 (version 8.0: 14/3/2012) inclusion criteria it is listed as 25-40 years. This has been corrected in the current protocol in all sections to read age range 20-40 years.
28 July 2014	Study assessments and procedures (Section 10.3): In the protocol (version 8.0: 14/3/2012) under section 10.3.1 Description of study assessments it states that height and weight will be measured at each visit. This has been amended that height (cm) and weight (kg) will only be assessed at the screening period. This is given the short duration of the study and the fact that there is no adjustment of dosing for weight, repeating this measure at each visit was felt to be unnecessary. In the text it says parathormone will be taken at screening, baseline, week 4, 8, 12, 16, 24 and end of study (week 28-30). This has been amended to show that parathormone will not be taken at week 12. Similarly vitamin D will not be measured at week 12. In the text to ensure uniformity where it refers to urea, electrolytes and creatinine this has been amended to U&E as this is the acronym used by the laboratory to incorporate all these measures and this has been added to the abbreviations section. This section has been re-ordered and rewritten to outline clearly what each study assessment involves (section 10.3.1), study end-point assessments (section 10.3.2) and what assessments are to be carried out at each visit (section 10.3.3) as there were some inconsistencies within the text and table 2: schedule of events it has also been updated. End of study visit was outlined in section 10.4 Definition of end of trial and this has now been moved to section 10.3.3.

28 July 2014	Discontinuation/Withdrawal of subjects from study treatment (Section 10.4): The current protocol (version 8.0: 14/3/2012) reads: Patients who discontinue the study will be requested to attend for all remaining study visits including end-point and end of study assessments. Patients who become pregnant will not have any endpoints assessed (MRI, Cliniical). The only exception to this requirement is when a subject withdraws consent for all study procedures. A modified visit schedule has been proposed for patients who discontinue the study drug. It is proposed that these patients attend an end of treatment visit within 2 weeks of stopping the medication and an end of study visit 4-6 weeks later. These patients will no longer require immunology or MRI scanning as part of the study protocol as these will be impossible to interpret in relation to the study objectives due to potential confounders (ie pregnancy, commencement of DMT's).
28 July 2014	Serious adverse event (Section 12.1.3): Planned hospitalisation for an elective procedure unrelated to the study drug does not need to be reported as an SAE.

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

None reported

Online references

http://www.ncbi.nlm.nih.gov/pubmed/28975037