Central Manchester University Hospitals NHS
NHS Foundation Trust

Phase II triple blind placebo controlled RCT of simvastatin treatment for autism in young children with Neurofibromatosis Type 1

Short title: Simv**A**statin in **N**eurofibromatosis **T**ype 1-**A**utism (SANTA)

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Chief Investigator: Professor Jonathan Green

Funder: CMFT

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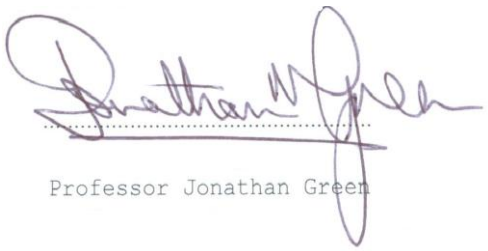
SAE/SUSAR reporting: Serious Adverse Events must be reported to the study sponsor within 24 hours of first knowledge. The study adverse event form should be completed and sent via fax to 0161 276 5766 or email to Adverse.Events@cmft.nhs.uk

SIGNATURES/PROTOCOL APPROVAL

Simv**A**statin in **N**eurofibromatosis Type 1-**A**utism (SANTA)

This document describes the SANTA trial and provides information about procedures for entering participants into it. The protocol should not be used as a guide for the treatment of participants outside the trial. Every care was taken in drafting this protocol, but corrections or amendments may be necessary, care must be taken to use the most up to date and approved version. This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) regulations 2004 *(if appropriate)* and ICH Good Clinical Practice guidelines. The trial will be conducted in compliance with the protocol, the Data Protection Act (DPA Z6364106), the Declaration of Helsinki, Human Tissue Act (2004), the Research Governance Framework (2005) and other regulatory requirements as appropriate.

Chief Investigator – Professor Jonathan Green, University of Manchester, Royal Manchester Children's Hospital
I Professor Jonathan Green as Principal Investigator for the SANTA trial to be conducted at Central Manchester and Manchester University Hospitals NHS Trust confirm that I will be responsible to ensure that all members of the local clinical trial team are appropriately trained on the trial protocol and have the relevant qualifications and experience to carry out their role in accordance with the trial protocol.



Professor Jonathan Green

Sponsor Representative: Dr Lynne Webster, CMFT

Table of Contents

LIST OF ABBREVIATIONS

Commonly used abbreviations – add or delete as applicable:

AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EU	European Union
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
ISF	Investigator Site File
MA	Marketing Authorisation
MHRA	Medicines and Healthcare products Regulatory Agency
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person for release of trial drug
RCT	Randomised Control Trial
REC	Research Ethics Committee
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
UAR	Unexpected Adverse Reaction

TRIAL SUMMARY

Title: Phase 2 triple blind placebo controlled RCT of simvastatin treatment for autism in young children with Neurofibromatosis Type 1.

Short title: SimvAstatin in Neurofibromatosis Type 1-Autism (SANTA).

Design:
Single site, 2 parallel groups, triple-blind (clinician/patient/assessor) phase II randomised trial of simvastatin (n=16) versus placebo (n=16) in a dose titration design.

Objectives:
The research aims are to test:

- The feasibility, safety and acceptability of treatment with simvastatin to children with NF1 and Autism Spectrum Disorder.
- The feasibility and acceptability of the assessment protocol.
- Treatment effects on intermediate and endpoint behavioural phenotype measures and imaging parameters.

Endpoints:
We will measure functional imaging parameters; blinded parent and clinician rated autism and behavioural symptoms; participant acceptability and adverse effects at baseline, 12 week primary endpoint and 16 week post-treatment. Intermediate assessment at 4 weeks will be made of physiological and biochemical parameters for adverse effects.

Cohorts:
This is a phase II feasibility study of children with NF1 autism. A total of 32participants (children between 4.5-10.5 years inclusive) will be identified and recruited from the NF1 clinics in Manchester, Liverpool, Leeds and Newcastle and through advertisements via the NeuroFoundation and NF wellbeing research group newsletters.

Inclusion criteria:
These criteria will be part of the screening assessments:
Children between 4.5-10.5yrs meeting diagnostic criteria for

i) Neurofibromatosis type 1 (National Institute of Health diagnostic criteria for NF1) and
ii) Autism (above diagnostic thresholds for ASD on ADI-R and ADOS-G).

Trial Treatment and methods:

After baseline monitoring, children will be randomised to receive either placebo or simvastatin at 0.5 mg/kg/day in a single daily dose for 4 weeks, and their status reviewed. Those showing no significant adverse effects (e.g. elevation of Liver Function Tests or Creatine Kinase) will be escalated to a dose of 1 mg/kg/day to a maximum of 30 mg/day in a single daily dose for a further 8 weeks. The trial intervention will end at 12 weeks. The participants will be followed up via a telephone call at 16 weeks (4 weeks after the end of the trial intervention) to monitor further progress, acceptability and any delayed adverse effects.

Summary diagram

	Screening & Baseline -6 to 0 weeks	**Day 1 Week 0**	**Dose titration Week 4**	**Treatment end-point Week 12**
Screening assessment	**x**			
Informed consent	**x**			
Inclusion/exclusion criteria	**x**			
Randomisation		**X**		
Allocation of study number	**x**			
Blood tests for adverse events		**X**	**X**	**X**
Blood sample for genotyping			**X**	
Blood sample for P-MAPK activity		**X**		**X**
Behavioural phenotype measures		**X**	**X**	**X**
Brain imaging		**x**		**X**
Dose titration			**X**	

Blood tests for monitoring adverse events will include lipids, liver function tests (LFT), Urea & Electrolytes (U&E) and Creatine Kinase (CK).
The behavioural phenotype measures will include Aberrant Behaviour Checklist-Community (ABC-C); Patient defined target symptoms and Clinician Global Impression of Improvement (CGI) and Conners Parent Rating Scale – Research (CPRS-R).

Post-intervention evaluation at 16 weeks(+/- 14 days) will consist of audio-recorded telephone follow-up screening for adverse events and qualitative interviews with parents on experience of trial, intervention, assessments including imaging procedures.

Trial duration per participant:
The trial duration per participant will be 16(+/- 14 days)weeks.

Estimated total trial duration:
The total trial duration will be 18 months.

Total number of participants planned:
32 participants will be included in this study randomised to either treatment or placebo arm.

Additional trials/ Sub-trials
Blood samples collected, as part of this study will be used to carry out the following sub-trials:

- PMAPK level as a peripheral biomarker for cognitive deficits of NF1: The aim of this exploratory study is to ascertain pre and post PMAPK levels as a potential biomarker for simvastatin treatment effects
- NF1 mutational analysis and evaluation of genotype-phenotype correlation: The aim of this study is to look at the mutations of the NF1 gene and correlate that with the clinical ASD phenotype.

INTRODUCTION

Background

Neurofibromatosis Type 1 and Autism Spectrum Disorder

Autism Spectrum Disorders (ASD) is a pervasive developmental disorder characterised by impairments in reciprocal social interaction, social communication combined with restricted interests and rigid, repetitive behaviours, with onset in early childhood. The prevalence of ASD in the general population is the region of 1% with boys being at increased risk.[1] It is a major public health concern because of its early onset, lifelong persistence, associated co morbidities and impairments.

The importance of genetic contribution in the aetiology of ASD is well known. The concordance of autism in monozygotic twin pairs is between 36%-91% compared with 10% in dizygotic pairs leading to heritability estimates of over 90%. Despite these heritability estimates, there is a lack of consensus about the neuropathological and neurophysiologic basis of autism and genetic studies have failed to identify clearly replicable causal loci, despite suggestive findings.[2] The lack of progress in untangling the genetic basis in idiopathic autism has spurred interest in the study of behavioural endophenotypes of ASD i.e. studying ASD in pathogenetically homogenous subgroups of patients.

One syndrome in which an association with ASD has been described is Neurofibromatosis Type 1 (NF1). NF1 is a common autosomal dominant genetic disorder with an estimated birth incidence of 1 in 2,699 and a prevalence of 1 in 4,560 [3]. Fifty percent of the cases are inherited while the rest are sporadic cases due to spontaneous mutation of the tumour suppressor gene located on long arm of chromosome 17 (17q11.2). Noted for somatic manifestations, the main morbidity from NF1 is actually in cognitive, social and behavioural difficulties. Clinical studies had suggested attention-deficit-hyperactivity disorder (ADHD) as the main psychopathology[4] along with social difficulties[5]; however Garg, Huson, and Green have recently undertaken the ***first population-based epidemiological study*** of the NF1 behavioural phenotype, showing in the screening phase (n=109) high prevalence (>40%) of Autism Spectrum Disorder (ASD) as well as comorbidity with ADHD.[6] A Manchester BRC research fellowship to Dr Garg (2011-12) allowed the confirmatory diagnostic evaluation second phase of this study using gold standard instruments (Autism Diagnostic Observation Schedule and Autism Diagnostic Interview), which found that 25% cases assessed met strict diagnostic criteria for ASD and another 21% met the partial criteria for ASD. This novel finding represents an important milestone in NF1 research.

From a *clinical perspective in NF1* it will be transformative for clinical services; re-focusing clinicians towards targeted treatments. From a *research perspective in autism*, it identifies NF1 as a single-gene disorder model of symptomatic autism with great potential significance; since the neuropathology of NF1 is so well understood in animal models as well as humans and because there is a putative intervention (statins) with theoretical justification and prior animal and human evidence of effect. Our finding on ASD prevalence in NF1 therefore opens the prospect of experimental medicine intervention of relevance to autism as well as NF1.

Biological basis of cognitive deficits in NF1

The NF1 gene encodes for protein neurofibromin, which is expressed in many tissues including neurons, Schwann cells and oligodendrocytes. Neurofibromin plays a key role in cellular transduction; signal regulation in synaptic plasticity, learning and memory by regulating the activity of RAS (Rat Sarcoma protein) bound guanosine triphosphate. Loss of neurofibromin in NF1 leads to increase in RAS activity and increased RAS/ERK signalling resulting in unregulated cell proliferation and differentiation. Experimentally the NF1 mouse model has been used to study the effects of diminished neurofibromin on cognitive functioning. Costa et al (2002) have shown that mice carrying a heterozygous null mutation of the *Nf1* gene (*NF1*$^{+/-}$) exhibit learning deficits associated with an increase in Ras activity, which leads to impairments in long-term potentiation caused by increased GABA-mediated inhibition. Additionally these learning deficits can be reversed by pharmacologically or genetically manipulating the excessive activity of Ras. RAS transforming activity requires iso-prenylation of RAS, which can be blocked by farnesyl transferase inhibitors and by 3-hydroxy-3methylglutaryl coenzyme A(HMG-CoA) reductase inhibitors.[7] Simvastatin, the drug proposed for this trial is a HMG-CoA reductase inhibitor. There is preliminary evidence that peripheral mononuclear P-MAPK activity is associated with cognitive deficits in the disorder.[8] It should be noted that promoter sequence of the NF1 gene and neurofibromin are both highly homologous in humans and mice.

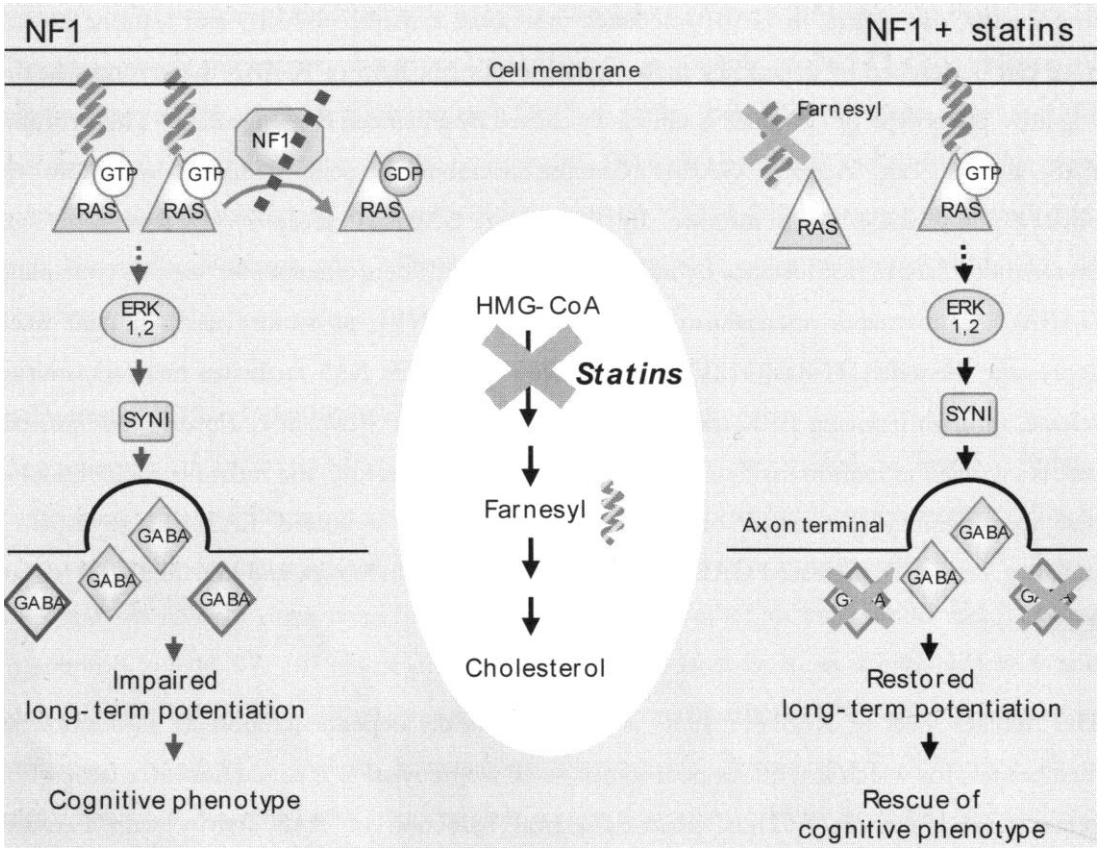


Fig1: Simplified overview of the proposed presynaptic mechanism underlying the cognitive phenotype of *Nf1* mice and NF1 patients. Left: Heterozygous loss of neurofibromin leads to elevated activity of RAS, which results in increase in release of GABA - causing disturbed long-term potentiation and leading to cognitive phenotype of NF1. Right- Reducing availability of farnesyl with statins normalised the GABA activity.[9]

Reviews of previous statin trials in NF1

Li et al (2005) used lovastatin to evaluate its benefits on learning deficits in *NF1*$^{+/-}$mice. *NF1*$^{+/-}$ mice treated with lovastatin demonstrated improved performance relative to mice on placebo on measures of attention, spatial learning and pre-pulse inhibition.[10]

In humans, Krab et al (2008) carried out a 12 week randomised, double-blind, placebo controlled trial to determine the effects of simvastatin on neuropsychological, neurophysiological and neuroradiological outcome measures in children with NF1.[9] Sixty-two children aged 8-16yrs were randomised to a dose-escalating regime of simvastatin (weeks 0-4, 10 mg/d; weeks 5-8, 20mg/d; and weeks 9-12, 20mg/d for children aged 8-12 years or 40 mg/d for children aged 13-16 years) or placebo. Although no significant differences were observed between the simvastatin and placebo groups on any primary outcome measures, the secondary outcome measure specifically

the object assembly scores showed significant improvement in simvastatin group; thus suggesting improvement in visual synthesis in the simvastatin group. Importantly there were no laboratory adverse events or serious adverse events reported. Acosta et al (2011) conducted a phase 1 study examining the safety and tolerability of lovastatin in children with NF1. This 12 week randomised controlled, double-blind trial of 23 children aged 10-17 years suggested improvements in verbal and non-verbal memory in the lovastatin group.[11] Minimal side effects were reported and no child experienced dose-limiting toxicity. Functional Magnetic Resonance Imaging (fMRI) of seven of the cohort showed normalisation of resting state functional connectivity (RSFC) in the Default Network core region following treatment.[12] This latter finding is particularly significant in relation to general autism development, where abnormalities in Default Network RSFC have been seen as a likely core process and repeatedly reported.[13]

TRIAL TREATMENT

Investigational medicinal product

Simvastatin is an HMG-CoA reductase inhibitor. It has a UK and US license for use in age 10 and above and there is extensive clinical experience of its use in younger children with other disorders such as familial hypercholesterolaemia and Smith Lemli Opitz Syndrome. Oral absorption of Simvastatin is 42.5%±42.5. It crosses the blood brain barrier. The only other available statin which crosses the blood brain barrier (lovastatin) is not licensed for use in children in Europe

Summary of known and potential risks and benefits

Please see Appendix 1 for simvastatin Expected Adverse Events and a full list of drug interactions. The current Summary of Product Characteristics and protocol will be used as the reference documents for classifying adverse events.

Procedure for drug administration

After randomisation and baseline monitoring, children will be given either simvastatin at 0.5 mg/kg/day in a single dose or placebo for 4 weeks. The formulation of simvastatin will be in liquid form and patients will be asked to take it orally. Medication will be provided to parents free of charge. They will be instructed to return all unused medication as well as empty bottles at each visit.

Parents and participants will be explained known and potential adverse effects, drug interactions and contraindications. After 4 weeks, children will

be seen and monitored for any adverse events. Those showing no significant adverse events (e.g. elevation of LFTs or CK) will be escalated to a dose of 1 mg/kg/day to a maximum of 30 mg/day in a single daily dose for a further 8 weeks.

RATIONALE FOR THE PROPOSED TRIAL

Research aims and hypothesis

The overall research aim is to determine whether treatment with statins improves the ASD phenotype in children with NF1 autism. This aim of this study is to test:

- Acceptability and feasibility of involvement for families of children with NF1 ASD
- The feasibility and acceptability of the assessment protocol
- Treatment effects on intermediate and endpoint behavioural phenotype measures and imaging parameters

The hypothesis is that treatment with statins in young children with NF1 autism will be:

- Feasible, safe and acceptable to families
- Associated with signals of change in brain functional connectivity and metabolites measured by fMRI and spectroscopy respectively, as well as other advanced imaging parameters
- Associated with signals of change in autism and other behavioural symptoms

Rationale for a statin trial in younger children with NF1 and ASD

Findings from statin studies in animal models and in older children give a strong biological and research rationale for investigation of statin treatment earlier in development, when greater neuroplasticity might increase intervention effects. Further, the brain imaging findings above need replication. The findings on ASD prevalence in NF1 link this work to the very active international research interest currently in early interventions for the general autism phenotype[14] and in biological models for early autism development. A trial of statin treatment in early-school age children with both NF1 and ASD is therefore a logical next step, with relevance to all these contexts and as preparation for a larger phase III trial. Experimental medicine trials in the context of this knowledge of the impact of statins on NF1 neuropathology promise eventually not only utility in developing treatments, but also insights into causal hypotheses regarding neurodevelopment in both NF1 and autism.[15]

Rationale and opportunities for Imaging

It is becoming increasingly recognised that imaging phenotypes of complex disease processes can only be expressed in the form of multi-parametric imaging data; our study will allow acquisition of advanced imaging data in one experiment, working towards an imaging model of autistic spectrum disorder in the developing brain.[16] We will undertake;
1) High resolution volume T1 and FLAIR acquisition of the whole brain to undertake voxel based morphometric assessment of regional cortical/grey/white matter volumes.[17,18]
2) fMRI assessment modified for younger age which builds on the RSFC fMRI work of our collaborators.[12]
3) Diffusion tensor assessment of the whole brain which has previously been used to assess children with autism as well as correlated with VBM analysis.[19,20]
4) Multi-voxel proton magnetic resonance spectroscopy (MRS) to look for changes in N-acetyl-aspartate (NAA) and Creatine (Cr) ratios pre and post treatment.
Increased RAS activity in NF1 leads to increased GABA-mediated inhibition and consequent impairment in long-term neural potentiation[7] and MRS can be sensitive to cortical GABA-ergic activity [21]; thus we will also undertake MRS analysis for GABA and glutamate activity as a potential marker of statin effect. Given that there are age related changes in metabolite levels, comparison across patients would not be possible, but with only a 12-week gap between spectroscopic assessment intra-patient assessment on both a global brain as well as region-specific analysis is likely to yield important data. The hypothesis is that analysis of the overall model will yield the imaging factors of highest importance with regard to outcome prediction in statin treatment of NF1 autism.

ASSESSMENT OF RISKS AND BENEFITS

Benefits

Every participant in this trial will be screened for ASD and will receive a report of the assessment. For children screened positive for ASD, parents if they wish will be able to use the report to seek a referral to a local paediatrician/Child and Adolescent Mental Health service for a formal diagnosis of Autism Spectrum Disorder. Having a formal diagnosis may help children struggling academically obtain education support.

For participants receiving simvastatin, it may have a direct benefit on their cognitive abilities. At the conclusion of the trial, participants will be sent a summary of the research findings with no personal identifying information. If

the results show that simvastatin is efficacious in treating ASD symptoms, this will lead to a larger study to determine its effectiveness.
This study will contribute to the understanding of the neurobiology of both autism and NF1 and thus potentially in the development of treatments for the same.

Risks

Simvastatin is not approved for use in children under the age of 10 years. Although clinical experience and research evidence of its use in conditions such as familial hypercholesterolemia and Smith-Lemli Opitz syndrome suggest that it is safe to use in the younger paediatric population, there are still risks of potential adverse effects to the participants. Comprehensive Adverse Event (AE) monitoring through parent report, clinician assessment and biochemistry will be undertaken at baseline, 4 weeks before dose escalation, at endpoint and follow-up. AEs will be reported in compliance with GCP and applicable regulatory requirements. A member of the research team will review blood results obtained from participants at 4 weeks and approve dose escalation on individual patient basis. This individual will remain blind to treatment allocation group. The trial team (AM, NW) have significant experience in the use of statin in the paediatric population.
The other risks include participants becoming distressed during the blood tests and the brain imaging. Experienced paediatric nurses at the NIHR WTCRF will take blood samples from the children and every step will be taken to minimise the distress. Play therapists will help explain the process of blood tests and brain imaging to the children through the use of pictures and social stories. In addition they will be prepared for the scan by playing of the scan noise at bedtime for two weeks prior to the procedure.

TRIAL OBJECTIVES AND ENDPOINTS

This aim of this study is to test:

- Acceptability and feasibility of involvement for families
- Feasibility of imaging in young children with NF1 ASD
- Intermediate and endpoint measurement

Endpoint outcome measures

- ASD symptoms as assessed by the behavioural phenotype measures
- Effect on neurocognitive functions as assessed by the judgement of line orientation task and paired associate learning task.

- Effect of 12 weeks of Simvastatin treatment on multi-parametric imaging findings including anatomical, physiological, spectroscopic and resting state functional connectivity in the default network as assessed at 3T MRI
- Qualitative interviews with parents on experience of trial, intervention, assessments including imaging procedures.

Exploratory

- Peripheral mononuclear P-MAPK activity as a marker for statin effect on cellular pathway and brain function (see section on sub studies for details)

TRIAL DESIGN

Purpose of the research

This is a phase II exploratory study of statin treatment in young children aged 4.5-10.5 years with NF1 and autism. The purpose of this study is to test acceptability and feasibility of involvement for families and the feasibility of imaging young children with NF1 autism.

Description of the design

This is a single site triple-blind (clinician, patient, assessor) randomised controlled phase II trial of simvastatin medication versus placebo. Primary endpoint will be at 12 weeks. The statin arm will include a single step dose escalation. Measurement will model and test the feasibility of a vertical integrated battery covering the behavioural symptoms, particularly ASD and ADHD, along with brain imaging parameters. The randomised design will provide the best chance of identifying preliminary signals of treatment effect on different levels of measurement and the preliminary data needed for a larger later trial application. A balanced non-treated control group is necessary because there is to date insufficient data on: i) the natural history of early development in this group; ii) the natural history of imaging data against which to judge any signal of treatment effect. There is no cross-over in this study.

Measures to avoid biases

Randomisation will be performed independently of the study team through the CMFT Clinical Trials Pharmacy using web based randomisation on a 1:1 ratio (active:placebo) using randomised blocks. To ensure allocation concealment from trial investigators, the randomisation list will be kept within pharmacy and will only be unblinded after initial analysis or in case of clinical necessity.

Investigational Medicinal Product (IMP) will be dispensed through the Clinical Trials Pharmacy in a bottle (simvastatin 20mg/5ml or placebo) labelled with instructions, complying with local SOPs and clinical trial regulations along with an oral syringe. Data will be double entered with treatment allocation encoded. Data entry and analysis regarding acceptability and feasibility of the trial will be performed blind to treatment allocation.

Description of the trial treatment including criteria for dose escalation

After baseline monitoring, children will be given simvastatin at 0.5 mg/kg per day in single daily dose for 4 weeks. Participants will be carefully monitored for adverse events. The safety of simvastatin will be evaluated using laboratory tests, clinical signs and reported adverse events. Those showing no significant adverse events will be escalated to a dose of 1 mg/kg/ day to a maximum of 30 mg/day in a single daily dose for a further 8 weeks. The medication or placebo will be given to participants' parents at week 0 and week 4 visits by the nursing team at CCRF who will be picking this up from pharmacy. Prescription of medication/placebo will only be dispensed for 4 weeks at a time (the shelf life of medication is for 30 days only). Participants will be sent the final 4 week supply of medication by post/courier. 'Significant adverse events' will include significant muscle symptoms or a rise in plasma transaminase or creatine kinase activity greater than two times the upper limit of normal.

Trial duration

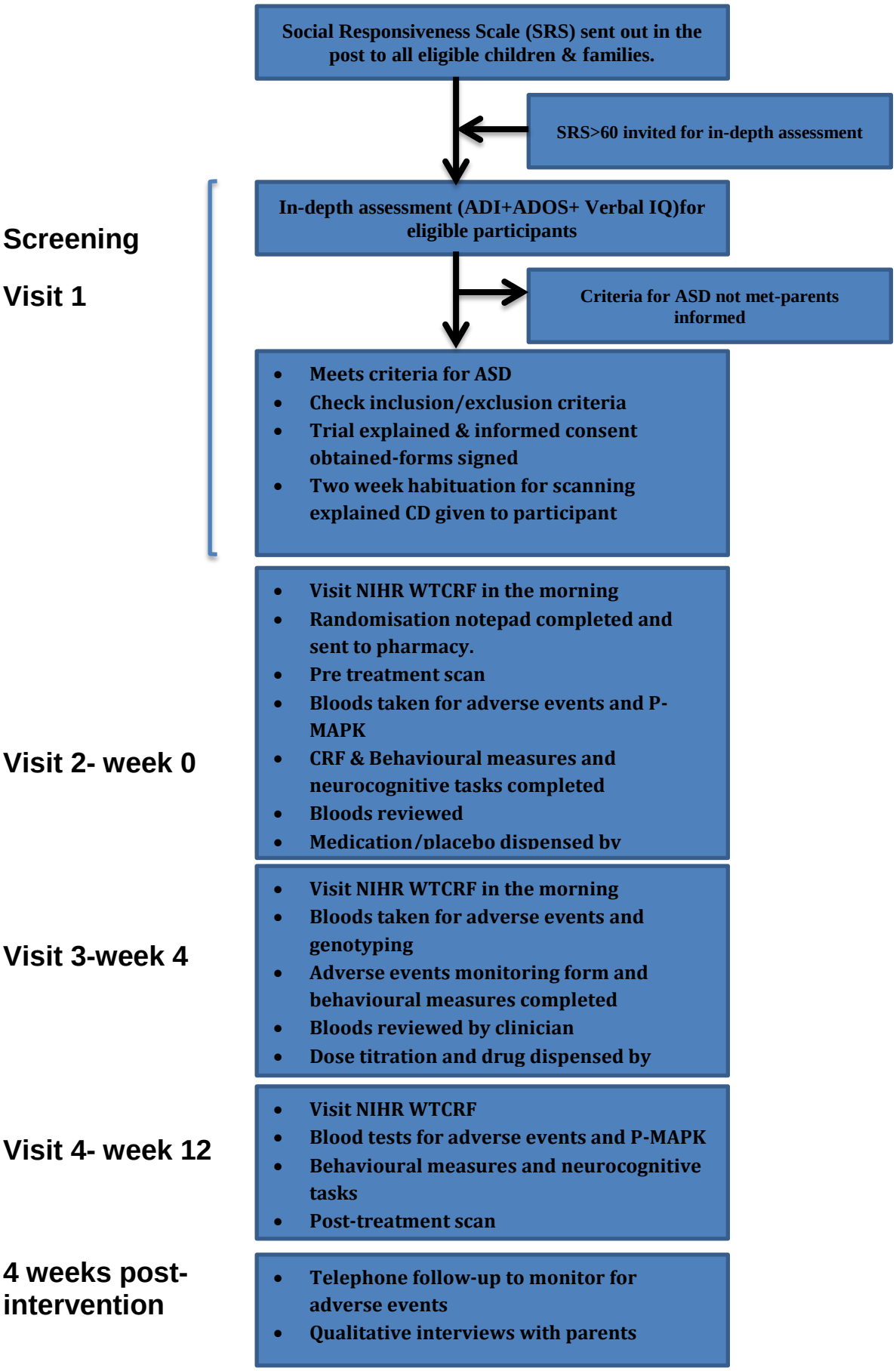
The trial duration per participant will be 16 weeks (+/- 14 days). The screening to assess participant eligibility for the trial will begin once the requisite approvals have been obtained.

Discontinuation criteria

Children who are found to have significant adverse effects from the medication will be withdrawn from the treatment. Blood tests including CK and LFT will be performed at baseline, 4 weeks (+/- 7 days) and 12 weeks (+/- 14 days). Participants who develop significant muscle symptoms or a rise in plasma transaminase or creatine kinase activity greater than two times the upper limit of normal will be withdrawn from the trial treatment. The decision to withdraw participants from treatment will be discussed with AM who is the statin expert on the trial team. Participants withdrawn from treatment will be followed up for the duration of the study.

Study flowchart

SANTA study flowchart



SELECTION OF TRIAL PARTICIPANTS

Inclusion criteria

Children between 4.5-10.5 yrs meeting diagnostic criteria for

1) Neurofibromatosis Type 1(National Institute of Health criteria).
2) Autism Spectrum Disorder (ASD) using Collaborative Programme for Excellence in Autism (CPEA) criteria, based on ADI-R and ADOS-G, age and IQ assessments). [22]
3) Patients who are on a stable dose of methylphenidate and/or dexamphetamine for at least three month prior to screening and who will remain on the same dose for the duration of the study.
4) Hepatic function: Participants with normal liver function defined as < 2 x upper limit of normal for age.
5) Renal function: Participants must have adequate renal function defined as GFR >60ml/min/1.73m2 calculated by 40 x height (in cm) / plasma creatinine (in micromol/l).
6) Informed consent.

Exclusion criteria

1) Severe learning disability defined as verbal IQ<50 as measured by the WASI.
2) Participants in active treatment for NF1 complication (e.g. chemotherapy for optic gliomas, Ilizarov frame for pseudarthrosis).
3) Individuals with abnormal liver function or renal function at baseline as defined in the inclusion criteria.
4) Parents of participants with insufficient English to complete the ASD screening assessments.
5) Participants taking psychotropic medication other than methylphenidate and/or dextroamphetamines. These participants are eligible if, as clinically indicated they discontinue medication for at least 30 days prior to screening and remain off these medications for the duration of the study.
6) Participants who have received any investigational drug within four months of screening.
7) Participants who have recently taken simvastatin or any other statins. These participants will be eligible after a washout period of at least three months.
8) Participants with a clinically significant unrelated illness, which in the judgment of the principal or associate investigator, would compromise the participant's ability to tolerate the medication or interfere with ability to participate in the required testing.
9) Low cholesterol (defined as total cholesterol < 90mg/dl).
10) Participants with planned surgery within 16 weeks of potential enrolment.

RECRUITMENT OF TRIAL PARTICIPANTS

Identifying Participants

Case finding will be done in the following way:
1) The parents of potentially eligible children on the NW NF1 register will be contacted.
2) Parents of eligible children known to the NF1 clinics in Leeds, Liverpool and Newcastle will be contacted.
3) The study will be advertised nationally via the Neuro Foundation newsletter and website www.nfauk.org alongside other organisations such as the NF wellbeing research group at Manchester Metropolitan University.

Dr Susan Huson (NF1 expert on the trial team) runs a nationally commissioned complex NF1 service for the North of England. She does regional clinics with the local NF1 teams in Leeds, Liverpool and Newcastle. She will lead the recruitment aspect of the study. Patients on the North West NF1 register have consented to receive information about research studies. Local doctors in Leeds, Liverpool and Newcastle will write to eligible patients initially. Participants interested in participating in the study will be requested to send the screening questionnaire and contact details to the research team. Only the direct care clinical team will have access to the patient details until participants interested send the screening pack to the research team.

Consenting Participants

Participants will be sent the initial information packs by their local care teams. The research team will contact eligible families (those that meet the screening criteria) to arrange baseline assessment. The informed consent process for the trial will take place before the detailed screening assessment (see below) for the eligible patients. Consent and eligibility will be recorded by one of the clinicians delegated to complete these tasks. Parents and participants will be given all the study information including the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial. The consenting clinician will stress that the patient/parent is completely free to refuse to take part or withdraw from the treatment or trial at any time. Parents and children will be provided with an opportunity to ask questions.

The conduct of the trial will be in accordance with the Principles of Good Clinical Practice. The parent's written informed consent to participate in the trial will be obtained after a full explanation has been given of the treatment options and the manner of treatment allocation. All the children will be

explained the study in a simple manner and children older than 7 years will be asked to sign the assent form. Participants' General Practitioners will be notified with parents' consent. Details of the informed consent discussions will be recorded in the participants' clinical notes including date, information given, initial discussion, the date consent was given, with the name of the trial and the version number of the Patient Information Sheet and Informed Consent Form. Throughout the trial the parent/participant will have the opportunity to ask questions about the trial and any new information that is relevant to the patient's continued participation should be shared with them in a timely manner. Additional consent will be obtained from the parents for genetic testing and for transfer of samples to the United States.

Screening assessments

Parents will be sent an initial screening questionnaire (SRS) along with the study information in the post by their direct clinical team. They will be asked to complete the SRS and return in a pre-paid envelope if they wish to participate in the study. Participants with high scores on the SRS (SRS T>60) will be invited for the baseline assessments. The study will be described in detail to participants and their parents/guardians at the initial visit. If the family and participant agree to participate, the applicable assent, consent forms will be signed. After informed consent, a baseline assessment will be performed. The baseline assessment will consist of parental interview using the ADI-R and the child assessment using the ADOS-G. Designated clinicians will carry out these assessments, which will take approximately three hours to complete. To be eligible, participants must meet the ADI-R and the ADOS-G threshold for ASD. If the child meets the criteria for the study, parents and participants will be given further information about the trial.

Ineligible and non-recruited participants

Only children meeting the criteria for ASD will be enrolled onto the trial. If the child fails to meet the screening criteria, parents will be verbally informed of the reason why the child did not meet the criteria. A screening and recruitment log will be maintained to record this information.

Registration/Randomisation procedures

Randomisation will be carried out at the beginning of the trial (week 0, visit 2- see study flowchart). Participant information will be collected and recorded on the randomisation notepad. This information will be sent to the CMFT Clinical Trials Pharmacy. The patient will be randomised by the pharmacy 1:1 to one of the two treatment groups: simvastatin or placebo. A web-based randomisation process will be used to ensure equal numbers in both treatment arms. Upon randomisation, participants will be given a trial specific

participant card which will have the trial title, IMP details, participant trial number and the contact details of the Principal Investigator and out of hours contact details in cases of emergency. To ensure allocation concealment, the randomisation list will be kept within the CMFT Clinical Trials Pharmacy and will only be unblinded after initial analysis or in case of clinical necessity.

Emergency Unblinding Procedures

Treatment allocation will be revealed for reasons of participant safety or when critical therapeutic decisions are contingent on knowing the assigned study medication. The decision to break the blind will be discussed with the Chief Investigator and Sponsor. If the blind is broken, the pharmacist will record key information such as the participant ID, date the blind was broken, reason for breaking the blind, name of the investigator who requested the blind be broken and name of the pharmacist who broke the blind.

Discontinuation/ withdrawal of participants and "stopping rules"

A participant will be taken off the trial treatment for the following reasons:

- The participant refuses further treatments and/or wishes to withdraw from the treatment or the trial;
- For participants concurrently taking stimulant medications (methylphenidate and/or dextroamphetamines), they will be withdrawn from the treatment if they stop or change the dose or type of stimulant medication before their final study visit;
- It is deemed in the best interest of the participant to stop the treatment by the research team;
- Serious protocol violation or non-compliance as determined by the principal investigator or research team;
- The development of significant drug toxicity
- The development of a concurrent serious medical condition that might preclude or contraindicate the administration of simvastatin;
- Death

Participants who cease study medication will be followed up for the entire duration of the study; the only exception to this being in those where there is withdrawal of consent by the participant/parent. A record will be made of any participants who cease study medication and the reason(s) for this.

TREATMENT DETAILS

Name of the IMP

Generic and trade name: Simvastatin

Drug supply

Study drug and placebo formulation will be provided by Rosemont pharmaceuticals http://www.rosemontpharma.com/, a specialist manufacturer of liquid drug preparations with a manufacturing license for IMP and placebo for use in clinical trials. Their input will be acknowledged in the trial.
Drug Product Manufacturer: Rosemont Pharmaceuticals Limited, Braithwaite Street, Leeds LS11 9XE. Marketing Authorisation – Simvastatin 20mg/5ml Oral Suspension – PL 00427/0146.
Manufacturer's Licence Number is MIA 427

Drug dosage, adverse effects and possible interactions

A list of possible interactions and adverse effects is provided in appendix 1.
The selection of maximum dosage of simvastatin of 30mg/kg is based on extensive clinical experience of its use in younger children with other disorders such as familial hypercholesterolaemia and Smith Lemli Opitz Syndrome, plus good information on dosing and clinical response in the 4.5-10.5yr age group.[23] It has been used in trials with older children and adolescents with NF1 in Europe. The dosing regimen is within recognised parameters for statins in young children in other disorders, within the experience of our statins expert member on the team (AM), and consistent with the international advice available.

Simvastatin has been shown to be safe and effective in the paediatric population. Adverse effects are not common or concerning in general at these dosages; usually involving muscle stiffness, which is rarely serious, or rarely gastrointestinal disturbance. There has been concern about very rare rhabdomyolysis, and CK will be measured as a sensitive marker for this. There has been theoretical concern about the effect of lowered blood lipids on CNS myelination in early development. However, the protocol will include monitoring blood lipids and the doses used are not expected to reduce blood lipids below the normal range. Furthermore the intensive imaging protocol will be able to give qualitative early indication of short-term effect on myelination.

Route of administration, dosage, dosage regimen and treatment period(s)

Simvastatin will be given orally in liquid form. The bioavailability of the medication increases under the influence of a meal. Parents will be instructed to give the child the medication once daily after their evening meal. Simvastatin is a substrate for cytochrome P450 isoform 3A4 (CYP3A4). Grapefruit juice contains one or more components that inhibit CYP3A4 and can increase the dose concentration. Parents and children will be instructed to avoid the consumption of grapefruit juice during the treatment. A complete list

of drug interactions/contraindications will be described to parents and participants.
The dose will be simvastatin 0.5 mg/kg/day in week 1. Bloods including LFTs, Renal Function Tests, CK and cholesterol will be checked at week 4. If the results of the bloods tests are normal, then the simvastatin dose will be titrated up to 1mg/kg/day. From week 4 to week 12, the participant will be on simvastatin 1mg/kg/day. The medication will be stopped after 12 weeks.
A similar procedure will be followed for participants randomised to the placebo arm.

Accountability procedures

The clinical trials pharmacy at Central Manchester University Hospitals NHS Foundation Trust (CMFT) will be responsible for the drug accountability, ordering and maintenance of IMP. General and patient accountability logs will be kept in pharmacy where date, amount, batch number and expiries will be captured for each supply and dispensing. These will be available for regulatory inspection and monitoring on request.

An accurate record of the date and amount of any expired or returned study drug destroyed will also be captured in these forms. Destruction of the IMP will be done in accordance to the sponsor's destruction policy.

Participant adherence

Parents will also be asked to maintain a diary card that will be reviewed at each visit to ensure compliance.

Overdose

To date, a few cases of overdosage have been reported; the maximum dose taken was 3.6g. All patients recovered without sequelae. There is no specific treatment in the event of overdose. In this case, symptomatic and supportive measures should be adopted.

Dose Changes and Modification of trial treatment

Participants will be withdrawn from the trial treatment if they suffer significant adverse effects from the medication. These will include rises in either plasma transaminase or CK values >2 upper limit of normal for age.

TRIAL METHODOLOGY

Trial assessments

Screening assessments	• Initial screening: The initial screening will be carried out using the Social Responsiveness Scale (SRS).[24] This will be sent in the post along with other study information. Only participants with a high score on the SRS (T>60) will be invited for the detailed screening. (Takes 15 minutes to complete) • Baseline assessment: The detailed screening will be carried out using the Autism Diagnostic Inventory -Research (ADI-R)[25] for parental interviews and the Autism Diagnostic Observation Schedule-Generic (ADOS-G)[26] for child assessments. Cognitive development will be assessed using verbal IQ from WISC[27] (Takes 3 hours to complete)
First trial visit (week 0)	• Behavioural phenotype symptoms will be assessed using Aberrant Behaviour Checklist-Community (ABC-C)[28], patient defined target symptoms[29] and Clinician Global Impression of Improvement (CGI)[30] and parents rated Conners questionnaire[31] to assess for hyperactivity symptoms • Cognitive functions will be assessed using the following neurocognitive measures: Judgement of line orientation task and paired associate learning task (20 minutes) • Blood tests for plasma lipids, liver function tests, renal function tests and creatine kinase, mononuclear P-MAPKinase activity. • Brain Imaging will be undertaken on the 3T MRI scanner at the CCRF without contrast injections or sedation, with support from CCRF staff, nursing and play specialists • Participants will be given stickers to positively reinforce the successful completion of the task. They will also be given a token of appreciation for their time and effort on the trial
Treatment procedures at	• Behavioural phenotype measures described

4 week visit (+/- 7 days)	above • Blood tests plasma lipids, liver function tests, renal function tests and creatine kinase, genotyping • If required brain imaging will be undertaken on the MRI scanner at the CRF without contrast injections or sedation, with support from CRF staff, nursing and play specialists (e.g. if scan at week 0 was incomplete or inadequate)
Procedure at 8 weeks (+/- 7 days)	• Nursing team at Wellcome CCRF will pick up medication or placebo from Pharmacy and this will be sent by courier to participants' homes.
Trial treatment endpoint at 12weeks (+/- 14 days)	• Brain Imaging will be undertaken on the 3T MRI scanner at the WTCRF without contrast injections or sedation, with support from CCRF staff, nursing and play specialists. • Behavioural phenotype measures described above • Blood tests plasma lipids, liver function tests, renal function tests and creatine kinase, mononuclear PMAPkinase activity • Participants will be given a token of appreciation for their time and effort on the trial.
Post treatment evaluation (4 weeks (+/- 14 days) post-trial)	• Audio recorded telephone follow-up screening for adverse events • Qualitative interview with parents on experience of trial, intervention, assessments including imaging procedures.

Procedure for imaging

No contrast injections will be necessary for any of the imaging experiments being proposed, with a total scan time of 30-40 minutes per participant attendance. Imaging studies will be performed on the WTCRF 3T research scanner with experienced research radiographer and consultant paediatric neuroradiologist in attendance. The parents and neuroradiologist will be available and remain in the scan room during scanning as required. Since we will not be anaesthetising or sedating our patients we will collaborate with the play therapy team from the Royal Manchester Children's Hospital to maximise our imaging yield. We will employ a standard pre-imaging clinical preparation protocol which includes explanation for parents and children (for the latter specially designed 'social stories' with pictures to familiarise them with the procedure); and use of a CD habituation procedure to the scanner noise by

parents at bedtimes. These techniques have been used routinely with success at the Royal Manchester Children's Hospital and by our collaborators at New York University, Prof Castellanos and Dr DiMartino.
If it deemed appropriate, due to difficulties in week 0, the participant may require part of the scan or the entire scan to be performed at week 4.

Blood collection for DNA analysis and sub studies

MAPK level as a peripheral biomarker for cognitive deficits of NF1
This sub-study will be conducted in collaboration with Professor Alcino Silva and Carrie Bearden at Silvalab, UCLA, United States.[8] The aim of this exploratory study is to ascertain pre and post MAPK levels as a potential biomarker for simvastatin treatment effects. Blood samples will be collected from participants at baseline and at 12 weeks trial end point. Initial processing of these blood samples will be done in a Biobank laboratory at CMFT. Peripheral mononuclear cells will be isolated at RMCH from the blood sample and frozen blood pellets will be shipped to the laboratory of our collaborators in UCLA. These blood pellet samples will be anonymised labelled only with a unique participant trial number.

NF1 mutational analysis and evaluation of genotype-phenotype correlation
The aim of this study is to look at the mutations of the NF1 gene and correlate that with the clinical ASD phenotype. Genotype-phenotype correlations will help in understanding whether certain mutations are more commonly associated with ASD. Blood samples for this study will be collected at baseline only. Samples will be labelled with a unique participant trial number and the genotyping will be carried out in the CMFT genetics laboratory. We will collaborate with Professor Eric Legius in Leuven and Dr Maria Acosta in Washington to pool anonymous genotype data in order to look at its correlation with the ASD phenotype.

Measuring trial endpoints

Adverse events Formal SAE monitoring and blood screening for plasma lipids, liver function tests, Urea & Electrolytes, and Creatine kinase. Recording of potential adverse effects from clinical history at the time of study visits using standardised Case Record Form (CRF). Safety parameters will be assessed, recorded and analysed via follow-up of CRFs and Serious Adverse Event (SAE) forms. SAEs and SUSARs will be reported to the sponsor (CMFT) in accordance with the relevant SOPs.

Autism symptoms Assessed with the Aberrant Behaviour Checklist-Community (ABC-C)[28]; Patient defined target symptoms[29] and Clinician

Global Impression of Improvement (CGI).[30] ***Overactivity*** symptoms assessed using the standard parent-rated Conners questionnaire [31].

Cognitive symptoms (baseline and 12 week end point) Assessed using the judgement of line orientation task, paired associate learning task

Brain imaging (baseline, 12 week endpoint) Multiparametric imaging data will be acquired both at baseline and end of trial. The anonymised data will be analysed by Dr Stivaros, Professor Keane (Computer Science) in collaboration with Professor Castellanos (NYU) and Dr Dimartino (NYU. Dr Stivaros will analyse the data initially and discuss the imaging findings with the US group as the study progresses at meetings in the US which Dr Stivaros will be attending. Where imaging needs to be transferred prior to that it will be via a 256 bit encrypted 'DropBox' transfer of fully anonymous datasets.

Trial closure

Intervention endpoint is completion of 12 week dosing. The end of the trial will be when at the last follow-up contact with the last recruited participant. The end of the study will be when the biomarker data has been analysed. An end of trial notification will be submitted to the REC and Regulatory Authority within 90 days of this date.

STATISTICS AND DATA ANALYSIS

Statistical analysis will be based on an intention-to-treat approach using all randomised patients. The primary analysis will focus on tabulated and associated graphical summaries of feasibility indicators: patient recruitment, checks for absence of selective recruitment of participants; baseline balance of summary statistics and patient flow (as in CONSORT). Case level analysis of any adverse events or drop out from assessment will be undertaken. Qualitative analysis of the post treatment parent interviews will test acceptability to families and indicate areas of further development for a definitive trial.

This feasibility study is not powered for formal analysis of group treatment effect. The random allocation parallel group, placebo-controlled design will provide an opportunity to identify the variance in imaging parameters and symptom measures, which will be used to inform the power calculation in a planned larger phase III study application. Analysis will use appropriate linear regression models to estimate the effect of random allocation on autism and behavioural symptom outcomes at 12 weeks. The presentation of the analysis will focus on point estimates and associated 95% confidence intervals rather

than statistical significance. As this is a chronic condition, we will also examine within group change as a check for a clinically important within subject response. Analysis of the multiparametric imaging data will be undertaken by Dr Stivaros. Prof Castellanos and Dr Di Martino will collaborate on interpretation of the imaging results.

PHARMACOVIGILANCE

Safety Reporting

Adverse Events (AEs)

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after the study has commenced, even if not considered to be related to the investigational medicinal product. Medical conditions/diseases present before starting the study will only be considered as adverse events if they worsen after the start of the study. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The occurrence of adverse events will be sought by non-directive questioning of the participant during the study. Adverse events also may be detected when they are volunteered by the patient or through physical examination, laboratory test, or other assessment. As far as possible each adverse event will be evaluated to determine:

1. The severity (mild, moderate, severe)
2. Its relationship to the investigational medicinal product
3. Its duration
4. Action taken (no action taken; study drug dose adjusted/temporarily interrupted; study drug permanently discontinued; concomitant medication taken; non-drug therapy given; hospitalisation required)
5. Whether it is **serious**, where a serious adverse event (SAE) is defined as one which:
    - Is fatal or life-threatening
    - Results in persistent or significant disability/incapacity
    - Constitutes a congenital anomaly/birth defect
    - Requires prolonged hospitalisation (except where it is for routine treatment/monitoring, elective or pre-planned treatment not related to study, for social or respite reasons)
    - Is medically significant i.e. defined as an event that jeopardises the patient or may require medical or surgical intervention to prevent one of the above

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements (see below).

All adverse events will be recorded in detail and treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments,

changes in the frequency or nature of assessments, hospitalisation, or any other medically required intervention. Once an adverse event is detected it will be followed until its resolution, and assessments will be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the investigational medicinal product, the interventions required to treat it, and the outcome.

Evaluation of AEs and SAEs

Seriousness, causality, severity and expectedness will be evaluated for each AE. Cases that are considered serious, possibly, probably or definitely related to drug (i.e. serious adverse reactions, SARs) and unexpected (i.e. SUSARs) should be reported as described below.

Assessment of Seriousness

The Investigator should make an assessment of seriousness as defined above (see definitions).

Assessment of Causality

The Investigator must make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions. All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g. possibly, probably, definitely) to the study drug will be considered as ARs/SARs. If concomitant or rescue/escape drugs are given, the Investigator must also make an assessment of whether the AE/SAE is likely to be related to an interaction between the study drug and concomitant or rescue/escape drugs or whether the AE/SAE might be linked to either the study drug or concomitant or rescue/escape drugs but cannot be attributed to only one of these drugs. All AEs/SAEs judged as being related (e.g. possibly, probably, definitely) to an interaction between the study drug and concomitant or rescue/escape drugs, or any AE/SAE that cannot be attributed to only the study drug or the concomitant or rescue/escape drugs will also be considered to be ARs/SARs .

Unrelated: where an event is not considered to be related to the study drug.
Possibly: although a relationship to the study drug cannot be completely ruled out, the nature of the event, the underlying disease, concomitant medication or temporal relationship makes other explanations possible.
Probably: the temporal relationship and absence of a more likely explanation suggest the event could be related to the study drug.
Definitely: The known effects of the study drug or its therapeutic class, or based on challenge testing, suggest that study drug is the most likely cause.

Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event to the treatment should be considered and investigated.

Assessment of Severity

The Investigator will make an assessment of severity for each AE/SAE and record this on the Adverse Event (AE) Form according to one of the following categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: an event that prevents normal everyday activities.

Assessment of expectedness

If an event is judged to be an AR/SAR, the evaluation of expectedness will be made based on knowledge of the reaction and the relevant product information documented in the current Summary of Product Characteristics (SmPC).

Serious Adverse Event (SAE) reporting

Any SAE will be reported by one of the trial Investigators (Dr Garg; or nominated medical deputy in her absence), including a completed SAE form, within 24 hours of first knowledge to the Sponsor. The Chief Investigator of the study (Prof Green) will be informed. Dr Garg or medical deputy will ensure that the participant is appropriately treated. They will also determine whether the SAE is a SUSAR (Suspected Unexpected Serious Adverse Reaction). If it is deemed to be a SUSAR it will be reported immediately to the sponsor. The Regulatory Competent Authority (MHRA) and Research Ethics Committee will also be informed in accordance with Trial regulations. All Adverse Events including SAEs will be reported to the Trial Steering Committee. A Development Safety Update Report (DSUR) will be submitted by the sponsor (in conjunction with the Chief Investigator) to the MHRA and the Research Ethics Committee. Completed initial and follow-up Serious Adverse Event forms should be faxed to the sponsor on 0161 276 5766 and addressed 'For the attention of the Quality Manager'. Alternatively, scanned forms can be emailed to adverse.events@cmft.nhs.uk.

Adverse Reactions and Significant Adverse Reactions:

Side-effects and ARs listed in the SmPC which have been assessed by the researchers as causally related will be recorded but not reported to the sponsor. ARs requiring hospitalisation will be treated as SARs and will be reported to the sponsor.

Regulatory Reporting Requirements

The sponsor, or their delegate, has a legal responsibility to notify the Regulatory Competent Authority and the Research Ethics Committee that approved the trial. Fatal or life threatening SUSARs will be reported no later than 7 calendar days, with a further 8 days for follow up information. All other SUSARs will be reported no later than 15 calendar days after the sponsor is first aware of the reaction.

Follow up procedures

After initially recording an AE or recording and reporting an SAE, the Principal Investigator is required to follow each participant until resolution. Follow up information on an SAE should be reported to the sponsor. AEs still present in participants at the last study visit should be monitored until resolution of the event or until no longer medically indicated.

Criteria for premature termination of study

These criteria include new safety data, or concerns from safety data (number and nature of SUSARs); or evidence from other studies.

Pregnancy

Given the age of participants (4.5-10.5 years), it is extremely unlikely that any participant in this trial will become pregnant. In the event that pregnancy does occur in a participant an any time between commencement of the study and 28 days after completion or termination of the study the pregnancy will be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. Details of the pregnancy will be recorded on a Pregnancy Reporting Form. After that the health of the baby will be followed up at 12 and 24 months old. Any SAE experienced during pregnancy will be reported on the SAE Report form.

Elective treatment/surgery

Elective treatment/surgery or pre-planned treatment/surgery (scheduled prior study enrolment) for a documented pre-existing condition that did not worsen from baseline is not considered an adverse event (serious or non-serious).

Follow up procedures

Participants will be followed up at 16 weeks (four weeks after the end of trial treatment) to monitor for adverse events and in order to study their experience

of participating in the trial, experience of intervention assessments and imaging.

Annual Safety Report/ Development Safety Update Report (DSUR)

The Development Safety Update Report (DSUR) will be prepared and submitted by the sponsor in conjunction with the Chief Investigator. This will be submitted annually within 60 days of the Data Lock Point (DLP). The DLP will be set at the last day of the one-year reporting period.

TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

Oversight Committees

Oversight committees for this trial are:

- Trial Management Group (TMG)
- Trial Steering Committee (TSC)

The TMG will comprise members of the trial personnel and will meet at regular intervals (to be decided at the first meeting). The role of the TMG is to oversee the day-to-day management of the trial. Minutes of these meetings and resulting actions will be recorded.

The sponsor will arrange for a TSC to annually review the trial progress. The TSC will comprise of members of the study team, an independent Chair, independent clinical expert, independent statistician and sponsor representative. The TSC will review the progress of the trial including adherence to the protocol, patient safety and consider any new information relevant to the trial. On consideration of this information the TSC will recommend appropriate action, such as changes to the trial protocol, additional patient information or stopping of the study. The rights, safety and well-being of the trial participants should be the most important considerations in these deliberations.

Clinical Trials Pharmacy

The CMFT Clinical Trials Pharmacy will undertake randomisation of the trial participants and will be responsible for dispensing the trial medication/placebo. The pharmacy will hold the randomisation list and will assist in unblinding in case of a medical emergency.

TRIAL MONITORING

Data Collection

Data collection during pre-screening assessment: Parents will be sent a pre-screening assessment tool (Social Responsiveness Scale- see appendix for details) in the post during this stage. Parents will be asked to complete this assessment tool and send it back to the research team. After two weeks, the research team will telephone families in order to answer any queries/ assist with filling in the pre-screening tool.

Data collection during screening assessments: Standardised instruments (ADOS, ADI, WASI- please see appendix for details) will be used to collect data during the screening stage. Data will be obtained from parental interviews and child assessments. Only participant ID, date of interview, name of researcher conducting the interview will be recorded on the data collection tools. Experienced researchers trained in GCP and conducting interviews with children and families will collect data.

Data collection during the trial (Including baseline/during treatment/end of trial): Data will be collected on Case Record Form as well as several standardised behavioural scales which include Aberrant Behavior Checklist-Community (ABC-C)[28], patient defined target symptoms[29] and Clinician Global Impression of Improvement (CGI)[30] and parents rated Conners questionnaire[31] (please see appendix for details). Participants' cognitive functions will be assessed using the judgement of line orientation task and paired associate learning task and this will be recorded in the case record form. Senior nurses at the NIHR WTCRF will collect data during these stages.

Data handling and analysis

Designated researchers will enter data onto the CRFs. Data from this trial will be handled by the Wellcome Trust Children's Clinical Research Facility (CCRF), a full-time research facility dedicated to, and with substantial experience in, the conduct of clinical research. The CCRF at CMFT is dedicated solely to carrying out paediatric clinical research. The CCRF recognizes the responsibilities of a data management centre with respect to the ethical practice of research and the adequate protection of human subjects. Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998. Patient details including name, address, postcode, date of birth and parent's telephone numbers will be collected after screening and informed consent. A clinical trial prescription will be sent to CMFT Clinical Trials Pharmacy Department to allow the pharmacy to randomize the patient.

Participants will be identified using only their unique trial number, date of birth, hospital number and initials on the Case Report Form. The data will be stored on a secure computer database, and all personal information obtained for the study will be held securely and treated as strictly confidential. Any data processed outside of the NIHR WTCCRF will be anonymised.

Direct Access to Data

The Chief Investigator will permit trial-related monitoring, audits, REC review, and regulatory inspection and provide direct access to source data and documents.

Site Monitoring

Site monitoring will be conducted according to the study monitoring plan agreed by the Sponsor and study CI. Quality Control and Quality Assurance processes will be according to the applicable SOPs of the Sponsor.

CONFIDENTIALITY AND DATA PROTECTION

Confidentiality

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998. Patient details including name, address, postcode, date of birth and parent's telephone numbers will be collected after screening and informed consent. This information together with the Parent Consent Form will be faxed to CMFT Clinical Trials Pharmacy Department to allow the pharmacy to randomize the patient. Participants will be identified using only their unique trial number, date of birth, and initials on the Case Report Form.

Participants will be informed that their trial data and information will be securely stored at the NIHR WTCRF, and will be asked to consent to this. The data will be stored on a secure computer database, and all personal information obtained for the study will be held securely and treated as strictly confidential. Any data processed outside of the NIHR WTCRF will be anonymised. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee, Regulatory Authorities, or the REC. The Investigator and trial staff involved with this trial will not disclose or use for any purpose other than performance of the trial, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the trial. Prior written agreement from the Sponsor or its designee will be obtained for the disclosure of any confidential information to other parties.

Data Protection

All Investigators and trial site staff involved with the trial will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to those clinicians treating the participants. Computers used to collate the data should have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

TRIAL CONDUCT

Protocol Amendments

Any changes in research activity (except those necessary to remove an apparent, immediate hazard to the participant) will be reviewed and approved by the sponsor and Chief Investigator prior to submission in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to enrolment into an amended protocol.

Protocol Violations/ Deviations/ Serious Breaches

The Regulations and guidance state that no deviation must be made from an approved trial protocol, unless it is an urgent safety measure taken to protect a patient from immediate harm. The trial investigators are encouraged to contact the sponsor if a potential protocol deviation has occurred (or if an event has occurred and it is unclear whether it should be classified as a deviation) and the sponsor will advise as to what information and actions are required.
For Clinical Trials of Investigational Medicinal Products (CTIMPs), there is a legal requirement to report serious breaches of GCP or the trial protocol to the MHRA and REC within a defined timeframe. If a major deviation on a CTIMP meets the criteria for a serious breach, it is notified immediately to the Sponsor and reported to the REC and the MHRA within 7 days of confirmation. Complete investigations of breaches are fully documented, filed in the TMF and a copy sent to the sponsor.

TRIAL RECORD RETENTION

In line with the Medicines for Human Use (Clinical Trials) Regulations, once

data collection is complete on all participants, all data will be stored for at least 15 years, in accordance with the sponsor's SOP. Trial data will be stored within the NIHR WTCRF under controlled conditions for at least 3 years after closure. Long-term offsite data archiving facilities will be considered for storage after this time. Any queries or concerns about the data, conduct or conclusions of the trial can also be resolved in this time. Limited data on the participants and records of any adverse events may be kept for longer if recommended by an independent advisory board.

END OF TRIAL

The Chief Investigator and/or the TSC have the right at any time to terminate the trial for clinical or administrative reasons. The end of the trial will be reported to the REC and Regulatory Authority within the required timeframe (15 days) if the trial is terminated prematurely. Investigators will inform participants of any premature termination of the trial and ensure that the appropriate follow up is arranged for all involved. A summary report of the trial will be provided to the REC and Regulatory Authority within the required timeframe.

CONTINUATION OF DRUG FOLLOWING THE END OF TRIAL

The trial drug will not be continued following the end of the trial as this is an exploratory phase II study to look at the safety and acceptability of the use of statins in children with NF1 ASD.

PEER REVIEW

The sponsor has arranged for external peer review of this protocol prior to submission for REC and MHRA approval.

ETHICAL AND REGULATORY REQUIREMENTS

The trial will be conducted in accordance with the principles of the good clinical practice (GCP).

The sponsor will ensure that the trial protocol, participant information sheet, consent form, GP letter and submitted supporting documents have been approved by a research ethics committee prior to any participant recruitment. The protocol and all agreed substantial protocol amendments, will be

documented and submitted for ethical and regulatory approval prior to implementation. The CI and sponsor will ensure that the REC and the MHRA are notified that the trial has finished (either as expected or prematurely) within required timeframes with summary reports to be provided as required.

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APPENDIX 1: SIMVASTATIN –SUMMARY OF PRODUCT CHARACTERISTICS

The current Summary of Product Characteristics is available at http://www.medicines.org.uk/emc/

The current known adverse effects and drug interactions are listed below.

Adverse effects

The frequencies of the following adverse events, which have been reported during clinical studies and/or post-marketing use, are categorized based on an assessment of their incidence rates in large, long-term, placebo-controlled, clinical trials including HPS and 4S with 20,536 and 4,444 patients, respectively. For HPS, only serious adverse events were recorded as well as myalgia, increases in serum transaminases and CK. For 4S, all the adverse events listed below were recorded. If the incidence rates on simvastatin were less than or similar to that of placebo in these trials, and there were similar reasonably causally related spontaneous report events, these adverse events are categorized as “rare”.

In HPS involving 20,536 patients treated with 40mg/day of simvastatin (n = 10,269) or placebo (n = 10,267), the safety profiles were comparable between patients treated with simvastatin and patients treated with placebo over the mean 5 years of the study. Discontinuation rates due to side effects were comparable (4.8% in patients treated with simvastatin compared with 5.1% in patients treated with placebo). The incidence of myopathy was < 0.1% in patients treated with simvastatin. Elevated transaminases (> 3 x ULN confirmed by repeat test) occurred in 0.21% (n = 21) of patients treated with simvastatin compared with 0.09% (n = 9) of patients treated with placebo.

The frequencies of adverse events are ranked according to the following: Very common (> 1/10), Common (≥1/100, < 1/10), Uncommon (≥1/1000, < 1/100), Rare (≥1/10,000, < 1/1000), Very Rare (< 1/10,000), not known (cannot be estimated from the available data).

Blood and lymphatic system disorders:

Rare: anaemia

Psychiatric disorders:

Very rare: insomnia

Not known: depression

Nervous system disorders:

Rare: headache, paresthesia, dizziness, peripheral neuropathy

Very rare: memory impairment

Respiratory, thoracic and mediastinal disorders:

Not known: interstitial lung disease (see section 4.4)

Gastrointestinal disorders:

Rare: constipation, abdominal pain, flatulence, dyspepsia, diarrhoea, nausea, vomiting, pancreatitis

Hepato-biliary disorders:

Rare: hepatitis/jaundice

Very rare: hepatic failure

Skin and subcutaneous tissue disorders:

Rare: rash, pruritus, alopecia

Musculoskeletal, connective tissue and bone disorders:

Rare: myopathy* (including myositis), rhabdomyolysis with or without acute renal failure , myalgia, muscle cramps

*In a clinical trial, myopathy occurred commonly in patients treated with Simvastatin 80 mg/day compared to patients treated with 20 mg/day (1.0% vs 0.02%, respectively).

Reproductive system and breast disorders:

Not known: erectile dysfunction

General disorders and administration site conditions:

Rare: asthenia

An apparent hypersensitivity syndrome has been reported rarely which has included some of the following features: angioedema, lupus-like syndrome, polymyalgia rheumatica, dermatomyositis, vasculitis, thrombocytopenia, eosinophilia, ESR increased, arthritis and arthralgia, urticaria, photosensitivity, fever, flushing, dyspnoea and malaise.

Investigations:

Rare: increases in serum transaminases (alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase), elevated alkaline phosphatase; increase in serum CK levels.

Class Effects

• sleep disturbances, including insomnia and nightmares

• memory loss

• sexual dysfunction

• Diabetes Mellitus: Frequency will depend on the presence or absence of risk factors (fasting blood glucose ≥ 5.6 mmol/L, BMI >30kg/m^2, raised triglycerides, history of hypertension).

Children and adolescents (10-17 years of age)

In a 48-week study involving children and adolescents (boys Tanner Stage II and above and girls who were at least one year post-menarche) 10-17 years of age with heterozygous familial hypercholesterolaemia (n=175), the safety and tolerability profile of the group treated with simvastatin was generally similar to that of the group treated with the placebo. The long-term effects on physical, intellectual and sexual maturation are unknown. No sufficient data are currently available after one year of treatment

Drug Interactions

Interaction studies have only been performed in adults

Pharmacodynamic interactions

Interactions with lipid-lowering medicinal products that can cause myopathy when given alone

The risk of myopathy, including rhabdomyolysis, is increased during concomitant administration with fibrates. Additionally, there is a pharmacokinetic interaction with gemfibrozil resulting in increased simvastatin plasma levels (see below *Pharmacokinetic interactions* and sections 4.2 and 4.4). When simvastatin and fenofibrate are given concomitantly, there is no evidence that the risk of myopathy exceeds the sum of the individual risks of each agent. Adequate pharmacovigilance and pharmacokinetic data are not available for other fibrates. Rare cases of myopathy/rhabdomyolysis have been associated with simvastatin co-administered with lipid-modifying doses (≥1g/day) of niacin.

Drug interactions associated with increased risk of myopathy/rhabdomyolysis	
Interacting agents	**Prescribing recommendations**
Itraconazole Ketoconazole Posaconazole Erythromycin Clarithromycin Telithromycin HIV protease inhibitors (eg, nelfinavir) Nefazodone Ciclosporin Danazol Gemfibrozil	Contraindicated with simvastatin
Other fibrates (except fenofibrate)	Do not exceed 10 mg simvastatin daily
Amiodarone Amlodipine Verapamil Diltiazem	Do not exceed 20 mg simvastatin daily
Fusidic acid	Patients should be closely monitored. Temporary suspension of simvastatin treatment may be considered.
Grapefruit juice	Avoid grapefruit juice when taking simvastatin

Effects of other medicinal products on simvastatin

Interactions involving inhibitors of CYP3A4

Simvastatin is a substrate of cytochrome P450 3A4. Potent inhibitors of cytochrome P450 3A4 increase the risk of myopathy and rhabdomyolysis by increasing the concentration of HMG-CoA reductase inhibitory activity in plasma during simvastatin therapy. Such inhibitors include itraconazole, ketoconazole, posaconazole, erythromycin, clarithromycin, telithromycin, HIV protease inhibitors (e.g. nelfinavir), and nefazodone. Concomitant administration of itraconazole resulted in a more than 10-fold increase in exposure to simvastatin acid (the active beta-hydroxyacid metabolite). Telithromycin caused an 11-fold increase in exposure to simvastatin acid.

Therefore, combination with itraconazole, ketoconazole, posaconazole, HIV protease inhibitors (e.g. nelfinavir), erythromycin, clarithromycin, telithromycin and nefazodone is contraindicated. If treatment with itraconazole, ketoconazole, posaconazole, erythromycin, clarithromycin or telithromycin is unavoidable, therapy with simvastatin must be suspended during the course of treatment. Caution should be exercised when combining simvastatin with

certain other less potent CYP3A4 inhibitors: fluconazole, ciclosporin, verapamil, diltiazem (see sections 4.2 and 4.4).

Fluconazole

Rare cases of rhabdomyolysis associated with concomitant administration of simvastatin and fluconazole have been reported (see section 4.4).

Ciclosporin

The risk of myopathy/rhabdomyolysis is increased by concomitant administration of ciclosporin particularly with higher doses of simvastatin (see sections 4.2 and 4.4). Therefore, the dose of simvastatin should not exceed 10mg daily in patients receiving concomitant medication with ciclosporin. Although the mechanism is not fully understood, ciclosporin has been shown to increase the AUC of HMG-CoA reductase inhibitors. The increase in AUC for simvastatin acid is presumably due, in part, to inhibition of CYP3A4.

Danazol

The risk of myopathy and rhabdomyolysis is increased by concomitant administration of danazol with higher doses of simvastatin (see sections 4.2 and 4.4).

Gemfibrozil

Gemfibrozil increases the AUC of simvastatin acid by 1.9-fold, possibly due to inhibition of the glucuronidation pathway (see sections 4.2 and 4.4).

Amiodarone

The risk of myopathy and rhabdomyolysis is increased by concomitant administration of amiodarone with higher doses of simvastatin (see section 4.4). In a clinical trial, myopathy was reported in 6% of patients receiving simvastatin 80mg and amiodarone.

Therefore, the dose of simvastatin should not exceed 20mg daily in patients receiving concomitant medication with amiodarone, unless the clinical benefit is likely to outweigh the increased risk of myopathy and rhabdomyolysis.

Calcium Channel Blockers

• *Verapamil*

The risk of myopathy and rhabdomyolysis is increased by concomitant administration of verapamil with simvastatin 40 mg or 80 mg (see section 4.4). In a pharmacokinetic study, concomitant administration with verapamil resulted in a 2.3-fold increase in exposure of simvastatin acid, presumably due, in part, to inhibition of CYP3A4. Therefore, the dose of simvastatin should not exceed 20 mg daily in patients receiving concomitant medication with verapamil, unless the clinical benefit is likely to outweigh the increased risk of myopathy and rhabdomyolysis.

• *Diltiazem*

The risk of myopathy and rhabdomyolysis is increased by concomitant administration of diltiazem with simvastatin 80 mg. The risk of myopathy in patients taking simvastatin 40mg was not increased by concomitant diltiazem (see section 4.4). In a pharmacokinetic study, concomitant administration of diltiazem caused a 2.7-fold increase in exposure of simvastatin acid, presumably due to inhibition of CYP3A4. Therefore, the dose of simvastatin should not exceed 40mg daily in patients receiving concomitant medication with diltiazem, unless the clinical benefit is likely to outweigh the increased risk of myopathy and rhabdomyolysis.

• *Amlodipine*

Patients on amlodipine treated concomitantly with simvastatin 80 mg have an increased risk of myopathy. The risk of myopathy in patients taking simvastatin 40 mg was not increased by concomitant amlodipine. In a pharmacokinetic study, concomitant administration of amlodipine caused a 1.6-fold increase in exposure of simvastatin acid. Therefore, the dose of simvastatin should not exceed 40 mg daily in patients receiving concomitant medication with amlodipine, unless the clinical benefit is likely to outweigh the increased risk of myopathy and rhabdomyolysis.

Niacin (nicotinic acid)

Rare cases of myopathy/rhabdomyolysis have been associated with simvastatin co-administered with lipid-modifying doses (≥ 1g/day) of niacin (nicotinic acid). In a pharmacokinetic study, the co-administration of a single dose of nicotinic acid prolonged-release 2g with simvastatin 20mg resulted in a modest increase in the AUC of simvastatin and simvastatin acid and in the C_{max} of simvastatin acid plasma concentrations.

Fusidic acid

The risk of myopathy may be increased by concomitant administration of

fusidic acid with statins, including simvastatin. Isolated cases of rhabdomyolysis have been reported with simvastatin. Temporary suspension of simvastatin treatment may be considered. If it proves necessary, patients on fusidic acid and simvastatin should be closely monitored (see section 4.4).

Grapefruit juice

Grapefruit juice inhibits cytochrome P450 3A4. Concomitant intake of large quantities (over 1 litre daily) of grapefruit juice and simvastatin resulted in a 7-fold increase in exposure to simvastatin acid. Intake of 240ml of grapefruit juice in the morning and simvastatin in the evening also resulted in a 1.9-fold increase. Intake of grapefruit juice during treatment with simvastatin should therefore be avoided.

Colchicine

There have been reports of myopathy and rhabdomyolysis with the concomitant administration of colchicine and simvastatin in patients with renal insufficiency. Close clinical monitoring of such patients taking this combination is advised.

Rifampicin

Because rifampicin is a potent CYP3A4 inducer, patients undertaking long-term rifampicin therapy (e.g. treatment of tuberculosis) may experience loss of efficacy of simvastatin. In a pharmacokinetic study in normal volunteers, the area under the plasma concentration curve (AUC) for simvastatin acid was decreased by 93% with concomitant administration of rifampicin.

Effects of simvastatin on the pharmacokinetics of other medicinal products

Simvastatin does not have an inhibitory effect on cytochrome P450 3A4. Therefore, simvastatin is not expected to affect plasma concentrations of substances metabolised via cytochrome P450 3A4.

Oral anticoagulants

In two clinical studies, one in normal volunteers and the other in hypercholesterolaemic patients, simvastatin 20 - 40mg/day modestly potentiated the effect of coumarin anticoagulants: the prothrombin time, reported as International Normalized Ratio (INR), increased from a baseline of 1.7 to 1.8 and from 2.6 to 3.4 in the volunteer and patient studies, respectively. Very rare cases of elevated INR have been reported. In patients taking coumarin anticoagulants, prothrombin time should be determined

before starting simvastatin and frequently enough during early therapy to ensure that no significant alteration of prothrombin time occurs. Once a stable prothrombin time has been documented, prothrombin times can be monitored at the intervals usually recommended for patients on coumarin anticoagulants. If the dose of simvastatin is changed or discontinued, the same procedure should be repeated. Simvastatin therapy has not been associated with bleeding or with changes in prothrombin time in patients not taking anticoagulants.

APPENDIX 2: ASSESSMENT MEASURES

Screening measure

- **Social Responsiveness Scale (SRS)**

The SRS is a standard measure of ASD symptoms consisting of 65 items rated on a 4 point likert scale (from 1= never true to 4= almost always true). It includes five dimensions: social awareness, social cognition, reciprocal social communication, social motivation and autistic mannerisms, summed into raw and T scores (the latter based on population norms). SRS Total T scores of 76T or higher is in the severe range associated with a clinical diagnosis of ASD. SRS has sensitivity of 0.85 and specificity of 0.75 for identifying independent consensus expert clinician diagnosis of ASD in validation studies [32], and sensitivity 0.78 and specificity 0.68 with positive predictive value to ASD diagnosis of 0.63 in a large independent longitudinal study (n=119).[1]Scores of between 60T-75T are considered as being in the mild to moderate range indicating deficiencies in reciprocal social interaction which may be clinically significant and below 59T are considered as being in the normal range. SRS scores are found to be generally unrelated to IQ and show good discrimination between ASD and other childhood psychiatric disorders. They are highly correlated (*r*=0.65-0.77) with results on the gold-standard Autism Diagnostic Interview-Revised (ADI-R).

Phenotype assessment measures

- **Autism Diagnostic Interview-Revised (ADI-R)**

The ADI-R is an investigator based parent interview about an individual's early childhood and current communication and social development and restricted, repetitive, stereotyped behaviours and interests. Algorithm scores are generated based on behavioural descriptions of the individual at 4-5 years for some items and at any time in their lives for other items. ADI-R is considered to be the gold standard instrument for the assessment and diagnosis of pervasive developmental disorders. It had good test-retest and inter-rater reliability >0.9 and concurrent validity (agreement with independent clinical formulation) of 0.74.

- **Autism Diagnostic Observation Schedule- Generic (ADOS-G)**

The ADOS-G is a semi-structured, standardized observational assessment of communication, social interaction, play and imaginative skills and repetitive behaviours. It consists of four modules each appropriate to different developmental age and expressive language skills. Scores on individual items range from '0' (no evident abnormality) to '3' (marked abnormality) and each

module provides scores on four domains of communication, social interaction, imagination and restricted, repetitive behaviours. It has established cut-offs for both autism and ASD. It has good concurrent validity with the ADI-R.

- **Wechsler Abbreviated Scale of Intelligence (WASI)**

The WASI is a reliable brief measure of intellectual ability widely used in clinical, educational and research setting for ages 6-89 years. It has four sub-tests which include matrix reasoning, block design, vocabulary and similarities. The four sub-tests can be combined for a full scale IQ. For this trial only the vocabulary and similarities sub-tests of the WASI will be used to assess child verbal IQ. The average reliability coefficient of WASI is 0.96-0.98, inter-rater reliability 0.98 for vocabulary and 0.99 for similarities.

Behavioural phenotype measures at baseline, 4 weeks and 12 weeks

- ***Clinical Global Impression Scale (CGI)***

This is a standardized assessment tool that allows the clinician to rate the severity of illness, change over time, and efficacy of medication, taking into account the patient's clinical condition and the severity of side effects. The CGI Scale is widely used in clinical psychopharmacology trials as an outcome measure. It consists of three global subscales formatted for use with the Global Scoring Sheet. The first subscale, Severity of Illness, assesses the clinician's impression of the patient's current illness state; it is often used both before and after treatment. Global Improvement subscale assesses the patient's improvement or worsening from baseline, which is usually the beginning of a clinical trial. The third subscale, the Efficacy Index, attempts to relate therapeutic effects and side effects by deriving a composite score that reflects both the therapeutic effect and the concomitant adverse reactions or side effects.

Scores on the Severity of Illness subscale range from 1 = not ill at all to 7 = among the most extremely ill. The Global Improvement subscale also goes from 1 = very much improved to 7 = very much worse. The Efficacy Index involves locating a rating on a matrix of therapeutic versus side effects with scores ranging from 0 = marked improvement and no side effects to 4 = unchanged or worse and side effects outweigh therapeutic effects. It takes a clinician only 1–2 minutes to score the CGI Scale after a clinical interview.

- **Parent Rated target symptoms**

This approach has been used in several drug trials in autism/ADHD research carried out by the Research Units of Pediatric Psychopharmacology. Parents are asked for chief complaints/or symptoms in their child of most concern to them. Clinician's blind to treatment allocation are asked to rate the parent-defined symptom on a 9 point scale at predetermined intervals during the trial.

This approach is described as being a reliable, sensitive, efficient and consumer-friendly way to assess treatment effects.

- **Aberrant Behaviour Checklist-C (ABC-C)**

The ABC-C is a symptom checklist for assessing problem behaviours of children and adults with mental retardation . It has 58 items which resolve into 5 subscales- irritability and aggression, lethargy and social withdrawal, stereotypic behaviour, hyperactivity and non-compliance and inappropriate speech. It is widely used in paediatric psychopharmacology trials and has good documented psychometric properties.

- **Connors Parent Rating Scale-Research**

Conner's Parent Rating Scale–Revised:(CPRS) is a standard measure for ADHD symptoms. It consists of 27 items rated on a 4 point likert scale (from 0= not true at all to 3= very much true) in 4 subscales: oppositional, hyperactivity, cognitive problems and ADHD index. An ADHD index T score of greater than 65 is considered clinically significant, showing sensitivity of 92.3% and specificity of 94.5% for identifying clinical consensus diagnosis for ADHD in validation studies.

<u>Cognitive assessment measures at baseline and 12 weeks</u>

- **Judgement of line orientation task**

The judgement of line orientation task is a widely used measure of visuospatial judgement that was originally conceptualised by Dr Arthur L Benton et al. This test measures accuracy of angular orientation based on judgements about a pair of angled lines that visually match an identical pair immersed within a semicircular array of 11 lines. Patients are asked to say which two lines from the array in the bottom of the page are exactly in the same position and point in the same direction as the two lines at the top of the page. The original format has 30 items but more recent versions are shorter and can have 15 items. This will be a computerised task.

- **Paired associates learning task**

This test assesses visual memory and new learning. It takes around 10 minutes to administer. Boxes are displayed on the screen and are opened in a randomised order. One or more of them will contain a pattern. The patterns are then displayed in the middle of the screen, one at a time and the participant must touch the box where the pattern was originally located. If the participant makes an error, the patterns are re presented to remind participants of their locations. The level of difficulty increases through the test.