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Randomized placebo-controlled study of lovastatin in children with neurofibromatosis type 1

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Supplemental data at Neurology.org

ABSTRACT

Objective: To assess the efficacy of lovastatin on visuospatial learning and attention for treating cognitive and behavioral deficits in children with neurofibromatosis type 1 (NF1).

Methods: A multicenter, international, randomized, double-blind, placebo-controlled trial was conducted between July 2009 and May 2014 as part of the NF Clinical Trials Consortium. Children with NF1 aged 8–15 years were screened for visuospatial learning or attention deficits (n = 272); 146 children demonstrated deficits at baseline and were randomly assigned to lovastatin (n = 74; 40 mg/d) or placebo (n = 70). Treatment was administered once daily for 16 weeks. Primary outcomes were total errors on the Cambridge Neuropsychological Test Automated Battery Paired Associate Learning task (visuospatial learning) and the Score subtest from the Test of Everyday Attention for Children (sustained attention). Secondary outcomes measured executive function, attention, visuospatial skills, behavior, and quality of life. Primary analyses were performed on the intention-to-treat population.

Results: Lovastatin had no significant effect on primary outcomes after 16 weeks of treatment: visuospatial learning (Cohen $d = -0.15$, 95% confidence interval −0.47 to 0.18) or sustained attention (Cohen $d = 0.19$, 95% confidence interval −0.14 to 0.53). Lovastatin was well tolerated, with no increase in reported adverse events compared to placebo.

Conclusions: Lovastatin administered once daily for 16 weeks did not improve visuospatial learning or attention in children with NF1 and is not recommended for amelioration of cognitive deficits in this population.

ClinicalTrials.gov identifier: This study was registered at ClinicalTrials.gov (NCT00853580) and Australian New Zealand Clinical Trials Registry (ACTRN12607000560493).

Classification of evidence: This study provides Class I evidence that for children with NF1, lovastatin does not improve visuospatial learning or attention deficits. *Neurology*® 2016;87:2575–2584

GLOSSARY

CANTAB = Cambridge Neuropsychological Test Automated Battery; **CI** = confidence interval; **GABA** = γ-aminobutyric acid; **HMG-CoA** = 3-hydroxy-3-methylglutaryl coenzyme; **LDL** = low-density lipoprotein; **mITT** = modified intention-to-treat; **NF1** = neurofibromatosis type 1; **RAS** = Rat Sarcoma protein; **UAB** = University of Alabama.

With a birth incidence of 1 in 2,700, neurofibromatosis type 1 (NF1) is one of the most common autosomal-dominant neurodevelopmental disorders to affect the human nervous system.[1] It is caused by a mutation in the *NF1* gene encoding neurofibromin, a negative regulator of the Rat Sarcoma protein (RAS)-bound intracellular signaling cascade. Although NF1 is characterized by

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diverse cutaneous, neurologic, skeletal, and neoplastic manifestations, the most common clinical feature is cognitive impairment, with 80% of school-aged children presenting with moderate to severe deficits in at least one cognitive domain.[2] While intelligence is usually only mildly affected, specific impairments in attention, executive function, visuospatial perception, and spatial memory are common.[2,3]

Mice with a heterozygous inactivating mutation in the *Nf1* gene ($Nf1^{+/-}$) have been used to model the pathology underlying the human cognitive phenotype. Spatial learning and attention impairments have been associated with elevated RAS activity, increased activity-dependent γ-aminobutyric acid (GABA) release, and reduced synaptic plasticity. Pharmacologic reduction of RAS activity with lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, normalized synaptic plasticity and rescued the behavioral phenotype in $Nf1^{+/-}$ mice, providing a rationale for human clinical trials.[4]

Initial trials using statin medications in children with NF1 have been inconclusive. While 2 randomized controlled trials of simvastatin reported no treatment effect on cognitive outcomes,[5,6] studies evaluating lovastatin have been more promising. An initial open-label phase I trial of lovastatin demonstrated normalization of functional connectivity within the default mode network[7] with accompanying improvements in memory and attention.[8] More recently, results from a small randomized controlled trial in children and adults with NF1 reported beneficial effects of lovastatin on learning and memory.[9]

In the current study, we tested the hypothesis that 16 weeks of lovastatin will result in cognitive, behavioral, and quality-of-life improvements for children with NF1. The safety profile of lovastatin was also evaluated. This is the largest statin trial in NF1 and the first to limit participation to patients with a learning or attention impairment at baseline.[4]

METHODS

Classification of evidence. The primary research question was, Is lovastatin effective for treating visuospatial learning or attention deficits in children with NF1? This study provides Class I evidence that lovastatin 40 mg daily does not improve visuospatial learning or attention deficits in children with NF1 aged 8 to 15 years after 16 weeks of treatment.

Study design. This was a multicenter, double-blind, randomized, placebo-controlled, parallel-group trial conducted from July 2009 to May 2014 with participants recruited from 1 Australian and 10 US academic clinics affiliated with the NF Clinical Trials Consortium.

Participants and randomization. Patients were diagnosed by expert physicians according to NIH clinical diagnostic criteria for NF1,[10] were between 8 and 15 years of age at the time of screening, and demonstrated impaired performance on at least one primary outcome measure (≥1 SD below the population mean). Exclusion criteria included full-scale IQ < 70, symptomatic CNS pathology, significantly impaired vision/hearing, insufficient comprehension of English, low baseline total cholesterol (<90 mg/dL), and contraindicated medication to lovastatin. Children on psychotropic medication were initially excluded; however, in response to a slower-than-expected recruitment rate, the protocol was amended to include patients on a stable dose of psychostimulant medication (1 month before screening and for study duration). The lower age limit was reduced from 10 to 8 years at the same time.

Randomization was stratified by site with a 1:1 allocation using random block sizes of 4. The sequence was created by an independent statistician at the University of Alabama at Birmingham (UAB) using SAS (version 9.2). Only staff members at the UAB Data Center were unblinded and advised site pharmacists of the identification kit number to be allocated to each patient. Patients, investigators, and study coordinators were blinded to treatment assignment.

Study medication. Eminent Services Corporation (Frederick, MD) synthesized and encapsulated lovastatin and provided identical placebo. After a 2-week titration period of 20 mg once nightly, the dose increased to a fixed dose of 40 mg once nightly for weeks 3 through 16. Participants were instructed to swallow capsules whole.

Standard protocol approvals, registrations and patient consents. This study was conducted in accordance with Good Clinical Practice guidelines. Informed consent was obtained from all parents/guardians, and age-appropriate assent was obtained. The protocol was approved by institutional review boards at each site and registered at clinicaltrials.gov (NCT00853580) and Australian New Zealand Clinical Trials Registry (ACTRN12607000560493).

Study visits. Participants underwent screening procedures consisting of a medical history, a physical examination, laboratory tests, and cognitive assessment. Follow-up visits were scheduled at 4-week intervals and included a physical examination, adverse events (AEs) review, and pill count. Compliance was defined as taking at least 80% of medication over the 16-week period. AEs were documented and assessed according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 3). Clinical laboratory evaluations were conducted at each visit. Hematologic assessments included hemoglobin, hematocrit, leukocyte count, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelet count. Blood chemistry tests included electrolytes and glucose, as well as assessments of liver function (aspartate aminotransferase, alanine aminotransferase, and total bilirubin), renal function (creatinine and blood urea nitrogen), and muscle inflammation (creatine phosphokinase). Lipid profiles included high-density lipoprotein, low-density lipoprotein (LDL), triglycerides, and total cholesterol.

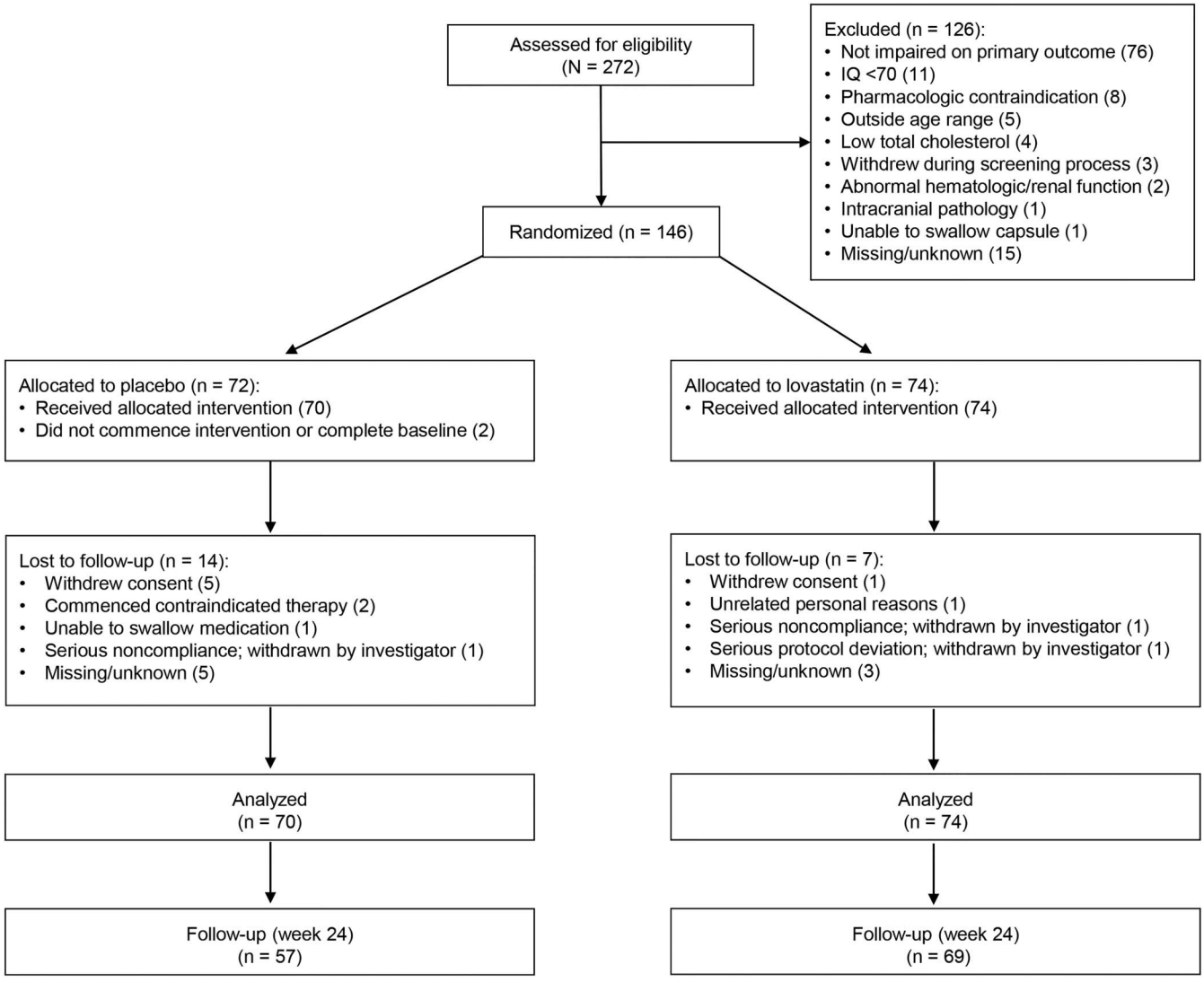
Efficacy outcomes were administered by psychologists at baseline and week 16. Participants were reassessed at week 24 to evaluate potential carryover effects.

Outcomes. Two primary outcomes were selected on the basis of cognitive abilities responsive to statin medication in preclinical trials.[4] Visuospatial learning was assessed with the Paired Associate Learning test from the Cambridge Neuropsychological Test Automated Battery (CANTAB),[3] and Score from the Test of Everyday Attention for Children[11] measured sustained attention. Both primary outcomes capture high basal rates of impairment in NF1.[2,3] Secondary outcomes assessed the effects of lovastatin more broadly across cognitive domains frequently affected in NF1, including attention, executive function, and visuospatial skills. Measures included Sky Search, Sky Search DT, and Creature Counting from the Test of Everyday Attention for Children[11]; the Conners Continuous Performance Task-II (commission and omission errors)[12]; Controlled Oral Word Association Test[13]; Judgment of Line Orientation task[14]; Wechsler Object Assembly[15]; and the following tests from the CANTAB: Spatial Working Memory (total between search errors), Stockings of Cambridge (mean number of moves, hardest problem), and Stop Signal Task (stop signal reaction time, last half). Parents and children completed questionnaires assessing emotional/behavioral functioning (Behavior Assessment System for Children, Second Edition; internalizing behaviors)[16] and quality of life (Pediatric Quality of Life Inventory; psychosocial score).[17] Parent ratings of functional executive behaviors were also obtained (Behavior Rating Inventory of Executive Function Global Executive Control[18]; appendix e-1 at Neurology.org).

Statistical analyses. To detect a clinically meaningful difference of half an SD with a 2-tailed significance level of $p = 0.05$ and power of 85%, a sample of 146 patients was required. A planned interim analysis was performed after 64 patients had completed the trial. Stopping rules were in place for futility, efficacy, and safety. The probability of rejecting the null hypothesis was assessed with a Lan-Simon-Halperin conditional power test. Using an assumed effect size of 0.5 (Cohen *d*) and an α level of 0.05, the trial would have been stopped if conditional power fell below 10%. Stopping criteria were not reached, and an independent Data and Safety Monitoring Board authorized the study to continue.

Primary analyses of efficacy and safety were performed on the baseline to posttreatment data of the modified intention-to-treat (mITT) population. The safety analysis included all randomized patients who received at least one dose of lovastatin or placebo. All patients who completed the baseline assessments were included in the efficacy analysis. Missing posttreatment values were imputed

Figure 1 **Patient screening, enrolment, follow-up, and analysis of A Randomized Placebo-Controlled Study of Lovastatin in Children With Neurofibromatosis Type 1 (STARS) trial**



for participants who did not complete the week 16 assessment with linear regression with age, sex, and the participants' baseline values used as predictors (20 imputations). A fully evaluable analysis was also conducted that included participants who completed both the baseline and posttreatment assessments and demonstrated no major protocol violations such as medication compliance below 80%.

For continuous efficacy variables, analysis of covariance was conducted to compare groups after treatment with adjustment for baseline scores, which minimizes potential regression to the mean.[19] Separate models were constructed for mITT and fully evaluable populations. A standardized measure of effect (Cohen *d*) of lovastatin on endpoints adjusted for baseline values was calculated using the mITT population. Because cholesterol levels have been linked to cognitive performance,[20] we investigated an exploratory multivariable model, comparing fully evaluable treatment groups after treatment and adjusting for baseline cognitive performance and baseline total cholesterol. Reliable change index analysis was also conducted on primary outcomes.[21] Possible carryover effects were examined by comparing cognitive scores at week 24, adjusting for posttreatment scores with analysis of covariance. All statistical tests were 2 tailed with the level of significance set at $p < 0.05$. Analyses were conducted with SAS (version 9.4), and Stata IC (version 13.1).

RESULTS From July 2009 to May 2014, 272 children were screened. Of these, 126 children were ineligible (figure 1). A total of 74 were randomized to receive lovastatin, and 72 received placebo. Baseline characteristics were similar between treatment conditions (table 1). Two participants allocated to placebo withdrew before beginning treatment and were not included in the mITT analysis, resulting in a total sample of 144 participants.

Table 1 Baseline characteristics of participants in A Randomized Placebo-Controlled Study of Lovastatin in Children With Neurofibromatosis Type 1 (STARS)

	Lovastatin	Placebo
No.	74	72[a]
Age, y	11.5 (2.25)	11.7 (1.95)
Sex, n (%)		
Male	43 (58)	45 (62)
Female	31 (42)	27 (38)
Race, n (%)		
White	61 (82)	56 (78)
Black	6 (8)	11 (15)
Other	7 (10)	5 (7)
Height, cm	144.5	145.0 (14.0)
Weight, kg	40.6 (13.6)	39.6 (14.4)
Head circumference, cm	55.9 (2.3)	55.6 (2.5)
FSIQ	91.4 (14.1)	89.2 (13.0)
Impairment of primary outcome, n (%)		
Visuospatial learning (PAL)	22 (30)	28 (40)
Attention (Score)	70 (95)	64 (91)
Psychostimulant medication, n (%)	11 (15)	11 (16)
Total cholesterol, mg/dL	160.1 (27.3)	159.4 (27.4)
LDL cholesterol, mg/dL	90.9 (24.8)	91.5 (24.2)
HDL cholesterol, mg/dL	54.4 (12.8)	54.5 (12.2)

Abbreviations: FSIQ = Full Scale Intelligence Quotient; HDL = high-density lipoprotein; LDL = low-density lipoprotein; PAL = Paired associate learning.
Data are mean (SD) or number (%).
[a] Two participants were excluded from efficacy and safety analyses because of failure to complete baseline assessment.

Eighty percent (56 of 70) of participants randomized to placebo and 91% (67 of 74) of participants randomized to lovastatin completed the week 16 assessment. There was no differential dropout by treatment group ($\chi^2 = 3.21$, $p = 0.07$). Missing week 16 values were individually imputed for 21 participants (14 placebo, 7 lovastatin). Median compliance per patient was 97.3% (interquartile range 92.4%–98.8%). For the fully evaluable sample, 2 participants were excluded for compliance rates <80%, resulting in 57 placebo and 67 lovastatin patients.

mITT analysis revealed no significant effect of lovastatin on the primary outcomes of visuospatial learning (Cohen $d = -0.15$, 95% confidence interval [CI] −0.47 to 0.18) or sustained attention (Cohen $d = 0.19$, 95% CI −0.14 to 0.53) after 16 weeks of treatment (table 2). There was no effect of lovastatin on any secondary outcome except Stockings of Cambridge, in which the lovastatin group demonstrated superior spatial planning compared with the placebo group (Cohen $d = -0.34$, 95% CI −0.68 to −0.01; figure 2). Analysis of the fully evaluable population produced similar results, with the lovastatin group demonstrating improved spatial planning after treatment (Cohen $d = -0.48$, 95% CI −0.85 to −0.10), indicating that multiple imputation for missing values had no unexpected influence on efficacy outcomes (table e-1). Multivariable analysis on the fully evaluable population adjusted for baseline levels of total cholesterol revealed similar results, with the lovastatin group exhibiting superior spatial planning compared with the placebo participants (Cohen $d = -0.47$, 95% CI −0.75 to −0.10). No other cognitive outcome significantly improved after treatment (data not shown). Reliable change index analysis revealed that the proportion of patients who improved in the lovastatin group was not significantly different from those on placebo (table e-2).

Analysis of the mITT population demonstrated no effects of group for any test on the week 24 follow-up data after adjustment for posttreatment scores (all $p > 0.056$; table e-3). Again, the fully evaluable data set reported identical results (data not shown).

Lovastatin was well tolerated. There was a mean of 4.3 (SD 4.8) AEs per participant, with comparable incidences of AEs in the placebo condition (table 3). At least one AE was reported by 65 of 74 (88%) patients in the lovastatin group and 63 of 72 (88%) patients in the placebo group.

Table 2 Efficacy outcomes at baseline and after treatment (week 16) for the intention-to-treat population in A Randomized Placebo-Controlled Study of Lovastatin in Children With Neurofibromatosis Type 1 (STARS)

	Control					Lovastatin							
		Baseline		Week 16			Baseline		Week 16				
Outcome	n	Mean	SD	Mean	SD	n	Mean	SD	Mean	SD	β	95% CI	p
Primary outcomes													
PAL[a]	68	17.0	15.4	11.7	11.7	73	15.8	22.4	10.3	8.4	−1.14	−4.53 to 2.24	0.51
Score[a]	66	5.7	2.4	6.8	2.5	73	6.0	2.0	7.3	2.1	0.36	−0.36 to 1.08	0.33
Secondary outcomes													
SWM[a]	68	47.9	16.2	41.6	17.0	72	47.0	15.9	40.4	17.2	−0.41	−4.89 to 4.06	0.86
SOC[a]	66	8.0	1.3	7.9	1.4	71	7.9	1.4	7.3	1.4	−0.53	−1.04 to −0.03	0.04
SST[a]	66	237.2	75.9	227.0	93.7	70	264.7	98.7	227.2	87.2	−14.72	−44.28 to 14.85	0.33
Sky search[a]	66	5.0	2.1	4.4	2.1	74	4.4	1.7	4.0	2.0	0.00	−0.59 to 0.59	0.99
Sky search DT[a]	66	9.6	18.6	6.6	11.6	73	9.9	15.5	7.0	15.6	0.32	−4.54 to 5.17	0.90
Creature counting[a]	66	3.8	2.3	4.6	1.8	74	4.0	2.2	4.5	1.9	−0.25	−0.81 to 0.30	0.37
CPT-II omission errors[b]	64	62.1	17.2	64.1	18.4	74	57.5	12.4	58.8	16.6	−1.67	−6.41 to 3.08	0.49
CPT-II commission errors[b]	64	56.9	6.9	55.5	7.4	74	54.7	10.5	54.2	10.5	0.22	−2.25 to 2.68	0.86
ADHD inattentive symptoms[b]	66	64.0	14.6	61.1	13.3	74	66.2	12.6	59.5	13.2	−3.05	−6.57 to 0.48	0.09
ADHD hyperactive/impulsive symptoms[b]	66	62.9	15.6	62.3	16.7	74	65.5	14.2	62.3	15.4	−1.92	−6.10 to 2.27	0.37
COWAT[a]	66	21.2	8.6	22.6	8.9	74	22.2	8.9	22.2	9.1	−1.32	−3.25 to 0.61	0.18
BRIEF GEC[b]	65	61.4	11.9	58.8	12.5	72	63.5	11.5	59.2	13.2	−1.52	−4.10 to 1.06	0.25
Judgment of line orientation test[a]	65	14.6	5.7	16.7	6.4	73	15.0	6.6	17.2	7.0	0.12	−1.43 to 1.67	0.88
Object assembly[c]	66	6.9	3.0	7.5	3.3	74	6.6	3.1	7.8	3.6	0.58	−0.32 to 1.48	0.20
Internalizing behaviors, parent report[b]	63	53.8	11.8	52.5	11.4	73	55.2	12.6	52.6	12.6	−0.97	−3.84 to 1.89	0.50
Internalizing behaviors, self-report[b]	63	51.2	8.7	49.2	8.5	72	50.9	10.2	47.9	10.0	−1.08	−3.26 to 1.10	0.33
Psychosocial quality of life, parent report[d]	63	64.6	18.1	68.1	16.6	72	62.8	15.7	69.2	16.0	2.34	−2.11 to 6.79	0.30
Psychosocial quality of life, self-report[d]	64	62.6	16.0	67.3	17.0	70	67.2	17.0	70.0	18.1	−0.81	−5.37 to 3.74	0.73

Abbreviations: ADHD = attention-deficit/hyperactivity disorder; BRIEF GEC = Behavior Rating Inventory of Executive Function Global Executive Composite; COWAT = Controlled Oral Word Association Test; CPT-II = Continuous Performance Test Second Edition; DT = Divided Attention; PAL = Paired Associated Learning; SOC = Stockings of Cambridge; SST = Stop Signal Task; SWM = Spatial Working Memory.

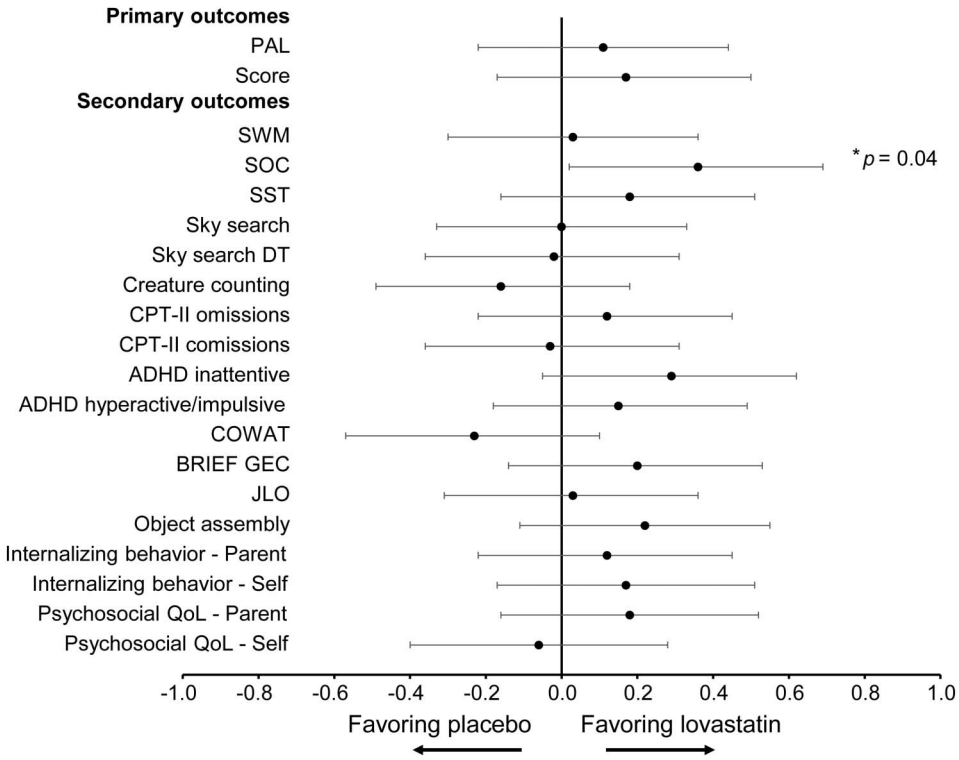
[a] Raw score.

[b] T score.

[c] Scaled score.

[d] Summary score.

Figure 2 Treatment effects on efficacy endpoints (week 16)



The standardized effect (adjusted Cohen *d*) of lovastatin on primary and secondary endpoints adjusted for baseline values. Results have been adjusted to uniformly indicate directionality of effect with the 95% confidence interval. ADHD = attention-deficit/hyperactivity disorder; BRIEF GEC = Behavior Rating Inventory of Executive Function Global Executive Composite; COWAT = Controlled Oral Word Association Test; CPT-II = Continuous Performance Test Second Edition; DT = Divided Attention; JLO = Judgment of Line Orientation; PAL = Paired Associated Learning; QoL = quality of life; SOC = Stockings of Cambridge; SST = Stop Signal Task; SWM = Spatial Working Memory.

Most AEs were mild or moderate (625 of 632, 99%).

After 16 weeks of lovastatin treatment, mean total cholesterol levels were reduced by 28.5 mg/dL (Cohen $d = -1.54$, 95% CI −1.94 to −1.13), a decrease of 15% over baseline levels (table e-4). As expected, this was due to a decrease in LDL, which was reduced by 25.6 mg/dL (Cohen $d = -1.61$, 95% CI −2.01 to −1.20), a decrease of 28% over baseline levels. Total cholesterol and LDL levels remained within normal limits throughout the study in all patients. The lipid profile of the placebo group did not change.

DISCUSSION This is the largest randomized, placebo-controlled, double-blind study of lovastatin as a targeted treatment for cognitive deficits in children with NF1. Our results showed that 16 weeks of lovastatin had no effect on the primary outcomes of visuospatial learning or attention. There were minimal indications of efficacy on secondary outcomes except for improvement on a computerized test of spatial planning (Stockings of Cambridge). Human lesion and functional neuroimaging studies suggest that performance on this task maps to the prefrontal cortex.[22] Because neurofibromin has been shown to regulate prefrontal inhibitory networks through activity-dependent GABA release in *Nf1*$^{+/-}$ mice, it is appealing to conclude that the mechanism of action of lovastatin is similar; however, because lovastatin had no effect on other endpoints regulated by frontostriatal circuitry such as spatial working memory and multiple uncorrected statistical comparisons were performed, this finding should be interpreted with caution.

High medication compliance and consistent findings between the mITT and fully evaluable patient populations indicate that the results are not due to poor compliance. Lovastatin was well tolerated in children with NF1. There were no unexpected AEs, and comparable side effects were reported in both treatment arms. Lovastatin reduced LDL and total cholesterol, but they remained within normal levels.

The effects of lovastatin in the *Nf1*$^{+/-}$ mouse model have pointed to specific endpoints for clinical trials, which we used in this study: visuospatial learning and attention. Despite the promise of preclinical data, the translation of these effects in randomized-controlled trials has been challenging. Our findings are consistent with 2 previous trials that reported no

Table 3 Summary of adverse events by severity, relationship, and body system

		Lovastatin (n = 74)		Placebo (n = 70)
AEs per participant, n[a]		4.3 (4.8)		4.3 (4.8)
Severity,[a] n (%)				
Mild		273 (49)		278 (51)
Moderate		41 (55)		33 (45)
Severe		5 (83)		1 (17)
Life-threatening		1 (100)		0 (0)
Death		—		—
Relationship,[a] n (%)				
Unrelated		127 (52)		118 (48)
Unlikely		118 (51)		114 (49)
Possible		74 (50)		75 (50)
Probable		1 (17)		5 (83)
Definite		—		—
	Grade 1–2	Grade 3+	Grade 1–2	Grade 3+
AE by body system[b]				
General	97 (45)	1 (100)	118 (55)	—
Cardiovascular	3 (50)	—	3 (50)	—
Digestive	50 (52)	—	46 (48)	—
Hematologic	20 (54)	2 (100)	17 (46)	—
Hepatic/biliary	20 (43)	2 (100)[c]	26 (57)	—
Musculoskeletal	14 (50)	1 (100)	14 (50)	—
Nervous	16 (37)	—	27 (63)	1 (100)
Respiratory	54 (60)	—	36 (40)	—
Skin/appendages	19 (50)	—	19 (50)	—
Special senses	13 (72)	—	5 (28)	—
Urogenital	8 (100)	—	—	—

Abbreviation: AE = adverse event.
Grade 1 = mild or asymptomatic AE not requiring intervention; grade 2 = moderate AE requiring minimal or noninvasive intervention; grade 3 = severe or medically significant AE not immediately threatening life; grade 4 = life-threatening requiring urgent intervention; and grade 5 = death related to AE.
[a] Data are mean (SD).
[b] Data are number (percent frequency per treatment group).
[c] Included one participant with a life-threatening AE that was due to elevated creatine phosphokinase. Administration of study drug was stopped for 7 days, and creatine phosphokinase levels normalized. Participant restarted study drug at a reduced dose of 50% and completed the study. Relationship with lovastatin was possible.

effect of simvastatin on cognitive performance in patients with NF1. These studies did not assess the efficacy of the same agent used in preclinical trials and did not exclude children with normal cognitive performance at baseline. We restricted participation to patients with baseline deficits on primary outcomes, thereby ensuring that we were treating only patients who might benefit from drug treatment. We also excluded patients with an intellectual disability to confirm that we were treating specific deficits in visuospatial learning or attention, which resemble behaviors and pathophysiologic mechanisms rescued in preclinical trials.[4] In contrast to our findings, results from a recent study of adults (n = 30; 80 mg/d) and children (n = 14; 40 mg/d) with NF1 for 14 weeks suggested possible beneficial effects of lovastatin on working memory, verbal memory, and adult self-reported internalizing problems.[9] Unfortunately, a high dropout rate (27%) resulted in only 32 evaluable patients (15 placebo, 17 lovastatin), reducing statistical power. Failure to analyze the ITT population and to adjust for baseline performance may also have resulted in overestimation of the effect size and led to nonrandom attrition of participants. Our sample size ensured adequate power to detect a medium treatment effect, and results indicate with

a reasonable degree of confidence that lovastatin is not an effective treatment for cognitive or behavioral deficits in children with NF1.

Reasons underlying the negative results of the current study are not immediately clear. Our dose was based on the maximum recommended daily dose for treatment of hypercholesterolemia in children. This dose achieved significant inhibition of the HMG-CoA reductase pathway in the liver, as evidenced by considerable reductions in blood cholesterol levels. While lovastatin is lipophilic and crosses the blood-brain barrier, it is possible that our dose was not sufficient to produce a therapeutic effect on human brain function. Although indirect evidence of improved memory and functional connectivity from a 12-week phase I study suggests that our dose may have been sufficient to inhibit RAS–mitogen-activated protein kinase activity,[7,8] this small open-label study was not powered for efficacy. Increasing the dose, especially in a pediatric population, would likely increase the risk of AEs, and the effect of statins on hormones critical in sexual development is unknown.

The adequacy of *Nf1*$^{+/-}$ mice used in preclinical studies to model the complexity of the human disease is also unclear. *Nf1*$^{+/-}$ mice exhibit no overt evidence of the structural neuroanatomic defects reported in humans,[23,24] including a large corpus callosum, volumetric abnormalities, and deficits in neuronal connectivity. These developmental abnormalities contribute to cognitive deficits and may have precluded a therapeutic response to lovastatin. It is critical to validate the relative contributions of neurofibromin, RAS, GABAergic neurotransmission, and deficient synaptic plasticity to the human phenotype. Although transcranial simulation and magnetic resonance spectroscopy studies have initiated this evidence base, identification of biomarkers linking neurofibromin expression to cognitive outcomes is still required.[25,26] Validated markers would enable pilot studies to demonstrate proof of principle and dose refinement of promising treatments to optimize clinical trial design, and they could prove useful in identifying young children at risk of cognitive problems, which might allow earlier treatment when the brain is potentially more responsive to change.

Our study is not without limitation. We restricted enrollment to patients aged 8 to 15 years because of the absence of safety data for lovastatin in younger children. Therefore, a potential therapeutic effect of lovastatin cannot be excluded in younger children, and an ongoing clinical trial is evaluating simvastatin on autism symptoms in children with NF1 aged 5 to 8 years (European Clinical Trials Database number 2012-005742-38). It is also possible that a 16-week treatment period was not long enough to observe clinically significant effects on cognitive performance. In selecting our treatment duration, we relied on preclinical data suggesting that lovastatin normalized plasticity and behavioral impairments in *Nf1* mice within days and findings from a phase I lovastatin trial suggesting improvements in functional connectivity 12 weeks after treatment.[7,8] Finally, while many of the cognitive outcomes used in our study have a long history of successfully detecting change in clinical trials, it is possible that some were not sufficiently sensitive within a pediatric NF1 context. Future studies should establish NF1-specific normative data and test-retest reliability to guide future selection of cognitive endpoints.

This is a negative study. Lovastatin does not improve visual learning or attention, and the finding of a benefit to spatial planning is modest at best. The current findings are not sufficient to recommend lovastatin as a treatment for cognitive deficits in children with NF1. Future research with second-generation mouse models that model the full spectrum of pathophysiologic mechanisms of NF1 in the human condition will be required to identify more effective treatments.

AUTHOR CONTRIBUTIONS

J.M.P., B.B., K.N.N., M.T.A., N.J.U., G.A.G., P.L.W., J.T., R.J.P., and B.K. contributed to the study concept and design. All authors except for A.C., S.J.C.H., G.C., P.L.W., and A.J.S. were involved in data acquisition for the study. A.C. and S.J.C.H. performed the statistical analyses, and G.C. provided statistical advice. A.J.S. assisted with the conception of the study and provided the stimulus for the formation of clinical research networks to test lovastatin in patients with NF1. J.M.P., B.B., and K.N.N. drafted the manuscript, which was critically revised for intellectual content and finally approved by all coauthors.

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