Neurofibromatosis Type 1 (NF1) Clinical Trials Consortium

Protocol for the
The NF1 STAtin Randomized Study (NF1 STARS)

A Randomized Placebo-Controlled Study of Lovastatin in Children with Neurofibromatosis Type 1

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1.0 Background and Significance

1.1 Neurofibromatosis and Cognitive Deficits

NF1 is a common autosomal dominant disorder with an incidence of 1 in 3,000 and is characterized by diverse cutaneous, neurological, skeletal and neoplastic manifestations (North, 1998). The most common neurological complication of NF1 in childhood is cognitive dysfunction (North, Hyman & Barton, 2002). Knowledge regarding the cognitive profile of children with NF1 has dramatically increased over the past 15 years. Many studies have shown a slight yet robust reduction of general intellectual function, with a downward shift in the distribution of IQ scores when compared to unaffected siblings, controls and normative data (Levine, Materek, Abel, O'Donnell & Cutting, 2006; Ozonoff, 1999). While general intellectual functioning is only mildly affected, one of the most telling areas indicative of cognitive deficits in NF1 is academic achievement, with up to 70 percent of school-aged children with NF1 underachieving (Brewer, Moore & Hiscock, 1997) and learning disabilities estimated to be present in 30 to 65 percent of children (North et al., 1997). Areas of difficulty appear to encompass a range of academic skills including reading, spelling and mathematics, with no one area predominately more affected.

As well as academic difficulties, studies consistently show children with NF1 have impaired performance on tasks of visual spatial function such as the Judgment of Line Orientation, a task associated with activation of the parietal and occipital lobes (Hyman, Shores & North, 2005; Kesler, Haberecht, Menon, Warsofsky & Dyer-Friedman et al., 2004; Levine et al., 2006). In addition, there is also a high incidence of executive dysfunction, including reduced cognitive flexibility, attention, working memory capacity, inhibition and planning; all functions thought to be mediated by the prefrontal cortex (Anderson, Anderson, Northam, Jacobs & Mikiewicz, 2002; Hyman et al., 2005). There is also a high incidence of attention deficit hyperactivity disorder (ADHD; Mautner, Kluwe, Thakker & Leark, 2002) and results from a recent study found that 63% of children with NF1 have poor sustained attention (Hyman et al., 2005). Other reported areas of impairment include verbal memory, language and psychomotor deficits (Ferner, Hughes & Weinman, 1996; Mazzocco, Turner, Denckla, Hofman, Scanlon et al., 1995).

Given that general intellectual functioning is not the key explanation for the pattern of observed academic failure, it is likely that these specific neurocognitive deficits contribute to or are responsible for the characteristic profile of children with NF1. As such, these NF1 associated deficits may negatively impact on daily living skills, academic performance, employment opportunities and quality of life (Graf, Landolt, Mori & Boltshauser, 2006; Page, Page, Ecosse, Korf, Leplege et al., 2006). Despite the significant negative impact of this disorder on cognitive and behavioral functioning, only one study has reported on treatment outcomes (Mautner et al., 2002). The results of this study demonstrated that stimulant medication was effective in treating the behavioral symptoms of ADHD in children with NF1. To date, no studies have examined the

effects of interventions on the cognitive functioning of children with NF1. Clearly, there is a need to investigate interventions that can improve cognitive impairments associated with NF1.

1.2. Biological Basis of Cognitive Deficits in NF1

Understanding the underlying genetic defect of NF1 has become critical in explaining the spectrum of clinical manifestations of this disorder, from benign and malignant tumor growth to cognitive dysfunction. The NF1 gene encodes a 250kDa protein called neurofibromin which is expressed in many tissues, including neurons, Schwann cells, and oligodendrocytes as well as in many non-neural cell types (Daston & Ratner, 1992; Daston, Scrable, Nordlund, Sturbaum, Nissen et al., 1992). Although the function of neurofibromin is not clearly understood, neurobiological evidence shows it plays a role in several biochemical processes, including adenylyl cyclase modulation (Guo, The, Hannan, Bernards & Zhong, 1997), microtubule binding (Xu & Gutmann, 1997) and mammalian target rapamycin signaling (Dasgupta, Yi, Chen, Weber & Gutmann, 2005; Johannessen, Reczek, James, Brems, Legius et al., 1995).

It is the effect of neurofibromin on ras, a protein implicated in cell proliferation and differentiation that has been most extensively investigated (e.g., Martin, Viskochil, Bollag, McCabe, Crosier et al., 1990; Weiss, Bollag & Shannon, 1999; Xu, Lin, Tanaka, Dunn, Wood et al., 1990). These studies suggest that neurofibromin contains a GTPase-activating protein domain that functions to accelerate the conversion of active GTP-bound ras to its inactive GDP-bound form. Loss of neurofibromin within a cell would thus result in constitutive activation of the ras signaling pathway, ultimately resulting in increased ras activity and unregulated cell proliferation and differentiation (Bollag, Clapp, Shih, Adler, Zhang et al., 1996; DeClue, Papageorge, Fletcher, Diehl, Ratner et al., 1992; Hiatt, 2001; Martin et al., 1990).

An important experimental model used to study the effects of diminished amounts of neurofibromin on cognitive functioning is the *Nf1* mouse model. Importantly, human and mouse forms of neurofibromin are highly homologous (98% sequence similarity) as are the promoter sequences of the gene, suggesting that both the biochemistry of the protein and the transcriptional regulation of the gene are conserved across species (Bernards, Snijders, Hannigan, Murthy & Gusella, 1993; Hajra, Martin-Gallardo, Tarle, Freedman, Wilson-Gunn et al., 1994). Silva and colleagues have demonstrated that mice heterozygous for a targeted mutation in the *Nf1* gene (*Nf1* +/- mice) exhibit cognitive deficits associated with enhanced GABA inhibition and reduced long-term potentiation (LTP; Costa, Federov, Kogan, Murphy, Stern et al., 2002; Costa & Silva, 2002; Silva, Frankland, Marowitz, Friedman, Laszlo et al., 1997). The *Nf1* +/- mouse model of cognitive dysfunction appears to mimic aspects of certain cognitive deficits in children with NF1 and is not related to tumor formation (Costa, Yang, Huynh, Pulst, Viskochil et al., 2001). These deficits primarily involve enhanced GABA-mediated inhibition affecting hippocampal-dependent and prefrontal-mediated tasks, including spatial memory and new learning, visual spatial attention, working memory and contextual discrimination (Costa et al., 2002; Li, Cui, Kushner, Brown, Jentsch et al., 2005).

1.3 Rationale for the Use of Lovastatin in Children with NF1

Lovastatin, a fungal antibiotic, is a specific inhibitor of the rate-limiting enzyme in cholesterol biosynthesis (a non-selective HMG-CoA reductase inhibitor), and is widely used in the treatment of hyperlipidemia in humans (Xu, McGuire, Blaskovich, Sebti & Romero, 1996). It is one of the first-generation statins and therefore has a large body of safety and pharmacokinetic data from clinical trials and general use for hyperlipidemia (Corsini, Maggi & Catapano, 1995b). Previous studies have shown that Lovastatin can inhibit small GTPase (including ras) isoprenylation and activity (Sebti, et al., 1991; Mendola and& Backer, 1990; Sebti, Tkalcevic & Jani, 1991).

Although it is difficult to draw direct correlations between the murine *Nf1* model and the neurocognitive changes in children, excessive ras activity and learning impairment observed in *Nf1*+/- mice suggests a possible substrate in part for the cognitive deficits demonstrated in children with NF1. As such, therapeutic interventions designed to decrease ras function have been proposed as treatments for NF1 (Weiss et al., 1999).

A recent study by Li and colleagues (2005) used Lovastatin to evaluate its benefit on cognitive deficits in *Nf1*+/- mice. *Nf1*+/- and wild-type mice were treated with either Lovastatin or placebo before completing a task of attention, visual spatial learning or pre-pulse inhibition. Mice completing the pre-pulse inhibition or visual spatial learning tasks were injected with 10 mg/kg of Lovastatin subcutaneously for three days before training and *Nf1*+/- mice completing the attention task were administered 0.15 mg of Lovastatin orally, once per day. *Nf1*+/- mice treated with Lovastatin demonstrated improved performance relative to mice on placebo on all cognitive and behavioral measures as follows:

- Attention – using a lateralized reaction time task, mice treated with Lovastatin were significantly more accurate at detecting the location of an unpredictable target stimulus than mice in the placebo group. This measure of divided visuospatial attention is dependent on the pre-frontal cortex;
- Spatial learning – using the hidden version of the Morris Water Maze (Morris, 1984), mice treated with Lovastatin spent significantly more time searching in the target quadrant of the maze compared with *Nf1*+/- mice given the placebo. This task is sensitive to lesions in the hippocampus as well as other structures within the limbic system (Mogensen, Moustgaard, Khan, Wortwein & Nielsen, 2005; Steffenach, Witter, Moser & Moser, 2005), suggesting the observed improvement in the Lovastatin group maybe due to increased LTP (a hippocampus-related learning process; Lynch, 2004), which has been shown to be defective in *Nf1* +/- mice (Costa et al., 2002; Silva et al., 1997);
- Pre-pulse inhibition (PPI) – *Nf1*+/- mutant mice treated with Lovastatin had an increased PPI when compared to the placebo group. PPI is a measure of sensory gating of environmental stimuli, so that a startle reflex is inhibited if it is preceded by a weak prestimulus. PPI has been shown to be significantly reduced in children with ADHD (Castellanos, Fine, Kaysen, Marsh, Rapoport et al., 1996), which has a high co-morbidity

with NF1 (38-46% of NF1 children satisfy DSM-IV diagnostic criteria for ADHD; Hyman et al., 2005; Hyman, Shores & North, 2006).

Interestingly, the dose of Lovastatin effective in *Nf1*+/- mice did not affect cognitive function in control mice, suggesting Lovastatin (which inhibits ras activation) has a beneficial effect on cognitive function in the setting of abnormal ras activation (as in NF1), but not when normal inhibition of ras activation is intact.

In addition to these cognitive experiments, Li and colleagues (2005) examined the effects of Lovastatin at a biochemical level. Treatment with Lovastatin was found to decrease ras activity in *Nf1*+/- mice as well as reverse LTP deficits.

Collectively, these results provide strong evidence that Lovastatin can reverse the cognitive and physiological deficits observed in *Nf1*+/- mice to levels comparable to wild-type control mice (Li et al., 2005). Critically, this indicates that the cognitive deficits observed in *Nf1*+/- mice are not caused by irreversible developmental abnormalities since they have been reversed with acute Lovastatin treatment in mutant mice. These experiments outline important parallels between the cognitive deficits in mice and humans with NF1 and have relevant implications for the development of treatment strategies for these deficits through modification of ras activity or GABA mediation (Costa, et al., 2002, 2001).

In summary, the highly homologous nature of neurofibromin in mice and humans make murine models of NF1 an appealing medium to study the pathologies associated with NF1, such as altered molecular pathways and cognitive function. Studies employing mouse models of NF1 have been crucial in identifying an increase in ras activity and GABA-mediated inhibition as key mechanisms behind the observed cognitive deficits (Weeber & Sweatt, 2002). Crucially, studies have also shown that pharmacological intervention (Lovastatin) not only decreased ras activity and GABA-mediated inhibition, but also reversed cognitive deficits. This suggests that similar effects of Lovastatin may also be observed in children with NF1-associated cognitive deficits. The aim of this study is to examine the efficacy of Lovastatin on the cognitive deficits of children with NF1. The use of oral Lovastatin in this study will be the first biologically-based, molecularly targeted treatment for children with NF1 and cognitive deficits.

1.4 The Tolerability and Safety of Lovastatin in Children with NF1

The safety and tolerability of Lovastatin in children with NF1 has recently been examined in a Phase I open label study. Lovastatin was orally administered to children with NF1 aged 10-17 years with normal cholesterol levels. Doses of 20, 30 or 40 mg/day were administered using a conventional Phase I design, with dose escalation to determine maximum tolerated dose. This study enrolled a total of 23 children with a diagnosis of NF1 aged between 10-17 years. The 20mg and 30mg cohorts included 3 patients each and the 40mg cohort contained 17 patients. All patients were clinically stable and those with symptomatic intracranial lesions were excluded. All three groups were found to tolerate the dose well for the duration of the study (3 months) and no significant side effects specifically related to Lovastatin were reported. Medication was very

well tolerated in all patients with minimal side effects. Side effects described include: headache, nausea and dizziness. Quantitatively, Lovastatin did not have a significant effect on liver or pancreatic enzymes, creatine phosphokinase (CPK), myoglobinuria, complete blood count (CBC), renal function, or lipid profiles, with all laboratory results within normal parameters for all patients. Lovastatin did have a significant effect on total cholesterol levels, with a 18% decrease in mean levels (range ↓28% to ↑8%) from the pre-treatment to post-treatment. However, different subtypes of cholesterol (LDL, HDL, VLDL) stayed within the normal limits. The most significant changes were observed in LDL levels, with changes between increments of 11% to decrease in 45% compared with the initial value. The decrease in cholesterol levels were independent of dosage levels and all groups remained within the normal limits (Acosta et al, submitted 2010)

1.5 Lovastatin for Treatment of Familial Hypercholesterolemia in Children

Evidence from clinical studies indicates the safety of Lovastatin (up to 40mg/day) for the treatment of familial hypercholesterolemia (heFH) in children. The safety and effectiveness of Lovastatin in 132 adolescent boys (10 to 17 years of age) with heFH has been evaluated in a doubled-blind, placebo-controlled study of 48 weeks duration (Stein, Illingworth, Kwiterovich Jr., Liacouras, Siimes et al., 1999). Adolescent boys were randomized to Lovastatin (n=67) or placebo (n=65). The dose of Lovastatin was started at 10 mg/day and increased at eight week intervals (8 weeks and 16 weeks) to 20 and 40 mg/day. Treatment with Lovastatin was considered safe, and the side-effect profile of the two groups (Lovastatin and placebo) was not significantly different. Additionally, Lovastatin did not have a significant effect on growth hormonal, and nutritional status at 24 and 48 weeks. No significant differences in increase serum levels of CPK and/or aspartate amino transferase (AST) were observed between the groups. Compared to placebo, the low density lipoprotein (LDL) cholesterol levels of boys receiving Lovastatin decreased significantly by 17%, 24%, and 27% receiving dosages of 10, 20, and 40 mg/d respectively (Kwiterovich, Jr., 2001; Stein et al., 1999).

The safety, tolerability and efficacy of Lovastatin was also evaluated in 54 adolescent girls (10 to 17 years) who were enrolled in a double-blind, randomized, placebo-controlled study over 24 weeks (Clauss, Holmes, Hopkins, Stein, Cho et al., 2005). Similar to the findings in boys, Lovastatin was efficacious at reducing LDL cholesterol by 23% to 27%, total cholesterol by 17% to 22% and apolipoprotein B (Apo B) by 20% to 23% at weeks 4 and 24 respectively. Importantly, Lovastatin was also found to be safe and well tolerated with no clinically significant alterations in vital signs, anthropomorphic measurements, hormone levels, menstrual cycle length or tests of liver and muscle function.

In another randomized double-blind trial, the efficacy, safety, and tolerability of Lovastatin was evaluated in 69 adolescent boys (9 to 17 years) with heFH (Lambert, Lupien, Gagne, Levy, Blaichman et al., 1996). Following a four-week placebo run-in period, the participants were randomized to receive active treatment with Lovastatin (10, 20, 30, or 40 mg/day) for eight weeks. Three children who received 30 or 40 mg/day of Lovastatin experienced transient AST elevations. A total of 10 children reported 13 adverse clinical events while receiving Lovastatin

including headache, fatigue, heartburn, jaundice, muscle cramps, chest pain, lymph node enlargement, and cold-and flu-like symptoms. Adverse events did not appear to be dose related (Lambert et al., 1996). There were no serious adverse experiences reported. None of the reported clinical adverse events or laboratory abnormalities required early withdrawal from the study.

In general, Lovastatin is considered a safe medication, and has been approved for the treatment of familial heFH in children 10 to 17 years old (Clauss et al., 2005; Stein et al., 1999). These studies, combined with results from the aforementioned Phase I study (Acosta et al., submitted), indicate that Lovastatin in doses of 40mg/day should be safe and well-tolerated in children with NF1.

1.6 Potential Cognitive Effects of Lovastatin in Individuals with Hypercholesterolemia

Very few studies have examined the potential cognitive effects of Lovastatin in humans. In one study, cognitive effects were assessed in adults with heFH who were randomized to receive 20 mg Lovastatin (n=98) or placebo (n=96) over a 6-month period (Muldoon, Barger, Ryan, Flory, Lehoczky et al., 2000). Results from this blinded randomized control trial indicated that at 6 months follow-up, control participants had improved on all five domains of cognitive functioning (attention, psychomotor speed, mental flexibility, working memory and memory recall), most likely due to practice and/or learning effects. Participants on Lovastatin improved only on measures of memory recall. Further sub-analysis indicated that only participants treated with Lovastatin with a lower mean LDL cholesterol level at follow-up (mean 109 ± 11mg/dL (2.79 ± 0.28mmol/l)) had a small decrease in cognitive function from baseline. However, total serum cholesterol in the lower range (<200mg/dL (5.13mmol/l)) has been associated with poorer performance on cognitive measures which place demands on abstract reasoning, attention/concentration, word fluency and executive functioning (Elias, Elias, D"Agostino, Sullivan, Wolf et al., 2005). Therefore, it plausible that lower cholesterol levels contributed to poorer cognitive performance observed by Muldoon et al. (2000).

Other studies that have examined cognitive performance of adults with heFH when treated with Lovastatin generally report no significant effect on measures of cognitive performance (Kostis, Rosen & Wilson, 1994; Gibellato, Moore, Selby & Bower, 2001). However, one study found that after four weeks of treatment with Lovastatin, patients with heFH performed significantly better on one cognitive task (digit symbol substitution) when compared to baseline (Gengo, Cwudzinski, Kinkel, Block, Stauffer et al., 1995). There were no significant changes for other cognitive tasks.

In summary, no studies have examined the potential cognitive effects of Lovastatin in individuals without heFH. The results from studies of individuals with heFH suggest no significant cognitive effects of Lovastatin. This is consistent with observations in control mice, in that the dose of Lovastatin effective in reversing cognitive deficits in *Nf1*+/- mice did not affect cognitive function in control mice (Li et al., 2005). However, it is important to consider that our current study is targeting a population that has a metabolic alteration that is susceptible to modification

from Lovastatin. Thus, the anticipated cognitive benefits of Lovastatin in children with NF1 are not necessarily expected in individuals with a normal metabolic pathway.

1.7 Use of Lovastatin for the Treatment of Other Conditions

Lovastatin has been used in several other clinical trials, including Phase I studies of adult participants with recurrent or metastatic squamous cell carcinoma of the head, neck, or cervix and in other solid tumors with maximum doses of between of 7.5 mg/kg and 45mg/kg/day (Knox, Siu, Chen, Dimitroulakos, Kamel-Reid et al., 2005; Thibault, Samid, Tompkins, Figg, Cooper et al., 1996). Thibault and colleagues (1996) conducted a Phase I trial to characterize the tolerability of Lovastatin administered at progressively higher doses to cancer patients. They were treated with a 7-day course of Lovastatin given monthly (over a 20 month period), with doses ranging from 2 to 45 mg/kg/day. They found cyclical treatment with Lovastatin reduced cholesterol concentrations by up to 43% when compared with pre-treatment levels. Very high doses of Lovastatin (25 mg/kg/day) were very well tolerated for seven days. Myopathy was the dose limiting toxicity and found to be prevented by ubiquinone supplementation (Thibault et al., 1996). In another Phase I study for treatment of cancer in adults, Lovastatin has been used in doses up to 7.5 mg/kg/day for up to 28 days with a good tolerability and limited side effects (Knox et al., 2005). In this study, the major side effects were related to myopathy and renal insufficiency (Knox et al., 2005). Doses even higher up to 45 mg/kg/day have been used for a seven day cycle per month up to 20 months with similar findings (Thibault et al., 1996). Current studies are not conclusive about the benefits of Lovastatin in cancer however in most of the cases, the dosage exceeded the maximum recommended dose for the standard treatment of heFH (current dose recommended in adults is up to 80 mg/day). Doses used in the aforementioned studies have been calculated in most cases by kg/weight (up to 45 mg/kg/day).

Lovastatin also has been used in patients with adrenoleukodystrophies to decrease very long chain fatty acids, where dosage was increased from 20mg to 40mg in two weeks (Pai, Khan, Barbosa, Key, Craver et al., 2000). No significant changes in cholesterol levels and no correlation between the dose and decrease of cholesterol levels were shown (Pai et al., 2000). Side effects were reported in 2-3%, including weakness, abdominal pain, constipation, diarrhea, dyspepsia, flatulence, nausea, myalgia, muscle cramps, dizziness, headache, skin rash, and blurred vision (Pai et al., 2000). One patient had adverse events severe enough to discontinue Lovastatin. Lovastatin-induced myopathy or marked elevation of CPK is expected to occur in <1:500 participants undergoing such therapy for heFH (Pierce, Wysowski & GrossPierce, 1990; Marais & Larson, 1990).

1.8 Comparisons between the Nf1 Murine Model and Children with NF1

While results from *Nf1*+/- mice provide evidence that Lovastatin may be effective in treating cognitive deficits in children with NF1, it is difficult to make direct comparisons between the Nf1 murine model and the neurocognitive functioning of children with NF1. The precise mechanism underlying the Lovastatin reversal of the cognitive phenotype in *Nf1*+/- mice is not completely understood. In addition, results from animal studies are limited in their direct

application to humans due to biological and cognitive differences. A recent systematic review of the treatment effects between animal experiments and human clinical trials for six different interventions found disconcordance for three interventions (Perel, Roberts, Sena, Wheble, Briscoe et al., 2006). The lack of concordance may be due to bias, random error or the failure of animal models to mimic human disease adequately (Perel et al., 2006).

Cognitively, the capabilities of mice are limited to basic levels of functioning and it is not possible to assess the full effects of Lovastatin using this model. Thus, the potential effects of Lovastatin in children with NF1 may not be fully realized by the evidence provided by animal studies. It is possible that the neurocognitive functions targeted by Lovastatin could be underestimated in children with NF1. For example, it is possible that the action of Lovastatin on LTP (i.e., it has shown to increase LTP in *Nf1* +/- mice relative to placebo controls) could form and strengthen neural connections not only in the hippocampus (associated with learning and memory), but also induce similar effects of neural connectivity and plasticity throughout the brain of children with NF1, resulting in significant improvements across a variety of cognitive domains.

1.9 Rationale for Outcome Measures: Neuropsychological Assessment

Neuropsychological assessment plays a critical role in identifying and monitoring central nervous system (CNS) dysfunction in children with developmental, acquired, and medical disorders, including NF1. Objective standardized tests assessing general cognitive ability and specific neuropsychological domains are reliable and valid measures that are sensitive to changes in CNS function, including cognitive improvements from pharmacological treatments (e.g. Bedard, Martinussed, Ickowicz & Tannock, 2004; Tucha, Mecklinger, Laufkotter, Klein, Walitza et al., 2006). As a result, neuropsychological test scores have been extensively used as cognitive outcome measures in clinical trials, including those with children (e.g., Bedard et al., 2004; Kempton, Vance, Maruff, Luk, Costin et al., 1999; Mehta, Goodyer & Sahakian, 2004; Mohr & Brouwers, 1991). When selecting neuropsychological tests to be used in clinical trials, various guidelines should be followed (Ruff & Crouch, 1991). First, tests should be selected that will assess the specific cognitive domains that are affected and may be changed by treatment. Second, as the tests need to be administered repeatedly, it is optimal that alternate forms be used where possible to minimize test-retest effects. Finally, the tests should be standardized measures with documented reliability and validity.

The selected measures in this study are drawn from both traditional pencil-and-paper neuropsychological tests as well as two computerized tasks which are sensitive to detecting treatment effects – the Cambridge Neuropsychological Testing Automated Battery (CANTAB, reviewed by Luciana, 2003) and the Conners‟ Continuous Performance Test-II (CPT-II; Conners, 2000). The CANTAB is a neuropsychological assessment battery that consists of 22 stand-alone subtests (we will administer 4 subtests only), developed to assess aspects of visual spatial learning, executive function, attention, semantic/verbal memory, decision making and response control. The CANTAB was selected as an outcome measure for a number of reasons. First, it has a number of subtests, including a visual spatial learning task that may potentially be

affected by Lovastatin. Second, the CANTAB has evolved from animal behavior paradigms to facilitate cross-species studies of cognition. As such, it has strong theoretical underpinnings. Third, it has been designed with a neural systems approach in mind, being extensively validated in patients with damage in specific areas of the brain. Fourth, it has extensive functional neuroimaging data available, outlining the neural substrates involved in many of the subtests, providing a useful confirmation of the neuroanatomical basis of the tests. Fifth, the tests are entirely computerized, enabling automatic recording of response accuracy and latencies via a touch-screen apparatus. This increases the consistency of data collection between sites. In addition, there are five equivalent forms available, minimizing retest practice effects. Subtests from the CANTAB start at a simple level and gradually increase in difficulty. Finally, research suggests that computerized batteries, in particular the CANTAB, are sensitive to subtle neurocognitive changes and may be better able to detect subtle cognitive dysfunction and changes over time than traditional paper-and-pencil neuropsychological measures (Bedard et al., 2004; Froestl, Gallagher, Jenkins, Madrid, Melcher et al., 2004; Makdissi, Collie, Maruff, Darby, Bush et al., 2001; Nestor, Scheltens & Hodges, 2004).

In this study, the primary outcome measures have been selected based on the effects of Lovastatin in murine models of NF1 and the cognitive functions that are frequently impaired in children with NF1. Therefore, the primary outcome measures assess visual spatial learning and sustained attention. Visual spatial learning will be assessed using the Paired Associate Learning (PAL) subtest from the CANTAB (see Section 5.1.4.1). Preliminary findings indicate that 46% (13/28) of children with NF1 performance on the PAL is impaired (>1SD below normative mean; Payne, Barton & North, unpublished data). Sustained attention will be assessed using the Score! subtest from the Test of Everyday Attention for Children (TEA-Ch). Previous findings indicate that 63% (50/80) of children with NF1 have impaired performance (>1SD below normative mean) on the Score! subtest (Hyman et al., 2005).

Since evidence from the murine model of NF1 suggests that Lovastatin may improve functions that rely on prefrontal cortex, such as response inhibition and the effect of Lovastatin on cognitive impairments is not known, a broader battery of neuropsychological tests have been included as secondary outcome measures. These include multidimensional measures of attention (selective, divided and switching attention from the TEA-Ch; see Hyman et al., 2003, 2005, 2006), executive functioning (e.g. response inhibition, planning and organization) and visuospatial ability, as well as quality of life.

The other computer-based measure, the CPT-II, has a significant history of use in identifying attentional deficits and the effects of stimulant medication (e.g., Boonstra, Kooij, Oosterlaan, Sergeant, & Buitelaar, 2005; Conners, Epstein, Angold, & Klaric, 2003). CPTs have been used in previous studies of children with NF1 (Ferner et al., 1996; Mautner et al., 2002; Mazzocco et al., 1995). Results from a recent study using the Kiddie‟s CPT, indicated that children with NF1 made significantly more omission (inattentive) errors than the control group (Irle, Shores, Watt & North, unpublished data).

1.10 Summary and Overview of Study

The most common neurological complication of NF1 in childhood is cognitive deficits for which there are no effective specific pharmacological treatments. Studies indicate that Lovastatin can reverse the biochemical, electrophysiological and cognitive deficits observed in *Nf1*+/- mice to levels comparable to wild-type control mice. *Nf1*+/- mice treated with Lovastatin demonstrated improved performance on tasks of spatial learning and memory (as measured by the Morris Water maze), attention, and pre-pulse inhibition. These tasks are hypothesized to reflect the cognitive deficits observed in children with NF1. The Morris Water Maze involves mechanisms of learning and memory, which relies on hippocampal functioning. While visual-spatial deficits have been reported in children with NF1 (e.g., Schrimsher, Billingsley, Slopis & Moore, 2003), no studies have reported on the visual spatial learning abilities in this cohort.

Lovastatin, which targets the inhibition of ras, is a logical choice as a potential therapeutic intervention for children with NF1 and cognitive deficits. Clinical trials indicate that Lovastatin is effective and safe to treat heFH in adults and children. Results from a recently completed Phase I trial indicate that Lovastatin is safe to be used in children (10-17 years) with NF1 (Acosta et al., submitted).

Currently, no studies have examined the effect of Lovastatin on cognitive functioning in individuals with NF1. This study will determine the efficacy of Lovastatin as a treatment for the cognitive deficits observed in children with NF1. We will conduct a multi-center randomized, double-blind, Phase II trial with two treatment arms (Lovastatin/placebo) for 16 weeks. Based on predictions from the NF1 murine mouse model, it is expected that children with NF1, who are impaired on a visual spatial learning task and/or an attention task, will benefit from Lovastatin.

2.0 Research Plan

2.1 Specific Aim

The specific aim of this study is to determine whether Lovastatin significantly improves visual spatial learning and/or sustained attention in children with NF1.

2.2 Secondary Aims

2.2.1. To evaluate the effect of Lovastatin on measures of executive function, behavior and quality of life in children with NF1 and cognitive deficits;

2.2.2. To further evaluate the toxicity and tolerability of Lovastatin in children with NF1 and cognitive deficits.

2.3 Hypotheses

It is hypothesized that Lovastatin will improve the visual spatial memory and/or attention deficits in children with NF1. This is based on studies demonstrating that Lovastatin has significantly improved impairments in visual spatial memory and attention in the NF1 murine model.

It is further expected that Lovastatin will be safe and well tolerated over a 16-week period.

3.0 Research Design and Methods

3.1 Study Design

This is a prospective multi-center; randomized, placebo-controlled Phase II study to determine the efficacy of Lovastatin on visual spatial learning and/or attention abilities of children with NF1 aged 8 to less than 16 years. In addition, the effect of Lovastatin on secondary measures of executive function, visual spatial skills, behavior and quality of life will be assessed. Participants will be randomized to 16-weeks of treatment with Lovastatin or a matched placebo. It is plausible and ethical to employ a placebo group as no standard therapy with established efficacy is being withheld. There is no cross-over in this study due to a lack of data concerning the length of possible washout effects. The Lovastatin dose will begin at 20 mg once daily/continuous dosing and escalate over a two-week period to 40 mg once daily/continuous dosing and continue at this dose for 14 weeks. Participants will be carefully monitored for side effects. The safety of Lovastatin will be evaluated using laboratory tests, clinical signs and adverse effects, which will be monitored at regular intervals over the 16-week period. Primary and secondary outcome measures will be administered at baseline, 16 weeks post-treatment and at follow-up, 8 weeks after cessation of treatment to determine any carry-over effects. The safety of Lovastatin will also be evaluated, with regular monitoring of side-effects during the trial.

4.0 Study Population

4.1 Human Subject Selection

This is a Phase II study involving children with NF1 (aged 8 years to less than 16 years at time of screening) with evidence of cognitive impairment, defined as having a score of at least one standard deviation or more below the population mean on a measure of visual spatial learning and/or attention.

A total of 142 participants with NF1 aged 8 years to less than 16 years will be enrolled in the study. The age limits were selected on the basis that Lovastatin has been shown to be safe and well tolerated in children aged between 10 and 17 years old. Lovastatin has been extensively used in children 10-17 years of age with hypercholesterolemia, with minimal side effects. Also, results from Phase 1 study indicate that Lovastatin is safe and well tolerated in children with NF1 aged 10-17 years (see section 1.4). Despite the lack of preliminary data in terms of safety using Lovastatin in children aged 8 and 9 years of age, we do not anticipate higher risk of side effects in this age group compared with children 10 years and older. During the phase 1 study (Acosta et al., submitted), 4 patients aged 10 years participated in the study. In all cases, no side effects or

significant changes in metabolic parameters were observed in this group compared with the older patients. In addition, metabolic pathways related to the metabolism of drugs, including Cytochrome P450 complex, are approximately developed up to 70% after the perinatal period (Lange, Pharmacology). It is considered fully developed by 5 years of age (Anderson et al 2009). Adjustments in the doses are related with changes in weight mainly. We do not expect changes in toxicity in this younger age group and those patients will be monitored closely as per study protocol during the study for immediate identification of any risk(s) or side effect(s).

In addition, one of the primary outcome measures (attention) only has normative data for up to 15 years 11 months. Therefore, the maximum age limit for participants at time of enrolment is 15 years 11 months so that normative data can be used to determine whether participants are impaired. The pediatric NF1 population is an ideal group in which to study the cognitive effects of Lovastatin because it represents an opportunity for early pharmacological intervention of cognitive deficits.

Participants will be recruited from the following sites*:

- Neurogenetics Clinic, The Children‟s Hospital at Westmead, Sydney, Australia. The Neurogenetics Clinic in Sydney caters for families with NF1 and services a population base of approximately 4 million. There is a wide referral base and the clinic caters for over 1300 individuals with NF1, 70% of whom are under 20 years of age, with all socioeconomic groups represented.

- Children‟s Hospital Boston/Dana-Farber Cancer Center**.** The multidisciplinary NF1 Program at CHB/DFCI works towards the overall goal of conception, development and conduction of collaborative pilot, Phase I and II clinical evaluations of promising therapeutic agents and approaches for the prevention, diagnosis, management and treatment of NF1, committed to: 1) ensuring that more than 12 patients with NF1 are available yearly for Consortium studies, 2) acting as a resource in areas where our expertise will ensure success of the Consortium, 3) proposing novel studies that meet the Consortium‟s goals, and, 4) developing and expanding a genomic and tissue bank. 5) Continuing to develop expertise in clinical and basic science research that will lead to new areas of treatment and management of patients with NF1.

- Children‟s National Medical Center**.** The Neurofibromatosis Program of the Children‟s National Medical Center (CNMC), designated as a Center of Excellence by the Children‟s Tumor Foundation, is a well-established, multi-disciplinary program caring for children and adults with NF1. The program is multi-faceted and has proven expertise in clinical care, clinical/translational research, basic research and advocacy. Commitment/Experience in NF1 Clinical Research: The CNMC program‟s personnel have extensive experience in coordinating care in clinical trials for children with neurofibromatosis.

- Cincinnati Children‟s Hospital Medical Center. The Cincinnati Neurofibromatosis Center, founded in 1986, is a multidisciplinary center dedicated to the diagnosis, clinical care,

and medical management of adults and children with NF1 and NF2. It is one of the largest NF centers in the country, with over 600 patients with NF1 evaluated and over 400 annual patient visits. The NF Center is housed at Cincinnati Children‟s Hospital Medical Center (CCHMC), with an adult program based in the Internal Medicine/Pediatrics

- Children‟s Hospital of Philadelphia. The Penn Joint Neurofibromatosis Program (NFP) constitutes a multi-institutional, multidisciplinary approach to the diagnosis and management of children and adults with neurofibromatosis type 1 (NF1) at The Children's Hospital of Philadelphia (CHOP) and the Hospital of the University of Pennsylvania (HUP).

- The University of Alabama at Birmingham**.** The UAB Neurofibromatosis Center includes both clinical and research activities dedicated to all forms of neurofibromatosis. Components include a Neurofibromatosis Clinic that serves individuals with NF1, NF2, and schwannomatosis at all ages; a full range of consultation services; expertise in conduct of clinical trials and natural history studies; a unique NF1 and NF2 genetic testing laboratory that serves the entire NF community; basic research focusing on mouse models and drug discovery.

- University of Chicago**.** The NF Program at the University of Chicago, established in 1989, is a large multi-disciplinary program for children and adults. The program offers exceptional care to patients of all ages and continues to demonstrate expertise in clinical care, clinical/translational research, basic research, and patient education and advocacy. Commitment/Experience in NF1 Clinical Research.

- University of Utah**.** The neurofibromatosis type 1 (NF1) research program and NF Clinic at the University of Utah have been established for over 20 years. The NF Clinic primarily focuses on the pediatric population seen at Primary Children‟s Medical Center, but adult patients are routinely managed by oversight of their health supervision and collaboration with an integrated network of experienced subspecialists.

- Washington University. Washington University School of Medicine (WUSM) has a multidisciplinary NF Center with a long history of commitment to and expertise in NF1 research and clinical care. The NF Center is focused on promoting pioneering laboratory research, facilitating collaborative basic science and clinical research, and translating innovative scientific discoveries to improved care for individuals with NF.

- Central Manchester University Hospitals. – Central Manchester University Hospitals NHS Foundation Trust, England – The Manchester Neurofibromatosis Centre was established in 1990 and has been providing diagnosis and management advice for families with all kinds of neurofibromatosis since then. The center has a long history of clinical and epidemiological research in NF1 and NF2, alongside molecular genetic research in NF2 and Schwannomatosis. The centre was nationally commissioned in April 2009 to be one of two English centers providing multidisciplinary care for patients with complex NF1. In April 2010, this was followed by national funding for NF2 care with 3 other English centers.

- University of Texas Southwestern Medical Center. – UTSW is a multidisciplinary clinic for patients with all types of neurofibromatosis throughout their lifetimes. Pediatric patients are seen at Children‟s Medical Center Dallas, one of the foremost pediatric hospitals in the nation, while adult patients are seen in the NCI designated Simmon‟s Cancer Center. UTSW has a strong history of basic laboratory, translational and clinical research in patients with neurofibromatosis.

*other sites may be added to help with accrual.

4.2 Inclusion Criteria

- Males or females aged between 8 years to less than 16 years at time of screening who meet NIH diagnostic criteria for NF1 (Appendix 1);
- Participants must have a full-scale IQ of 70 or above. In cases where there is a statistically significant difference between verbal IQ and performance IQ (.05 level as determined by Table B3 in the WASI manual), participants will be eligible if at least one of these quotients is 70 or above;
- Participants must have a cognitive impairment defined as having a score of at least one standard deviation or more below the population mean on one or more of the primary objective outcome measures (i.e., impaired on a measure of visual spatial learning and/or sustained attention);
- Participants must be medically stable;
- Participants who are on a stable dose of methylphenidate and/or dextroamphetamines for at least one month prior to screening and who will remain on the same dose for the duration of the study (see Section 4.3 for exclusion criteria regarding other psychotropic medication, i.e. medication capable of affecting the mind, emotions or behavior)
- Hepatic function: Participants must have adequate liver function defined as at least 3 of the 4 liver function tests to be < 2 x upper limit of normal for age according to institution standards (AST, ALT, Direct bilirubin, Indirect bilirubin [computed from total bilirubin and direct bilirubin]) and no tests should be >5x upper limit of normal for age;
- Renal function: Participants must have adequate renal function defined as serum creatinine ≤ 1.5x upper limits of normal for age, and BUN ≤ 1.5x upper limits of normal for age or a creatinine clearance of greater than 70 ml/m/1.73m^2;
- Hematologic function: Participants must have an absolute neutrophil count of > 1,000, a hemoglobin ≥10 gms/dl, and a platelet count > 100,000 on study entry;
- Participants must sign all required documents, including informed assent and HIPAA documents;
- Female participants of childbearing age should not be pregnant, must have a negative pregnancy test before initiation of treatment, and take appropriate birth control precautions to participate in this study.

4.3 Exclusion Criteria

- Full-scale IQ less than 70. In cases where there is a statistically significant difference between performance IQ and verbal IQ (.05 level), patients will be excluded if both quotients fall below 70;
- Individuals that are not cognitively impaired on at least one of the primary objective outcome measures;
- Individuals with insufficient English to complete the assessments;
- Participants taking psychotropic medication other than methylphenidate and/or dextroamphetamines (see Section 4.2). Appendix 2 contains a list of common psychotropic medications. These patients are eligible if, as clinically indicated, they cease medication for at least 30 days prior to screening and remain off these medications for the duration of the study;
- Participants with intracranial pathology such as epilepsy, diagnosed head injury, hydrocephalus or progressive intracranial tumors (children with asymptomatic or static lesions will be eligible);
- Participants who are pregnant or breastfeeding;
- Participants who have received any investigational drug (other than sirolimus) within 30 days of screening;
- Participants who have recently taken sirolimus. These participants will be eligible after a washout period of at least three months;
- Participants who have recently taken Lovastatin. These participants will be eligible after a washout period of at least three months;
- Participants with significant hepatic, renal or hematologic function as previously defined;
- Participants with a history of neuromuscular disease, excluding hypotonias thought to be associated with NF1;
- 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 potentially interfere with the participant‟s ability to participate in the required testing;
- Low cholesterol (lower limit of a total cholesterol of 90mg/dl).

5.0 Study Procedures

An overview of the study procedures can be found in Appendix 3.

5.1 Pre-Treatment

The pre-treatment phase of this study consists of obtaining informed consent, an initial evaluation and enrollment process, as well as a screening for eligibility.

5.1.1 Screening and Informed Consent

The study will be described in detail to participants and their parents/legal guardians at an initial visit. If the participant and family agree to participate in the study, the applicable assent, consent and HIPAA documents will be signed (see Appendix 4). After informed consent has been obtained, a screening assessment will be performed. The screening assessment will include a medical history, physical examination and laboratory tests. The site psychologist will also administer the primary outcome measures (see section 5.1.4.1) and an abbreviated IQ measure which is estimated to take 50 minutes to complete. To be eligible to participate in the treatment phase, patients must have an impairment on at least one of the primary outcome measures of visual spatial learning or sustained attention (≥ 1SD below the normative mean) and an IQ equal to or above 70. Unless the patient has undergone an IQ test within six months of screening, IQ will be assessed using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). If the participant has recently completed an IQ test (within six months of screening), then this score will be used for the purposes of screening. Examples of acceptable IQ tests include (but are not limited to) the WASI, the Wechsler Intelligence Scale for Children 4th Edition (WISC-IV), and the Stanford-Binet Intelligence Scales 5th Edition (SB-V).

If the child fails to satisfy the screening criteria, parents will be verbally informed of the reason why the child did not meet the criteria. If the reason for exclusion was a Full-Scale IQ less than 70, parents will be verbally informed of the range of the child‟s IQ score and that it may indicate the presence of an intellectual disability. Parents will be advised that only a brief assessment of IQ was conducted and that the child‟s performance may vary due to motivation, attention and fatigue. It will be recommended to parents that their child undergo a full IQ assessment to obtain further information about their child‟s intellectual functioning. Upon request, results of the IQ test will be provided to parents in a written report.

5.1.2 History and Physical Examination

A complete medical history and physical examination (including neurological examination) will be performed initially to confirm each participant‟s NF1 diagnosis and to document any complications that they might have from the disease. Height, weight, head circumference, blood pressure, and pulse will be recorded. Patients will also be given a list of medications to avoid while on this study. To be eligible participants must have not taken any stimulant medications for at least 30 days or more and agree to remain off stimulant medication for the duration of the study.

5.1.3 Laboratory Studies

A pre-treatment enrollment laboratory test will be performed at least two weeks prior to commencing the study medication. The following studies will be performed:

- Hematology: Complete blood count (CBC);
- Chemistries: Blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), asparate aminotransferase (AST), CPK and, Lipid profile (HDL, Cholesterol, LDL, Triglycerides), total bilirubin and direct bilirubin (indirect bilirubin will be computed from total and direct bilirubin serum measurements);

- Urine Pregnancy Test: A urine pregnancy test will be performed on all females of childbearing age before commencing study medication and at weeks 8 and 16.

In order to ascertain an accurate lipid profile, participants will fast overnight before their laboratory tests are performed. This will apply to all lipid assessments conducted during the study period.

5.1.4 Baseline Assessment - Neuropsychological Outcome Measures

Within one month of enrollment and before initiation of treatment, participants will be administered all primary and secondary outcome measures as outlined in section 5.1.4.1. The child"s history will also be reviewed with the family to gather information about the child"s cognitive development and behavior.

5.1.4.1 Primary Outcome Measures

The effect of Lovastatin on cognitive functioning in children with NF1 has not been examined. Evidence from a murine model of NF1 suggests Lovastatin improves visual spatial learning and memory, and attention. As such, the following primary outcome measures are:

- Visual Spatial Learning and Memory

*CANTAB Paired Associate Learning (PAL)

PAL is a measure of arbitrary visuospatial associations. It involves the randomized „opening" of six boxes that are displayed on the screen, each revealing a different pattern. Immediately after presentation of the six patterns, each item reappears in the central box in random order and the participant has to indicate where each item was first seen. Measures include the number of patterns placed correctly on the first trial and the number of repeat presentations needed to learn all the associations correctly. Administration time for this measure is approximately 10 minutes.

- Attention

*Score! Subtest from the Test of Everyday Attention for Children (TEA-Ch: Manly, Robertson, Anderson & Nimmo-Smith, 1999).

The TEA-Ch is a normed and standardized multi-subtest battery that assesses various aspects of attentional function in children aged between 6 years and 15 years 11 months. The Score! subtest requires children to keep count of sounds they hear on a tape. This task does little to „grab" the child"s attention and is a measure of their ability to self-sustain his or her own attention. Previous findings indicate that approximately 63% of children with NF1 are impaired on Score! (Hyman et al., 2005). There are two alternate forms for this subtest and it takes approximately 8 minutes to complete.

5.1.4.2 Secondary Outcome Measures

- Attention

*Test of Everyday Attention for Children (TEA-Ch: Manly et al., 1999)

In addition to the Score! subtest, three other subtests from the TEA-Ch will also be used in this study: Sky Search (visual selective attention), Sky Search Dual Task (divided attention between visual and auditory information), and Creature Counting (attentional control/switching). The test is normed for children 6 to 15 years 11 months and there are two alternate forms available. Administration time for these three subtests is approximately 20 minutes.

*Conners‟ Continuous Performance Task – II (CPT-II: Conners, 2000)
CPT-II is a computerized measure of sustained attention and concentration. It is a 14-minute computerized test that requires the child to discriminate targets (X‟s) from nontargets (letters of the alphabet). Various measures calculated by the program include hit reaction time, omission and commission errors, change in reaction time speed and consistency. This test takes approximately 14 minutes to administer.

Parent version of the Conners ADHD/DSM-IV Scales (CADS: Conners, 1997)
The CADS-P is a published questionnaire that uses observer ratings to help assess the presence of inattentive and hyperactive/impulsive behaviors in children and adolescents and directly corresponds to the DSM-IV criteria for ADHD. The parent version of the questionnaire will be administered, which contains 26 items (12 items ADHD index, 9 inattentive items and 9 hyperactive-impulsive items). It takes approximately 5 to 10 minutes to complete. The CADS-P is a subjective outcome measure of attention.

- Executive Functions

*CANTAB Spatial Working Memory (SWM)
This is a test of the participant‟s ability to retain spatial information and to manipulate remembered items in working memory. It is a self-ordered task that also assesses heuristic strategy. Administration time for this test is approximately 10 minutes.

*CANTAB Stockings of Cambridge (SOC)
This is a test of spatial planning and problem solving (aspect of executive function), which is based on the „Tower of London‟ test (Shallice, 1982). Administration time for this measure is approximately 10 minutes.

*CANTAB Stop Signal Task (SST)
This is a classic stop signal response inhibition test that uses interleaved staircase functions to generate an estimate of stop signal reaction time. SST gives a measure of the participant‟s ability to inhibit a response. Administration time for this task is approximately 15 minutes.

Controlled Oral Word Association Test (COWAT; Benton & Hamsher, 1989)
The COWAT assesses the executive aspects of language processing, including verbal fluency and concept formation. It requires children to rapidly make verbal associations to specific letters of the alphabet (i.e., F, A and S). The test is normed for children between 7 to 16 years of age (Anderson, Lajoie & Bell, 1995). Administration time for the COWAT is approximately 5 minutes.

Behavior Rating Inventory of Executive Function-Parent - Report Form (BRIEF; Gioia, Isquith, Guy & Kenworthy, 2000; Guy, Isquith & Gioia, 2004)
The BRIEF is a published clinical questionnaire assessing executive function in the home and school setting. The Parent Form is completed by the child's parent or guardian. It contains 86 items, with eight clinical scales and two validity scales. Behavior descriptors of a child are rated on a three-point Likert scale: "never," "sometimes," "always." Items were developed to capture everyday behaviors associated with executive functions, and tap eight domains including: initiate, inhibit, shift, plan, organize, self-monitor, emotional control, and working memory. These scales are combined to form two broader indices (Behavioral Regulation and Metacognition) and a Global Executive Composite score. The Parent Form is normed for parents of children aged 5-18 years old. This questionnaire takes approximately 15 minutes to complete.

- Visual Spatial Skills

*Judgment of Line Orientation (JLO: Benton, Varney & Hamsher, 1976)
The JLO is a motor-free untimed measure of spatial perception and orientation. JLO requires participants to judge the spatial directionality and size of angles. Two alternative forms of this test are available. These tests take approximately 10 minutes to administer.

- Visual Perceptual Skills

Object Assembly (Wechsler Scale for Intelligence – Third Edition; WISC-III; Wechsler, 1991)
The Object Assembly test is a perceptual organization timed task, that is, a task that requires mentally organizing spatial information into meaningful part-whole relationships. For this task, participants are required to put together puzzle pieces to form line drawings of common objects. This test takes approximately 13 minutes to complete.

- Behavior

Behavior Assessment System for Children - Second Edition (BASC-II; Reynolds & Kamphaus, 2004)
The BASC-II is a multidimensional system used to evaluate the behavior and self-perceptions of children and young adults aged two through 25 years. The Parent Rating Scales is a comprehensive measure of the child's adaptive and problem behaviors in community and home settings, which takes 10 to 20 minutes to complete. It assesses various internalizing and externalizing problem behaviors as well as adaptive skills. The Self-Report of Personality is a personality inventory for children and adolescents, and young adults, which takes about 30 minutes to complete. It assesses various internalizing problems, hyperactivity/inattention, personal adjustment and school problems.

- Quality of Life

The Pediatric Quality of Life Scale (PedsQL: Varni, Seid & Kurtin, 2001)
The PedsQL 4.0 Generic Core Scales are multidimensional child self-report and parent proxy-report scales to assess health-related quality of life in children and adolescents aged 2 to18 years. There are parallel child and parent scales, which include self-report forms for children,

and proxy-report forms for parents. The PedsQL consists of four core scales: physical, emotional, social, and school. It takes 5 to 10 minutes to complete.

- Visuospatial Learning and Memory

Computer Generated Arena (CGA: Astur, Taylor Mamelak, Philpott & Sutherland, 2002)
The CGA is an optional computerized clinical outcome measure that will be performed after completion of all secondary outcome measures. It is a virtual environment task that has been developed as a "Human Morris Water Maze" (Astur et al., 2002; Kallai, Makany, Karadi & Jacobs, 2005). The task requires children to use a joystick controller to navigate a circular "arena" to locate an invisible target hidden on the floor. The walls of the arena contain cues, such as windows that remain in place. The fixed target must be located in subsequent trials, each time starting from a new location. This test takes 10-15 minutes to administer and will only be conducted at the following sites: The Children‟s Hospital at Westmead, Children‟s Hospital Boston and Children‟s National Medical Center.

In addition to these measures, Motor Screening (MOT) from the CANTAB will also be administered. MOT is a simple pointing task that measures psychomotor speed and introduces the participant to the touch-screen apparatus, ensuring they can touch the screen accurately, and can hear, understand and follow instructions. The mean latency to touch a cross is measured. MOT takes approximately 5 minutes to administer.

All primary measures will be administered at screening. The length of time required for the child to complete the remainder of the baseline neuropsychological assessment is estimated to be 128 minutes (98 minutes testing and 30 minutes to complete self-report questionnaires). It is estimated that parent questionnaires will take 55 minutes to complete (see Appendix 5). All neuropsychological assessments that will be used in this study are published tests that have normative data that is both reliable and valid for the age range in this study. These tests are all extensively used in clinical and/or research settings. All tests have standardized instructions for administration ensuring there will be no site variation in administration.

To minimize any test-retest effects for neuropsychological outcome measures, alternate forms (indicated by „*‟ in the above sections 5.1.4.1 and 5.1.4.2) will be used where possible, to minimize potential practice effects. Furthermore, the use of a placebo group will allow us to identify any potential practice effects and distinguish whether any changes in the treatment group are larger than those accounted by practice effects i.e. a treatment effect. In addition, statistical analysis of the data will be conducted to account for confounding variables such as practice effects (see Section 8).

5.1.5 Randomization

Following a successful screening period, the participant will be randomized to one of the two treatment groups: Lovastatin or placebo. Randomization will be implemented by the Data Coordinating Center for this trial utilizing a permuted blocks approach.

Randomization will be stratified by clinical site, to ensure adequate balance between the treatment groups, overall and at each site. Password protection will be implemented to ensure that only certified clinical center personnel are allowed to randomize patients and access the database. At the time of randomization, each participant will be assigned a unique medication kit identification number that will be used throughout the duration of the study.

5.1.6 Masking Procedure and Labeling

In order to preserve the double-masking of the trial, only the Data Coordinating Center, the drug distribution center, and the pharmacist at each participating site will be unmasked. The drug distribution center will be provided with the randomization sequence and treatment assignment directly by the Data Coordinating Center.

Both treatments (Lovastatin and placebo) will be distributed from the Pharmacy Department at each site. Except for the pharmacist, all staff will be blinded to the treatment sequence at each site.

The success of blinding will not be evaluated. While the CONSORT checklist previously stated that the success of blinding should be evaluated this statement has recently been removed from the CONSORT checklist as it was deemed that testing for blindness may not generate valid answers (Sackett, 2007).

5.2 Treatment Visits

5.2.1 Dose Schedule and Prescription

Eligible participants will be dispensed 20mg of Lovastatin or the placebo at the initiation of treatment. Participants will be given directions to take the medication once daily after dinner time. Given that Lovastatin has a strong odor; participants will be required to swallow the capsule whole without breaking the seal. Participants will take one tablet (20mg Lovastatin) per dose for two weeks; and increase to two tablets per dose during the following weeks (40 mg Lovastatin). Participants will continue on the same dosage for a total of 14 weeks. In order to assess medication compliance, participants will be required to complete pill diary forms. All participants will be provided with a complete list of medications and products that have detrimental interactions with Lovastatin that must be completely avoided while being treated with Lovastatin (see Appendix 6).

5.2.2 Clinical Evaluation

Throughout the treatment phase of the study, participants will be clinically evaluated every 4 weeks (± 4 days) by a neurologist or a qualified physician at each site. At each visit, an interim medical history and physical examination (including a neurological examination) will be performed. Height, weight and body surface area will be obtained. Participants will be assessed for toxicity/side effects and safety labs will be performed as specified in section 5.1.3 every 4 weeks (± 4 days). Medication compliance will also be assessed at these visits. Pill diaries will be

reviewed and a pill count will be performed and recorded. At the end of the study or the treatment period, all unused medication will be collected and dispensed of properly on a per site basis according to clinical procedures and guidelines.

5.2.3 Off-Study Criteria (Note: See Section 6.6 for modifications for toxicity information)

A participant will be taken off the study for the following reasons:

- The participant refuses further treatments and/or wishes to withdraw from the study;
- For participants concurrently taking stimulant medications (methylphenidate and/or dextroamphetamines), they will be withdrawn from the study 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 study 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, such as recurrent grade 3 or 4 toxicity after dose reduction; and/or persistent grade $\geq$2 toxicity for >10 days without administration of the drug;
- The development of a concurrent serious medical condition that might preclude or contraindicate the administration of Lovastatin;
- Death.

5.3 Post-Treatment

The following tests and procedures will be performed at the end of the 16-week treatment period (unless participants have come off treatment early):

- Medical history and physical examination;
- Laboratory studies as specified in section 5.1.3 including CBC, BUN, creatinine, ALT, AST, direct and total bilirubin, CPK and complete lipid profile;
- All outcome measures will be re-administered 16 weeks (± 4 days) after treatment commenced (and the day it is terminated). In exceptional situations where participants are unable to attend the post-treatment assessment at week 16 (± 4 days), the post-treatment assessment will be conducted earlier, but as close to week 16 as possible. If a participant is withdrawn from the study early, the battery will still be given, if it is at least four weeks since the baseline assessment (unless the reason is non-compliance of medication). It is estimated that it will take the child approximately 148 minutes (118 minutes testing and 30 minutes self-report questionnaires) to complete the primary and secondary outcome measures. It is estimated that parent questionnaires will take 55 minutes to complete.

5.4 Follow-up

To determine any carry-over effects in the present study, a follow-up assessment will be conducted 8 weeks (± 7 days) post cessation of treatment. As treatment status will be blinded, all participants will be re-administered all outcome measures and laboratory tests (excluding pregnancy test) at follow-up. While no existing data exist on the carry-over effects of Lovastatin, in patients with cancer who were given high dosages of Lovastatin (2-45mg/kg/day)

approximately 30 days after the cessation of Lovastatin, cholesterol levels returned back to normal levels (Thibaulet et al., 1996).

All study personnel that have responsibility for direct participant related activities will be intensively trained in the relevant specific procedures before contact with participants, which includes providing informed consent/assent, family/child interviews, the administration and scoring of tests, maintenance of confidentiality, data collection and handling, and procedures for handling difficulties that might arise during the study. All study personnel who have direct contact with study participants or their data, will sign a confidentiality form, indicating their understanding of appropriate procedures for maintaining participant confidentiality. Doctoral-level neuropsychology staff and physicians will provide the relevant training and ongoing supervision of all study personnel.

6.0 Pharmaceutical Information

6.1 Pharmacokinetics

Lovastatin is one of the first-generation statins and therefore has a large body of safety and pharmacokinetic data from clinical trials and general use for hyperlipidemia (Corsini, et al., 1995a).

1. It is a lactone that is readily hydrolyzed in vivo to the corresponding b-hydroxyacid, a potent inhibitor of HMG-CoA reductase. Inhibition of HMG-CoA reductase is the basis for an assay in pharmacokinetic studies of the b-hydroxyacid metabolites (actives inhibitors) and, following base hydrolysis, active plus latent inhibitors (total inhibitors) in plasma following administration of Lovastatin;
2. Following an oral dose of 14C-labeled Lovastatin in men, 10% of the dose was excreted in urine and 83% in feces. Plasma concentrations peaked at two hours and declined rapidly to about 10% of peak by 24 hours post dose;
3. Lovastatin is highly bounded (>95%) to human plasma proteins. Animal studies demonstrated that Lovastatin crossed the blood brain and placental barriers;
4. The major active metabolites present in human plasma are the b-hydroxyacid of Lovastatin, its 6‟-hydroxy derivative, and two additional metabolites. Peak plasma concentrations of both active and total inhibitors were obtained within two to four hours of dose administration;
5. The recommended dose in children 10 to 17 years old is 10-40 mg/day (Merck & Co., Inc, 2007);
6. It is metabolized in the liver by the cytochrome P450 isoenzyme CYP3A4, with less than 10% being excreted renally (Kantola, Kivisto & Neuvonen, 1998);
7. With a once a day dose regimen, plasma concentrations of total inhibitors over a dosing interval achieved a steady state between the second and third days of therapy and were about 1.5 times those following a single dose (Vree, Dammers, Ulc, Horkovics-Kovats, Ryska et al., 2003). When Lovastatin is given under fasting conditions, plasma concentrations of total inhibitors were on average about two thirds those found when

Lovastatin was administered immediately after a standard test meal (Hsu, Spinler & Johnson, 1995; (Merck & Co., Inc, 2007).

6.2 Drug Company/Supply/Distribution

Based on an estimate from Eminent Corporation, funds included in the budget will be used to purchase Lovastatin and its matching placebo and distribute Med Kits to participating sites. Eminent Corporation will also be responsible for study medication reconciliation and shipping.

6.3 Procedures/Recommendations for Drug Supply and Distribution

Lovastatin and its matching placebo will be obtained and distributed to participating sites by a subcontractor to the Operations Center. A 20mg tablet of Lovastatin or an identical tablet (placebo) will be provided for each participant. Medications, including the placebo, will be provided to the participants free of charge. Participants will be instructed to return all unused medications as well as empty bottles at each clinic visit. All unused medicine will be disposed of properly at each individual site.

Lovastatin is administered orally. The bioavailability of the tablet increases under the influence of a meal. Based on these findings, it is advised to take the tablets immediately after a meal. One dose per day after dinner time will be instructed to parents, since evening doses are more effective than the same dose given in the morning (Merck & Co., Inc, 2007). Lovastatin 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. Participants 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.

Compliance will be monitored by recording the number of pills dispensed to families from the pharmacy and then comparing that to the number of pills that are returned at each monthly visit. Participants will be asked to return all unused pills at each visit. A pill diary will also be maintained by each participant and reviewed at each follow-up visit to ensure compliance with the medication.

6.4 Drug Dosage and Possible Interactions

The selection of the maximum dose of Lovastatin to be used in this study, 40 mg/day, is based on the tolerability of the drug when used for heFH and the results of the Phase I study examining safety and tolerability of Lovastatin in children with NF1 (Acosta et al., submitted). Participants will commence at 20 mg/day for two weeks and escalate to the 40 mg/day dose in week 3.

Approximately 40% of children with NF1 are diagnosed with comorbid ADHD, many of whom are treated with psychostimulant medication such as methylphenidate and/or dextroamphetamines. In order to minimize recruitment bias and ensure recruitment targets are met, patients taking methylphenidate and/or dextroamphetamines will be eligible for this study.

The predominant metabolic pathway of methylphenidate is de-esterification to form pharmacologically inactive d- or l-ritalinic acid (Patrick et al 1987). Metabolism of methylphenidate when used by oral administration undergoes a stereoselective clearance to ritalinic acid (CES1A1 is the major enzyme responsible) before entering systemic circulation (Sun et al, 2004). Methylphenidate does not use the microsomal cythochrome P450 in the liver for its metabolism (Sun et al 2004; Patrick et al 1987). Dextroamphetamines are metabolized by the cythrochrome P450 in liver. However the isoenzyme responsible for its metabolism is the CYP2D6 (Adderall Medication Guide, Shire, Nov. 2010). Instead, lovastatin is metabolized by the CYP3A4 (Ritalin® Medication Guide, Novartis, 2010). There are not described interactions or competitive mechanism between dextroamphetamines and lovastatin.

Although limited by a small sample size, results of the Phase I study provide some evidence for the inclusion of patients on stimulant medication (Acosta et al., submitted). A total of 23 patients with NF1 (10-17 years of age) completed 12 weeks of treatment with Lovastatin. Nine patients had a diagnosis of ADHD and five of these patients were taking a stable dose of stimulant medication during the course of the study. Calculation of Reliable Change Index for individuals indicated significant improvements on both verbal and nonverbal memory measures in approximately 40% of participants regardless of whether they were off or on methylphenidate.

The list of psychotropic medications is quite extensive. As many psychotropic medications may interact with the metabolism of Lovastatin, e.g. Fluoxetine, patients taking psychotropic medications other than stimulant medications will be excluded from the study. Also, participants on other medications to control behavior (including Straterra) and anticonvulsants will not be eligible to participate in the study as these medications are known to affect cognition and learning and, thus, confound the results of the study. However, these participants may be eligible if they cease medication for at least 30 days prior to screening and remain off these medications for the duration of the study. Importantly, all participants must still demonstrate impairment on either of the primary outcome measures at the screening assessment and satisfy all other inclusion criteria to be enrolled in the study.

6.5 Toxicity and Adverse Event Monitoring

6.5.1 Lovastatin Side Effects

Lovastatin has been shown to be safe and effective in a pediatric population for treatment of familial heFH with minimal toxicity. The FDA has approved it for treatment in children and it is believed to be safe to extend to other potential uses. Side effects reported have been considered reversible once the medication has been discontinued. The most frequent side effects of Lovastatin are described below by systems. Presentation is less common in children than in adults, and includes:

Muscular: Muscle pain, tenderness, weakness, increase of CPK, cramps, rhabdomyiolisis (rare)
Hematologic: Thrombocytopenia

Gastrointestinal: Abdominal pain, flatulence, diarrhea, constipation
Hepatic: Transient elevation in serum transaminases
Dermatologic: Rash (rare)
Constitutional: Fatigue, asthenia, flu-like symptoms, headache, dizziness, insomnia

6.5.2 Frequencies of side effects

The frequencies of adverse events are ranked according to the following: Very Common (>1/10), Common (≥1/100, <1/10), Uncommon (≥1/1,000, <1/100), Rare (≥1/10,000, <1/1000), and Very Rare (<1/10,000).

Common >1% and < 10% (less than 1 person in every 10 but more than 1 person in every 100): Gastrointestinal disorders: constipation, dyspepsia

Uncommon 0.1% to 1% (less than 1 person in every 100 but more than 1 person in every 1,000): Skin and subcutaneous tissue disorders: itching; elevated transaminases.

Rare 0.01% to 0.1% (less than 1 person in every 1,000):

- Eye disorders: blurred vision.
- Gastrointestinal disorders: abdominal pain, diarrhea, dry mouth, flatulence, nausea, vomiting.
- General disorders and administration site conditions: weakness.
- Hepatic disorders: yellowing of the skin and eyes (cholestatic jaundice), hepatitis.
- Metabolism and nutrition disorders: loss of appetite.
- Musculoskeletal, connective tissue and bone disorders: muscle weakness (myopathy), tenderness and muscle pain, muscle cramps. Participants will be told to promptly report any unexplained muscle pain, tenderness or weakness. If myopathy is diagnosed or suspected, then Lovastatin therapy will be immediately discontinued.
- Nervous system disorders: dizziness, absence of the sense of taste, headache, tingling sensation, tingling and numbness of the feet and legs.
- Psychiatric disorders: insomnia, psychic disturbances including anxiety, sleep disorders.
- Skin and subcutaneous tissue disorders: alopecia, spotted or diffuse redness of the skin including Stevens - Johnson syndrome, redness and swelling of the skin, shedding of the skin.
- An apparent hypersensitivity syndrome has been reported rarely which has included one or more of the following features: anaphylaxis, angioedema, lupus-like syndrome, polymyalgia rheumatica, dermatomyositis, vasculitis, thrombocytopenia, leukopenia, eosinophilia, hemolytic anemia, positive anti-nuclear antibodies, erythrocyte sedimentation rate increase, arthritis, arthralgia, urticaria, asthenia, photosensitivity, fever, flushing, chills, dyspnea and malaise, and other liver function test abnormalities including elevated alkaline phosphatase and bilirubin; increase in serum CPK levels.

6.5.3 Breaking the Blind

Treatment assignment may be revealed only for reasons relating to the participant‟s safety or when critical therapeutic decisions are contingent on knowing the assigned study medication. Except in the most pressing circumstances, a decision to break the blind must be discussed with the Operations Center, Chair of the Protocol Committee or the Chair‟s designee from the Protocol Committee and permission in writing must be obtained before the blind may be broken. Withdrawal of a participant from the study is not a sufficient reason to break the study blind.

If the blind is broken, the pharmacist will record key information at the time the blind is broken. Information to be collected includes the participant number and initials, medication kit number, date the blind was broken, reason for breaking the blind, the name of the investigator who requested the blind be broken, the name of the pharmacist who broke the blind, and the names of the Clinical Review Committee members who authorized breaking the blind. A member of the Clinical Review Committee will fax or e-mail written permission to break the blind to the pharmacist and to the Operations Center.

6.6 Modifications for Toxicity

Participants who experience grade 2 toxicity related to Lovastatin will have Lovastatin withheld until the toxicity resolves (grade $\leq$1) and then restarted at the same dose level. If the grade 2 toxicity recurs, the dose will be withheld again until the toxicity resolves (grade $\leq$1) and then reduced to 50% of the dose. If the grade 2 toxicity recurs, the participant will be taken off the protocol. The participant will be evaluated by the principal investigator.

Participants who experience grade 3 or 4 toxicity related to Lovastatin will have their dose withheld. If the toxicity returns to grade $\leq$1 within 10 days, the participant may resume Lovastatin at a dose reduced by 50%. If the toxicity persists at grade $\geq$2 for >10 days without administration of Lovastatin or the grade 3 or 4 toxicity recurs at the lower dose, the participant will be removed from the study.

6.7 Adverse Event Monitoring (UK sites please refer to Appendix 10)

Participants will be monitored closely for medication side effects with a diary and with laboratory testing as indicated in the Study Procedures section (see of the protocol (see Section 5). Adverse events will be addressed immediately as described in Section 6.8. Participants will also be closely followed clinically at least once per month while on the study medication by the site physician. The Side Effects Checklist and Adverse Event Monitoring Form will be used to assess participants at every visit or phone contact.

Participants will be monitored for adverse events regularly throughout the study. Adverse events are any unfavorable or unintended sign (including abnormal laboratory findings), symptom or disease temporarily associated with the use of medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated,

unlikely, possible, probable, or definite). Life-threatening adverse events are any adverse event that places the participant at an immediate risk of death from the reaction. Serious adverse events are any event occurring at any dose level that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization, prolongation of an existing hospitalization, a persistent/significant disability/incapacity, and other medically significant events. Unexpected adverse events not listed above may also occur.

All observed or volunteered adverse events will be recorded on the Adverse Event form. Events involving adverse drug reactions, illnesses with onset during the study or exacerbation of pre-existing illnesses will be recorded. Objective test findings, such as abnormal lab values, that result in drug dosage changes will also be recorded.

6.8 Adverse Event Reporting

Adverse events will be graded according to the Cancer Therapy Evaluation Program‟s; Common Terminology Criteria for Adverse Events Version 4 (CTCAE), which can be downloaded from http://ctep.cancer.gov. A severity grading of side effects related to Lovastatin use can be found in Appendix 7. The principal investigator at each site will determine whether the adverse event is related or unrelated to the medical treatment. If deemed to be related, the principal investigator will determine whether the adverse event is expected or unexpected. Details of the event (expected or unexpected), and whether the side effect and severity (i.e. toxicity) were included in informed consent (yes/no), will be recorded on the adverse event module form.

A justification will be recorded on the adverse event module if the observed toxicity was not included in the informed consent. For all adverse events, the investigator must pursue and obtain information to determine the outcome of adverse events and to assess whether it meets the criteria for classification of a serious adverse event. All serious adverse events require immediate notification to the Operations Center. All adverse events, whether serious or otherwise, will be followed until resolution (the patients health has returned to his/her baseline status) or until the event has stabilized (investigator does not expect any further improvement or worsening of the adverse event). Participants withdrawn from the study due to adverse events will be followed by the investigator until resolution. All grade 3, 4 and 5 adverse events will be reviewed by the Clinical Review Committee.

All adverse events will be tabulated by treatment arm, severity grade and reported to the NF1 Data and Safety Monitoring Board (DSMB). The occurrence of adverse events will be reviewed by the DSMB at 2-month intervals to monitor patient safety. Summary details of the occurrences and grade of adverse events will be distributed to each site at 2-monthly intervals. It will be the responsibility of the site principal investigator to disclose this information to the site‟s institutional review board. In addition, all unexpected and serious adverse events will be forwarded to the medical monitor, Tena Rosser, M.D. She will serve as a patient advocate, will be independent of the clinical study team, and will report to the DSMB. She will oversee the progress of the protocol, especially issues of individual subject/patient management and safety. The monitor will provide an unbiased written report of each event.

6.9 Classification of Adverse Events by Severity and Relationship to Study Drug Administration

- **Adverse Event** – Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease regardless of whether it is considered related to the study medications (attribution of unrelated, unlikely, possible, probable, or definite).
- **Life-Threatening Adverse Event** – Any adverse event that places the participant, in view of the investigator, at immediate risk of death from the reaction.
- **Serious Adverse Event** – Any adverse event occurring that results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, or a persistent or significant disability/incapacity. Important medical events that do not result in death, are not life-threatening, or do not require hospitalization may be considered serious adverse experiences when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in the definition.
- **Hospitalization -** All hospitalizations (or prolongation of existing hospitalization) for medical events equivalent to CTCAE grade 3, 4 or 5 must be reported.
- **Attribution** – The determination of whether an adverse event is related to a study treatment. Attribution categories:
  - Definite – The adverse event *is clearly related* to the study medication.
  - Probable – The adverse event *is likely related* to the study medication.
  - Possible – The adverse event *may be related* to the study medication.
  - Unlikely – The adverse event *is doubtfully related* to the study medication.
  - Unrelated – The adverse event *is clearly NOT related* to the study medication.

6.10 Expedited Adverse Event Reporting

All serious adverse events or unexpected adverse events that are life threatening or fatal regardless of attribution, including death within 30 days of the last dose of study treatment, should be reported to the DCC within 24 hours or by the next business day.
All reportable adverse events must be reported to the Operations Center within 10 working days. All fatal or life-threatening events will be reported to the Operations Center within 24 hours with a written report to follow within 10 working days.

6.11 Risks and Protection from Risks

Individual risks in this study are associated with the use of Lovastatin, laboratory tests (blood drawn) and with neuropsychological testing.

Lovastatin has been shown to be safe and effective in a pediatric population for treatment of familial HeFH. The FDA has approved Lovastatin (40mg/day) for treatment of HeFH in children and it is believed to be safe to extend to other potential uses. The most frequent side effects of Lovastatin are muscular pains, weakness and tenderness, and gastrointestinal conditions such as

abdominal pain, flatulence, diarrhea and constipation, and are reversible once the medication has been discontinued. Participants will be provided with a list of drug and food items that they should avoid while on study medication. All participants will be monitored for adverse events as described in Section 6.7. Participants who experience adverse events related to the study medication will have study medication withheld and/or modified as outlined in Section 6.6. Additionally, there will be regular monitoring and clinical evaluation.

In the 24 patients that were evaluated for the Phase 1 Lovastatin study, few patients‟ total cholesterol dropped below 100, and in all cases, the HDL:LDL ratio was within normal limits. In all cases total cholesterol returned to baseline after the treatment. From a clinical perspective, decreased LDL is one of the desirable outcomes of using Lovastatin. There is no evidence of increase risk or harmful consequences for a temporal decrease of LDL levels. Since this is a 16 week study (plus follow-up), transient decrease in lipid profile levels will not change the risk for this patient population.

Since the long-term effects of lowering lipid levels in the children on Lovastatin are unknown at this time, having normal range cholesterol (cholesterol $\geq$ 90 mg/dL) is a criteria for enrollment. In addition, a lipid profile is checked at baseline and at weeks 4, 8, 12, 16 and 24. If levels drop below 80 mg/dL, the site should notify the protocol team for guidance. If other lipid profile levels drop below normal values, the site should also notify the protocol team for guidance.

Despite the lack of preliminary data in terms of safety using Lovastatin in children aged 8 or 9 years of age, we anticipate no changes or higher risk of side effects, compared with the older children. Date from a phase 1 mentioned before (Acosta et al., submitted), demonstrated no differences in tolerability or side effects in children 10 years of age compared with older children. In addition, metabolic pathways related with metabolism of drugs, including Cythrome P450 complex, are fully developed by 5 years of age (Anderson et al 2009). We do not expect changes in toxicity in this younger age group and those patients will be monitored closely during the study to early identify any risk(s) or side effect(s). Any metabolic alteration or adverse event observed in this specific group age will be notified to the Protocol team for careful evaluation.

There is some pain and a small risk of bruising associated with blood collection, which can be minimized by applying a topical anesthetic cream and having experienced blood collectors.

There are minimal risks associated with neuropsychological assessments. All the tests in this study are routinely used in clinical and/or research neuropsychological evaluations. As such, they are not expected to result in any undue stress in the child. Some children, particularly younger children, may become tired during the assessment. If this happens, the child can take short breaks. At the beginning of the session, the child will be told by the psychologist that they should try their best. They will also be told that they may find some questions easy and others a little harder, but not to worry if they cannot answer all questions, as some are for older children. The majority of tests have a discontinue rule, so that testing is stopped if the child makes a certain number of consecutive errors. This ensures that the child does not become anxious or frustrated. If a participant worries that they are unable to answer a question, the test administrator will

reassure the child that he/she is not expected to answer all the questions and it is only expected that he/she try his/her best on the task. All test administrators will have psychological training and experience with interacting and assessing children. If for some reason the child states that he/she does not want to continue, the assessment will cease. We will then consult with the parent and child to ascertain whether the testing will continue.

There are an estimated six partial or whole days that a child could miss school while participating in this trial. We will recommend to the parent (parents, legal guardians) that he/she should discuss school policies for excused absences and make necessary arrangements for make-up work.

7.0 Statistics

Determination of sample size: No data exist on the effects of Lovastatin in children with NF1. A clinical and meaningful difference for primary efficacy measures is defined as an effect size of one half of a standard deviation, which is classified as a treatment effect of medium magnitude (Cohen, 1988). A sample size of 64 participants per treatment group (128 total) will be sufficient to detect an effect size of half a standard deviation at the two-sided 0.05 significance level with power of 0.80 (Peat, Mellis, Williams & Xuan, 2001). To allow for a 10% dropout rate prior to the 16-week point, a total of 142 participants will be enrolled.

Interim analyses: Interim analyses will be done under the purview of the DSMB, which will set the content and frequency of analyses. We will propose a Charter containing a data monitoring plan that will include stopping guidelines. We expect these to be the Lan DeMets modifications of the O'Brien Fleming boundaries (Lan & DeMets, 1983; O'Brien & Fleming, 1979)). It is expected that the DSMB will review this charter during its first meeting.

Data analysis: Demographic and baseline characteristics will be summarized for each group using descriptive statistics. Intention-to-treat analyses will be conducted for the primary outcomes measures. An intention-to-treat analysis provides an unbiased, conservative and consistent estimate of a treatment effect (Heritier, Gebski & Keech, 2003).

Analyzing change scores from baseline to post-treatment does not control for baseline imbalance because of the regression to the mean (Vickers & Altman, 2001). Also, with changes scores, if the treatment is effective, the statistical significance of the treatment effect will depend upon the correlation between baseline and follow-up scores. Therefore, to determine whether there has been a significant change from baseline to post-treatment, primary and secondary outcomes will be analyzed using ANCOVA, which is a better approach than analyzing change scores with independent t-tests (Vickers & Altman, 2001).

In addition, at an individual case level to determine whether test-retest change scores from baseline to post-treatment are reliable and clinically meaningful, standardized regression based

change scores (RBC) will be also be calculated. RBC can account for measurement error, differential practice effects and regression to the mean (Sherman et al., 2003). However, RBC scores do not necessarily indicate whether a significant change from baseline has occurred (Sherman et al., 2003). Similar statistical analysis will be conducted for scores from post-treatment to follow-up. Linear mixed models may also be conducted to examine changes over time. In addition, a number of sub-group analyses are planned to help identify individuals more likely to benefit from, or be harmed by, the treatment. In regard to treatment effects, definition of subgroups will rely on baseline data, not data measured after randomization. Such subgroups might include age (including comparison of 8-9 years to 10 years and older), gender, and presence of ADHD (on or off stimulant medication) (if there are sufficient cases). Exploratory data derived through subgroup analyses will serve primarily to generate new hypothesis for subsequent studies.

8.0 Data Management

Data sets will follow all standards as per Data Coordinating Center‟s guidelines and recommendations. All paper charts, forms, and information associated with this study will be kept in a locked cabinet at the clinical sites. Access to this cabinet is available only to investigators on the study and research assistants. All participant information from this study will be strictly confidential. On entry to the study, participants will be assigned a unique PIN that will be used throughout the study. All data stored in computer systems will be password protected and stored using participant PINs only (see next section for more information regarding database security). Participant names will not be used in any computer databases associated with this project. A list of participant names and coordinating PINs will be kept separately on the password-protected principal investigator‟s computer. This information will be kept in accordance with the requirements of the Department of Defense.

8.1 Medical Monitor and Data Safety Monitoring Plan

The Data Safety and Monitoring Plan for this study will follow the NIH guidelines. The External Advisory Board (EAB) will serve as external reviewers and advisors to DoD and the Governing Body. The EAB will review and approve the protocol with respect to ethical and safety standards. Its primary responsibility will be to monitor the emerging results of the trial to assess treatment representatives are *ex officio* members of the EAB. The EAB will determine the content and frequency of safety reports it will review and will periodically review interim analyses of data collected for this study.

In addition, the DSMB will protect the integrity of the study by ensuring that recruitment targets can be met within the study timelines. This will be achieved by comparing actual recruitment against planned and by monitoring participant drop-out rates. In the event that the number of accrued patients is less than 20% of the planned value, the DSMB will advise on appropriate remedial action. Possible remedial actions may include, but are not limited to, the extension of the recruitment period, review and amendment of inclusion criteria.

Dr. Tena Rosser will be the medical monitor for this study. She is a qualified physician and is not associated with this protocol. She will work closely with the Principal Investigator to monitor the participants‟ treatment while on this study. The medical monitor is required to review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject related deaths associated with the protocol, providing an unbiased written report of the event. At minimum, the medical monitor should comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should also indicate whether she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or the medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the USAMRMC ORP HRPO.

It is expected that summaries of accrual, retention, and adverse events will be disseminated to the clinical centers periodically. To maintain the blind, adverse events will be aggregated across treatment arms.

8.2 Confidentiality

Data will be obtained and stored consistent with IRB and HIPAA guidelines. All participant charts, standardized study forms, and information associated with this study will be kept in a locked cabinet in the principal investigator‟s office. Access to this cabinet is available only to the principal investigator and the research team. All participant information from this study will be strictly confidential. On entry to the study, participants will be assigned a unique participant PIN that will be used throughout the study. All data stored in computer systems will be password protected and stored using PINS only. Participant names will not be used in any computer databases associated with this project. A list of participant names and coordinating PINs will be kept separately on the password protected principal investigator‟s computer. This information will be kept in accordance with the requirements of the Department of Defense.

9.0 Ethical and Regulatory Consideration

9.1 Consent/Assent/HIPAA Process and Documentation

The investigational nature and objectives of the study, procedures involved, treatments involved, medication side effects, risks, benefits and alternative therapies will be carefully explained to potential participants and their parents/legal guardians. Potential participants will be allowed to take their time in deciding whether to join the trial and will be given privacy to make their decision. They will also be encouraged to discuss their concerns or questions about joining the trial with their parents/guardian. Consent will be obtained from parents/legal guardians and assent will be obtained from children aged 8 years to less than 16 years, with signatures obtained as appropriate. This trial will be conducted in compliance with the Good Clinical Practice guidelines and the applicable regulatory requirements.

9.2 Benefits

For participants receiving Lovastatin, it may have a direct benefit on their cognitive abilities (namely their visual spatial learning and/or attention). It is possible that Lovastatin may additionally have a positive effect on secondary outcome measures. After the participant has completed the trial, a report of their neuropsychological assessment will be provided to the parents to assist the child in the school environment. This will include a summary of the child‟s cognitive strengths and weaknesses based on the neuropsychological assessments. If cognitive weaknesses are found, strategies will be offered with the aim to help parents and educators manage the cognitive difficulties. Recommendations for additional support/services will also be provided as necessary. 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 Lovastatin is efficacious in treating cognitive impairments, this will lead to a Phase III study to determine its effectiveness in the general NF1 community.

The current study will contribute to the treatment of cognitive impairments associated with NF1. It will provide additional information regarding the safety of Lovastatin in children with NF1 and cognitive deficits. As mechanisms related with cholesterol metabolism and similar pathways that imply Ras pathway activation has been implicated in other models of learning. These results may have a more generalized benefit. Through this study, there is the hope to gain a better understanding of the neurobiology and extent of cognitive difficulties in children with NF1.

9.3 Potential Biases and Problems

Challenges may exist in recruiting participants. The Recruitment section of the proposal details participant availability and recruitment measures for the clinical trial. At recruitment, the side effects of the medication, as well as its use in children with NF1 (Phase I), will be reviewed with parents. The system for monitoring side effects will also be discussed. Parents and children will have the opportunity to ask questions regarding use of the medication and will be able to withdraw from the study at any time.

Participants will be stratified to study medication by clinical site to minimize systematic differences in important or unknown confounders between the groups. The requirement for multiple visits may result in some attrition in the sample due to difficulties keeping each of the scheduled appointments. We have included a 10% attrition rate in the calculation of a sample size. To assist in the retention of participants, we will have regular contact with participants throughout the entire study. The investigators, study coordinator, and research assistant will work closely with the families to facilitate their return and maximize participation. All participants will be required to attend scheduled visits. This will allow us to monitor any side effects and may also indirectly assist in maintaining treatment compliance.

Lovastatin has been shown to be safe and effective in a pediatric population for treatment of familial heFH. Although unexpected adverse events may occur, frequent monitoring as well as phone contact with participants and their families will be done throughout the study.

9.4 Costs to Subjects

The study will not imply cost for participants and families. We will provide study medication during the trial; laboratories and neuropsychological testing that are part of the present study. Participants still have to pay for any other medical care that is not part of the study.

9.5 Conflicts of Interest

None.

9.6 Subject Compensation

Medication, laboratories and neuropsychological testing will be free of charge. Additional medical expenses not related with the study will not be covered.

9.7 Outside Consultants/Collaborators

9.8 Contractual Agreements

None**.**

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APPENDIX 1: National Institute of Health Criteria for Neurofibromatosis 1 (1988)

National Institute of Health Criteria Diagnostic for Neurofibromatosis 1
Two or more of the following 7 clinical features (NIH, 1988):
Six or more café au lait spots (>0.5 cm in prepubertal individuals and >1.5 cm in postpubertal individuals.
Axillary or groin freckling.
Optic glioma.
Two or more Lisch nodules.
A distinctive bony lesion.
One plexiform neurofibroma or two or more neurofibromas of any type.
A first degree relative with NF1 by the above criteria.

APPENDIX 2: List of Common Psychotropic Medications

Medication	Generic Name
Abilify	Aripiprazole
Clozaril	Clozapine
fluphenazine (generic only)	Fluphenazine (Prolixin)
Geodon	Ziprasidone
Orap (for Tourette's syndrome)	Pimozide
Risperdal	Risperidone
Seroquel	Quetiapine
Zyprexa	Olanzapine
Aventyl (tricyclic)	Nortriptyline
Celexa (SSRI)	Citalopram
Cymbalta (SNRI)	Duloxetine
Desyrel	Trazodone
Effexor (SNRI)	Venlafaxine
Elavil (tricyclic)	Amitriptyline
Lexapro (SSRI)	Escitalopram
Luvox (SSRI)	Fluvoxamine
Norpramin (tricyclic)	Desipramine
Pamelor (tricyclic)	Nortriptyline
Paxil (SSRI)	Paroxetine
Prozac (SSRI)	Fluoxetine
Remeron	Mirtazapine
Wellbutrin	Bupropion
Zoloft (SSRI)	Sertraline
Depakote	Divalproex Sodium (Valproic Acid)
Lamictal	Lamotrigine
Neurontin	Gabapentin
Tegretol	Carbamazepine
Topamax	Topiramate
Trileptal	Oxcarbazepine
Xanax	Alprazolam
Adderall	Amphetamine
Adderall XR	Amphetamine (Extended Release)
Concerta	Methylphenidate (Long Acting)
Daytrana	Methylphenidate Patch
Desoxyn	Methamphetamine
Dexedrine	Dextroamphetamine
Dextrostat	Dextroamphetamine
Focalin	Dexmethylphenidate

Focalin XR	Dexmethylphenidate (Extended Release)
Metadate ER	Methylphenidate (Extended Release)
Metadate CD	Methylphenidate (Extended Release)
Methylin	Methylphenidate (Oral Solution And Chewable Tablets)
Ritalin	Methylphenidate
Ritalin SR	Methylphenidate (Extended Release)
Ritalin LA	Methylphenidate (Long Acting)
Strattera	Atomoxetine
Vyvanse	Lisdexamfetamine Dimesylate
Catapress	Clonidine

APPENDIX 3: Study Evaluation Before, During, Post Treatment and Follow-Up

Observation	Pre-treatment	2 weeks*	4 weeks (2 titrated & 2 weeks full dose)	8 weeks	12 weeks	Post-treatment 16 weeks	Follow-up (8 weeks off medication)
Phone Calls		X					
Hx and Physical	X		X	X	X	X	X
CBC	X		X	X	X	X	X
Chemistries	X		X	X	X	X	X
Neuropsychological measures	X					X	X
Pregnancy testing	X			X		X	
Side-Effects/Toxicity (Check List)		X	X	X	X	X	X
Drug compliance		X	X	X	X	X	

NB. Chemistries: Blood urea nitrogen (BUN), creatinine, glucose, alanine amino transferase (ALT), aspartate amino transferase (AST), total and direct bilirubin, CPK, Lipid profile (HDL, Cholesterol, LDL, Triglycerides). Indirect bilirubin will be computed from total and direct bilirubin serum measurements.

*Use CRFs as a guide with questions for phone follow-up

APPENDIX 4: Consent/Assent

[Blank intentionally – add your site specific approved consent/assents]

APPENDIX 5: Outcome Measures: Neuropsychological Assessment

Domain	Measure	Age range	Time (mins)
Memory	*Paired Associate Learning (CANTAB)	7 yrs - adult	10
Attention	Test of Everyday Attention for Children	6 yrs – 15 yrs 11 mths	25
	*Score!		
	Sky Search		
	Creature Counting		
	Sky Search DT		
	Continuous Performance Test –II	6 yrs - adult	15
	Conners‟ ADHD/DSM-IV scale (parent)	3 yrs – 17 years	10
Executive Function	Stop Signal Task (CANTAB)	7 yrs – adult	10
	Spatial Working Memory (CANTAB)	7 yrs – adult	15
	Stockings of Cambridge (CANTAB)	7 yrs – adult	10
	Controlled Oral Word Association Test	7 yrs – adult	5
	Behavior Rating Inventory of Executive Function (parent)	5 – 18 yrs	15
Visual-spatial	Judgment of Line Orientation	7 yrs - adult	10
Visual-perceptual	Object Assembly (WISC-III)	6 yrs – 16yrs 11mths	13
Motor	Motor Screening (CANTAB)	7 yrs – adult	5
Intelligence	Wechsler Abbreviated Scale of Intelligence	6 yrs – adult	30
Behavior	Behavior Assessment Scale for Children – II (parent & child)	2 – 25 yrs	20
Quality of Life	Pediatric Quality of Life Inventory (parent & child)	2 – 18 yrs	10

*Primary outcome measures.

All tests of the comprehensive test battery are administered at baseline, post-treatment and follow-up except for the Wechsler Abbreviated Scale of Intelligence, which will only be administered at screening.

APPENDIX 6: Drugs & Other Components That Interact With Lovastatin

AVOID WHILE TAKING LOVASTATIN

CYP3A4/5 – Inducers	
Carbamazepine (Tegretol)	Phenytoin (Dilantin)
Dexamethasone	Primidone (Mysoline)
Ethosuximide (Zarontin)	Progesterone (all progestins)
Glucocorticoids	Rifabutin
Griseofulvin	Rifampin
Nafcillin	Rofecoxib
Nelfinavir (Viracept)	St John's wort
Nevirapine (Viramune)	Sulfadimidine
Oxcarbazepine (Trileptal)	Sulfinpyrazone
Phenobarbital	Troglitazone
Phenylbutazone	Rifapentine
	Modafinil
CYP3A4/5 – Inhibitors	
Amiodarone	Metronidazole
Anastrozole	Mibefradil
Azithromycin (Zithromax)	Miconazole
Cannabinoids	Nefazodone
Cimetidine (Tagamet)	Norfloxacin
Clarithromycin	Norfluoxetine
Clotrimazole	Omeprazole (Prilosec)
Cyclosporine	Oxiconazole
Danazol	Paroxetine
Delavirdine (Rescriptor)	Propoxyphene
Diethyldithiocarbamate	Roxithromycin
Diltiazem	Quinidine
Dirithromycin	Quinine
Disulfiram	Quinupristin and dalfopristin
Entacapone	Ranitidine
Erythromycin	Ritonavir (Norvir)
Ethinyl estradiol	Saquinavir (Invirase)
Fluconazole (Diflucan)	Sertindole
Fluoxetine	Sertraline
Fluvoxamine	Troleandomycin
Gestodene	Valproic acid (Depakote)
Grapefruit juice	Verapamil
Indinavir (Crixivan)	Voriconazole
lsoniazid	Zafirlukast (Accolate)
ltraconazole	Zileuton
Ketoconazole	

ADAPTED from Cytochrome P-450 Enzymes and Drug Metabolism: Lacy CF, Armstrong LL, Goldman MP, eds. Drug Information Handbook 8th ed. Hudson, OH; LexiComp Inc. 2000: 1364-1371.

APPENDIX 7: Common Terminology, Criteria for Adverse Events (CTCAE)

Items selected according to most common or serious side effects associated with Lovastatin

Adverse Event	0	1	2	3	4
DERMATOLOGIC					
Allergic reaction/ hypersensitivity (including drug fever)	none	transient rash, drug fever <38°C (<100.4°F)	urticaria, drug fever >38°C (>100.4°F), and/or asymptomatic bronchospasm	symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis
Rash/desquamation	none	macular or papular eruption or erythema without associated symptoms	macular or papular eruption or erythema with pruritus or other associated symptoms covering <50% of body surface or localized desquamation or other lesions covering <50% of body surface area	symptomatic generalized erythroderma or macular, papular or vesicular eruption or desquamation covering >50% of body surface area	generalized exfoliative dermatitis or ulcerative dermatitis
HEMATOLOGIC					
Hemoglobin (Hgb)	WNL	<LLN - 10.0 g/dL <LLN - 100 g/L <LLN - 6.2 mmol/L	8.0 - <10.0 g/dL 80 - <100 g/L 4.9 - <6.2 mmol/L	6.5 - <8.0 g/dL 65 - <80 g/L 4.0 - <4.9 mmol/L	<6.5 g/dL <65 g/L <4.0 mmol/L
Platelets	WNL	<LLN - 75.0 x 109 /L <LLN - 75,000/mm3	>50.0 - <75.0 x 109 /L >50,000 - <75,000/mm3	>10.0 - <50.0 x 109 /L >10,000 - <50,000/mm3	<10.0 x 109 /L <10,000/mm3
CONSTITUTIONAL					
Fatigue (lethargy, malaise, asthenia)	none	increased fatigue over	moderate (e.g., decrease	severe (e.g., decrease in	bedridden or disabling

		baseline, but not altering normal activities	in performance status by 1 ECOG level or 20% Karnofsky or *Lansky*) or causing difficulty performing some activities	performance status by >2 ECOG levels or 40% Karnofsky or *Lansky*) or loss of ability to perform some activities	
Dizziness/lightheadedness	none	not interfering with function	interfering with function, but not interfering with activities of daily living	interfering with activities of daily living	bedridden or disabling
Insomnia Note: This adverse event is graded when insomnia is related to treatment. If pain or other symptoms interfere with sleep do NOT grade as insomnia.	normal	occasional difficulty sleeping not interfering with function	difficulty sleeping interfering with function, but not interfering with activities of daily living	frequent difficulty sleeping, interfering with activities of daily living	-
Headache	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain: pain or analgesics severely interfering with activities of daily living	disabling
GASTRO-INTESTINAL					
Abdominal pain or cramping	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of	severe pain: pain or analgesics severely interfering with activities of daily living	disabling

			daily living		
Constipation	none	requiring stool softener or dietary modification	requiring laxatives	requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea participants without colostomy:	none	increase of <4 stools/day over pretreatment	increase of 4-6 stools/day, or nocturnal stools	increase of >7 stools/day or incontinence; or need for parenteral support for dehydration	physiologic consequences requiring intensive care; or hemodynamic collapse
Flatulence	none	mild	moderate	-	-
HEPATIC					
AST	WNL	>ULN - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN
ALT	WNL	>ULN - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN
Bilirubin	WNL	>ULN - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 10.0 x ULN	>10.0 x ULN
MUSCLE					
CPK (creatine phosphokinase)	WNL	>ULN - 2.5 x ULN	>2.5 - 5 x ULN	>5 - 10 x ULN	>10 x ULN
Muscle weakness (not due to neuropathy)	normal	asymptomatic with weakness on physical exam	symptomatic and interfering with function, but not interfering with activities of daily living	symptomatic and interfering with activities of daily living	bedridden or disabling
Myositis (inflammation/damage of muscle) Also consider CPK. Note: Myositis implies muscle damage (i.e., elevated CPK).	none	mild pain, not interfering with function	pain interfering with function, but not interfering with activities of daily living	pain interfering with function and interfering with activities of daily living	bedridden or disabling
Myalgia (muscle pain)	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain: pain or analgesics severely interfering with activities of daily living	Disabling

B. TIMETABLE

As outlined in the statement of work, the general timetable of this project is as follows:
0 – 12 weeks: Initiation of the study and subject recruitment
12 – 24 weeks: Continue subject recruitment and initiation of treatment and evaluation
24 - 36 weeks: Continue recruitment initiation and implementation of the clinical trial
36 to 72 weeks: Continue study/follow-up participants
72 to 96 weeks: Final data analysis and completion of the study

Months	0 - 3	3 - 6	6-9	9-18	18-24	24-36
Study Design/Ethics/Logistics	X					
Subject Recruitment/Initiation /Evaluation		X	X	X	X	
Implementation of Trial			X	X	X	
Follow-up Participants				X	X	
Begin and Complete Final Data Analysis						X

APPENDIX 8: List of Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
ALT	Alanine amino transferase
ANCOVA	Analysis of covariance
Apo	Apolipoprotein
AST	Aspartate amino transferase
BASC-II	Behavior Assessment System for Children, 2nd ed‟n
BRIEF	Behavior Rating Inventory of Executive Function
BUN	Blood urea nitrogen
CADS	Conners‟ ADHD/DSM-IV Scales
CANTAB	Cambridge Neuropsychological Testing Automated Battery
CBC	Complete blood count
COWAT	Controlled Oral Word Association Test
CPK	Creatine phosphokinase
CPT	Continuous Performance Task
CTCAE	Common Terminology Criteria for Adverse Events
DoD	Department of Defense
DSMB	Data and Safety Monitoring Board
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th ed‟n
EAB	External Advisory Board
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GDP	Guanosine diphosphate
GTP	Guanosine-5'-triphosphate
HDL	High-density lipoproteins
heFH	Hypercholesterolemia
HIPPA	Health Insurance Portability and Accountability Act
HMG CoA	3-hydroxy-3-methyl-glutaryl-Coenzyme A
IQ	Intelligence quotient
IRB	Institutional Review Board
JLO	Judgment of Line Orientation
LDL	Low-density lipoprotein
LTP	Long-term potentiation
MOT	Motor Screening
NF1	Neurofibromatosis type 1
NIH	National Institutes of Health
PAL	Paired Associate Learning
PedsQL	The Pediatric Quality of Life Scale
PPI	Pre-pulse inhibition
RBC	Regression based change
SD	Standard deviation
SGT	Solanidine UDP-glucose glucosyltransferase
SOC	Stockings of Cambridge
SST	Stop Signal Task
SWM	Spatial Working Memory
TEA-Ch	Test of Everyday Attention for Children
UDP-glucose	Uridine diphosphate glucose
VLDL	Very low density lipoprotein
WASI	Wechsler Abbreviated Scale of Intelligence
CGA	Computer Generated Arena

APPENDIX 9: Protocol Roster

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Elizabeth Davis, RN, MPH, CCRC, Clinical Coordinator – NF Operations Center

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APPENDIX 10: Adverse Event Monitoring – UK Sites

Participants will be monitored closely for medication side effects with a diary and with laboratory testing as indicated in the Study Procedures section (see of the protocol (see Section 5). Adverse events will be addressed immediately. Participants will also be closely followed clinically at least once per month while on the study medication by the site physician. The Side Effects Checklist and Adverse Event Monitoring Form will be used to assess participants at every visit or phone contact.

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, require therapy, or as specified on CTCAE, version 4.

The occurrence of adverse events will be sought by non-directive questioning of the patient 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, reported to the sponosor 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.
Unlikely: the adverse event *is doubtfully related* to the study medication.
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 make 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 based on CTCAE (version 4)

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 Summary of Product Characteristics (SmPC).

Serious Adverse Event (SAE) reporting
Any SAE will be reported by the Principal Investigator (including a completed SAE form) within 24 hours of first knowledge to the Sponsor. The Principal Investigator will ensure that the patient 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. An annual safety report will be sent by the Chief Investigator to the MHRA, the Ethics Committee and sponsor. 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.

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
It the event that pregnancy does occur in a patient 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.

All serious adverse events require immediate notification to the UK co-sponsor and US Operations Center. All adverse events, whether serious or otherwise, will be followed until resolution (the patients health has returned to his/her baseline status) or until the event has stabilized (investigator does not expect any further improvement or worsening of the adverse event). Participants withdrawn from the study due to adverse events will be followed by the investigator until resolution. All grade 3, 4 and 5 adverse events will be reviewed by the US Clinical Review Committee.

All adverse events will be tabulated by treatment arm, severity grade and reported to the US NF1 Data and Safety Monitoring Board (DSMB). The occurrence of adverse events will be reviewed by the DSMB at 2-month intervals to monitor patient safety. Summary details of the occurrences and grade of adverse events will be distributed to each site at 2-monthly intervals. In addition, all unexpected and serious adverse events will be forwarded to the US medical monitor, Tena Rosser, M.D. She will serve as a patient advocate, will be independent of the clinical study team, and will report to the DSMB. She will oversee the progress of the protocol, especially issues of individual subject/patient management and safety. The monitor will provide an unbiased written report of each event.