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## Protocol for a phase II, monocenter, double-blind, placebo-controlled, cross-over trial to assess efficacy of pyridostigmine in patients with spinal muscular atrophy types 2, 3 and 4 (SPACE trial)

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Keywords:	spinal muscular atrophy, SMA, neuromuscular junction, pyridostigmine, fatigability, cross-over

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**TITLE**

Protocol for a phase II, monocenter, double-blind, placebo-controlled, cross-over trial to assess efficacy of pyridostigmine in patients with spinal muscular atrophy types 2, 3 and 4 (SPACE trial)

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**STUDY DETAILS**

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## ABSTRACT

### Introduction

Hereditary proximal spinal muscular atrophy (SMA) is caused by homozygous deletion of the *survival motor neuron 1 (SMN1)* gene. The main characteristic of SMA is degeneration of alpha motor neurons in the anterior horn of the spinal cord, but recent studies in animal models and patients have shown additional anatomical abnormalities and dysfunction of the neuromuscular junction (NMJ). NMJ dysfunction could contribute to symptoms of weakness and fatigability in patients with SMA. We hypothesize that pyridostigmine, an acetylcholinesterase inhibitor that improves neuromuscular transmission, could improve neuromuscular junction function and thereby muscle strength and fatigability in patients with SMA.

### Methods and analysis

We designed a monocenter, placebo-controlled, double-blind cross-over trial with pyridostigmine and placebo to investigate the effect and efficacy of pyridostigmine on muscle strength and fatigability in patients with genetically confirmed SMA. We aim to include 45 patients with SMA types 2, 3 and 4, aged 12 years and older in the Netherlands. Participants receive 8 weeks of treatment with pyridostigmine and 8 weeks of treatment with placebo in a random order separated by a wash-out period of one week. Treatment allocation is double-blinded. Treatment dose will gradually be increased from 2 mg/kg/day to the maximum dose of 6 mg/kg/day in four daily doses, in the first week of each treatment period. The primary outcome measures are a change in the Motor Function Measure and repeated Nine-Hole Peg Test before and after treatment. Secondary outcome measures are changes in recently developed endurance tests, i.e. the Endurance Shuttle Nine Hole Peg Test, the Endurance Shuttle Box and Block Test and the Endurance Shuttle Walk test, muscle strength, level of daily functioning, quality of and activity in life, perceived fatigue and fatigability, presence of decrement upon repetitive nerve stimulation, and adverse events.

### Ethics and dissemination

The protocol is approved by the local medical ethical review committee at the University Medical Center Utrecht and by the national Central Committee on Research Involving Human Subjects. Findings will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of medical care and quality of life for SMA patients.

### Trial registration

- US registry NCT02941328 ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) registration date: October 21, 2016
- European registry 2011-004368-34 ([www.clinicaltrialsregister.eu](http://www.clinicaltrialsregister.eu)) registration date: November 3, 2014

- Dutch registry NL38048.041.14 ([www.ccmo.nl](http://www.ccmo.nl)) registration date: April 24, 2015

**Keywords** spinal muscular atrophy, SMA, neuromuscular junction, NMJ, pyridostigmine, cross-over, muscle strength, motor function, fatigability

## STRENGTHS AND LIMITATIONS

- This randomized double-blind, placebo-controlled cross-over trial will provide important information to clinicians and patients with spinal muscular atrophy about efficacy of treatment of fatigability, lack of endurance and diminished motor function with pyridostigmine.
- The cross-over design is an ideal design for this rare disease with striking variability, because participants will be their own controls, which reduces unsystematic variance, subsequently reducing the necessary sample size to detect systematic variance after treatment.
- Permuted block randomization ensures treatment group numbers are evenly balanced at the end of each block and at the end of the study with this relatively small number of participants.
- The use of tests that are still in the process of validation is a limitation of this protocol, however these tests can capture a dimension of SMA for which validated outcome measures are largely lacking.

## MAIN TEXT

### Introduction

Hereditary proximal Spinal Muscular Atrophy (SMA) is a motor neuron disease in children and adults caused by a homozygous deletion of the *survival motor neuron 1 (SMN1) gene*, resulting in a significant reduction of full length functional SMN protein.[1, 2] The main characteristic of SMA is the degeneration of alpha motor neurons in the anterior horns of the spinal cord, resulting in progressive muscle weakness of axial muscles and muscles of the arms and legs with a mild to severely reduced life expectancy in the majority of patients.[3-5] SMN protein is ubiquitously expressed and is involved in the pre-mRNA splicing pathway, ubiquitin and cytoskeleton homeostasis, endocytosis and axonal transport.[6-10] Although motor neurons are most sensitive to the disruption of cellular pathways caused by SMN deficiency, other cell types and tissues may be affected as well.[11, 12] Histological and electrophysiological studies have shown that sufficient levels of SMN protein are essential for the development, maturation and function of the neuromuscular junction (NMJ).[13, 14] SMN-deficient mice display both presynaptic (i.e. abnormal density and distribution of synaptic vesicles and abnormal accumulation of neurofilaments at the nerve terminal of the

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3 NMJ) and postsynaptic (i.e. shrinkage of motor endplates) abnormalities.[15-18] In patients  
4 with SMA type 1, abnormal aggregation of acetylcholine receptors at the muscle endplates  
5 has been reported.[18, 19] Nerve conduction studies with repetitive nerve stimulation (NCS-  
6 RNS) in patients with SMA types 2 and 3, a specific but not very sensitive test for NMJ  
7 dysfunction, showed an abnormal decremental response in 49% of patients.[13] SMA  
8 patients frequently complain of fatigability, which is defined as a decrease in performance  
9 over a given time or sustained measure of mechanical output,[20] in addition to muscle  
10 weakness.

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14 Intrathecal administration of SMN-specific anti-sense oligonucleotides that augment cellular  
15 SMN levels improves motor development in infants and children with SMA, but efficacy has  
16 not been tested in adults.[21, 22] There is a clear need for low cost treatment that is easy to  
17 administer in patients with longer disease duration. The finding of post-synaptic dysfunction  
18 of the neuromuscular junction in SMA suggests that patients may benefit from drugs that  
19 facilitate neuromuscular transmission. Acetylcholinesterase inhibitors may represent a new  
20 category of candidate drugs for the treatment of SMA. Pyridostigmine, an  
21 acetylcholinesterase inhibitor with relatively long half-life, is an FDA and EMA approved first  
22 line treatment of disorders of the post-synaptic neuromuscular junction, i.e. Myasthenia  
23 Gravis. Pyridostigmine inhibits the natural enzymatic breakdown of acetylcholine and thereby  
24 increases its biological availability at the neuromuscular junction enhancing neuromuscular  
25 transmission.[23]

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34 Our aim in this study is to investigate the efficacy and effect of pyridostigmine on muscle  
35 strength and fatigability in SMA. We designed a placebo-controlled, cross-over trial in  
36 patients with SMA types 2, 3 or 4, with double-blind treatment allocation. The crossover  
37 design is an ideal design for this rare disease with striking variability, as using participants as  
38 their own controls will reduce the unsystematic variance (error variance). This allows for  
39 easier detection of systematic variance following the intervention using fewer study  
40 participants. The short half-life of pyridostigmine excludes carry-over effects.

## 41 42 43 44 45 **Methods and Design**

### 46 *Study setting and design*

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48 We conduct this study at the neuromuscular department of the University Medical Center  
49 Utrecht, a tertiary referral center for neuromuscular diseases in The Netherlands. All  
50 members of the study team, consisting of physicians, physical therapists and nurses, have  
51 broad experience with SMA due to the national cohort study that is carried out in this center  
52 since 2010.[24]

This investigator-initiated, monocenter, placebo-controlled study has a cross-over, double-blinded design, with blinding of participants and investigators. The pharmacist is not blinded for allocation of treatment. The study protocol was designed using the recommendations of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. (See Additional file 1 for the SPIRIT checklist 2013 statement).

The study is currently ongoing; the first participant was included on November 25, 2015. We expect study completion by the end of 2017.

### Participants

The details of the inclusion and exclusion criteria are provided in Table 1. The main inclusion criteria are: a clinical diagnosis of SMA type 2, 3 or 4 and a genetically confirmed homozygous *SMN1* deletion and age 12 years or older. We recruit patients with SMA types 2-4 through the national SMA registry that contains detailed clinical information of approximately 300 patients.[24] To minimize selection bias, all eligible patients, based on known SMA type, are invited to participate.

**Table 1. Selection criteria**

<b>Inclusion criteria</b>	
	Clinical diagnosis of SMA type 2, 3 or 4 <ul style="list-style-type: none"> <li>• type 2: age at onset &gt;6 months and ability to sit unsupported but not to walk unsupported</li> <li>• type 3: age at onset &gt;18 months and the ability to walk unsupported at any point in life</li> <li>• type 4: age at onset <math>\geq</math>30 years and the ability to walk unsupported at any point in life</li> </ul> In case of discrepancy between age at onset and highest acquired motor milestone, the latter is used to define SMA type
	Genetically confirmed homozygous <i>SMN1</i> deletion
	Given oral and written informed consent when $\geq$ 18 years old and additional informed consent by the parents or legal representative in case of participants aged $\geq$ 12 till <18 years old
	Ability to perform at least 2 subsequent rounds of the Nine Hole Peg Test[27, 28]
	A maximum Motor Function Measure[25] score of 80%
<b>Exclusion criteria</b>	
	Known concomitant disorders of the NMJ (Lambert Eaton myasthenic syndrome, myasthenia gravis)
	Use of drugs that may alter NMJ function <ul style="list-style-type: none"> <li>• cholinergic medication (e.g. rivastigmine, neostigmine, galantamine, fysostigmine, succinylcholine)</li> <li>• non-depolarising musclerelaxans (e.g. (cis)atracurium, gallamine, mivacurium, pancuronium, rocurpnium, vecuronium)</li> <li>• other antagonizing medication of prydostimine (procainamide, quinidine, propranolol,</li> </ul>



	lithium, chloroquine, hydroxychloroquine, aminoglycoside antibiotics, clindomycine, polymixine)
	SMA type 1
	Apprehension for participation in nerve conduction studies
	Inability to meet study visits
	Mechanical gastro-intestinal, urinary or biliary obstruction
	Clinical significant alterations of blood tests drawn within 14 days prior to start of study entry
	Electrocardiophysiology abnormalities known as a contraindication for pyridostigmine use
	Current pregnancy or breast-feeding
	Known allergy to bromides
	Severe bronchial asthma (in case of uncertainty of diagnosis, we will contact the treating pulmonologist or physician)

We will not replace withdrawn or unblinded participants. We will not include them in the study again once dropped-out and we will not re-use their identification number and treatment.

With permission of the participant, we will plan a follow-up by phone for at least one week. If there is an adverse event that is still present after one week, follow-up will be longer.

#### *Sample size calculation*

We aim to recruit 45 participants with SMA types 2-4 based on two power calculations that we performed based on the cross-over design using pilot data on repeated measures of the total score of the MFM test (unpublished data). First, we calculated the within-participant standard deviation, and next the standard deviation of the difference between subsequent measurements of the participants.

Calculation 1: If a total of 40 participants will enter this two-treatment cross-over study, the probability is 80 % that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.093 units. This is based on the assumption that the within-participant standard deviation of the response variable is 1.7 units.

Calculation 2: If a total of 40 participants will enter this two-treatment cross-over study, the probability is 80 % that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.409 units. This is based on the assumption that the standard deviation of the difference in the response variables is 3.1 units.

Both calculations show similar results in terms of the detectable difference based on 80% power, a two-sided significance level of 0.05 and 40 participants in total in the trial. Five



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3 additional participants will be recruited to compensate for potential dropouts. The total  
4 number of included participants will therefore be 45.  
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### 7 *Intervention and participant timeline*

8 Once the investigator confirms eligibility, the participant is assigned to one of the two  
9 treatment groups (pyridostigmine or placebo) and will cross-over during the trial.

10 Randomization to a treatment order (i.e. to start with either pyridostigmine or placebo) is  
11 done through a permuted 4-block design by the pharmacist, who is the only one not blinded  
12 for treatment allocation. Permuted block randomization ensures treatment group numbers  
13 are evenly balanced at the end of each block and at the end of the study with this relatively  
14 small number of participants.  
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20 *Figure 1* shows the participant timeline. At the screening visit we investigate whether  
21 participants are eligible for participation in the trial concerning all in- and exclusion criteria.  
22 As a safety measure, participants are screened for clinical significant alterations in blood  
23 tests (sodium, potassium, hemoglobuline, hematocrite, c-reactive protein (CRP), urea,  
24 creatinine, estimated glomerular filtration rate (eGFR), aspartate transaminase (AST),  
25 alanine transaminase (ALAT), gamma glutamyltranspeptidase (GGT), anti-acetylcholine  
26 receptor (AChR) antibodies and beta-HCG levels as a pregnancy test.), and they are  
27 screened for EKG alterations to ensure that included participants have no kidney  
28 dysfunction, liver function alterations, bradycardia or arrhythmias and/or a present  
29 pregnancy. If any of the screening tests results in a clinical significant alteration, the  
30 participant is excluded from study participation. The study schedule is presented in *Table 2*.  
31 At the start of the study, participants are randomized to one of two intervention groups  
32 (double-blinded; A or B). Each participant receives pyridostigmine (tablet) and placebo  
33 (matching tablet with no pharmacological ingredients) in consecutive periods.  
34 Group A starts with 8 weeks of treatment with pyridostigmine at a final dose of 6mg/kg a day.  
35 After a one week wash-out they start an 8-week period with placebo treatment.  
36 Group B starts with 8 weeks of treatment with placebo. After a one week wash-out they start  
37 an 8-week period with pyridostigmine at a final dose of 6mg/kg treatment a day.  
38 Each treatment is given 4 times a day and dosage is gradually increased in the first week of  
39 each treatment period to minimize side-effects; starting at 2 mg/kg a day in the first 3 days  
40 after the first administration. When this dose is well tolerated, the dose is increased to  
41 4mg/kg a day during day 4 up till day 7. When this dose is well tolerated, the dose is  
42 increased to the maximum dose of 6mg/kg a day after one week. In case of unfavorable  
43 side-effects of the medicinal product at 6mg/kg a day, the participant continues to use the  
44 highest achievable dose (2 or 4 mg/kg/day). The investigator gives the approval for increase  
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of the dosage after the first three days and after seven days by phone, after checking for invalidating side effects. If there are side effects the investigator can decide to not increase the dosage or to (temporarily) stop the medication depending on the extent of the side effects. In case of severe side-effects, the investigator can decide to intervene.

Pyridostigmine can cause a cholinergic crisis when overdosed due to the parasympathomimetic induction. Symptoms of a cholinergic crisis are excessive salivation, urinary urgency, diarrhoea, muscle weakness, fasciculations, cramps of striated muscles and respiratory problems. In case of symptoms of diarrhoea, excessive salivation and or sweating atropinesulphate can be given orally, 0.125 mg 1-2 per day. In case of severe symptoms, these symptoms can be treated with intravenous 1-2 mg atropinesulphate on slow infusion and supportive care of respiratory function, if needed. When it's necessary to unblind the treatment of a participant, for example in medical emergencies, this is done by the on-call pharmacist. The rest of the study team remains blinded.

**Table 2. Trial schedule of enrolment, interventions, and assessments**

TIMEPOINT	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
	-V <sub>1</sub>	0	V <sub>1</sub>	V <sub>2</sub>	Wash-out	V <sub>3</sub>	V <sub>4</sub>	V <sub>x</sub>
<b>ENROLMENT:</b>								
Eligibility screen	█							
Informed consent	█							
Inclusion		█						
Allocation		█						
<b>INTERVENTIONS:</b>								
[Intervention group A]			█	█		█	█	
[Intervention group B]			█	█		█	█	
<b>ASSESSMENTS:</b>								
Blood tests	█							
EKG	█							
<b>FUNCTIONAL TESTS</b>								
MFM	█		█	█		█	█	
R9HPT	█		█	█		█	█	
MRC scale	█		█	█		█	█	
ESNHPT	█		█	█		█	█	
ESBBT	█		█	█		█	█	
ESWT	█		█	█		█	█	

<b>PATIENT REPORTED OUTCOME MEASURES</b>							
SMA-FRS							
SF-36 / PedsQL							
FSS							
Fatigability questionnaire							
<b>NERVE CONDUCTION STUDIES</b>							
NCS-RNS							

**Legend Table 1.** Participants are asked to take the study medication 1-1.5 hour prior to the test battery on the day of their study visit to ensure the maximum effect of the pyridostigmine is measured. The tests are performed in the same order at each visit.

*Abbreviations: V=visit, EKG=Electrocardiography, MFM=Motor Function Measure,[25] R9HPT=Repeated Nine-Hole Peg Test,[Stam et al, submitted data] MRC= Medical Research Council Scale,[29] ESNHPT=Endurance Shuttle Nine-Hole Peg Test, ESBT=Endurance Shuttle Box and Block Test, ESWT=Endurance Shuttle Walk Test, [Bartels et al, in progress] SMA-FRS= SMA-Functional Rating Scale, SF-36=36-Item Short Form Health Survey,[43] PedsQL= Pediatric Quality of Life inventory,[45] FSS=fatigue severity scale,[52] NCS-RNS=nerve conduction studies with repetitive nerve stimulation*

To monitor therapy adherence, we inquire participants about any problems taking the medication and we manually count residual study medication and compare this to the expected amount based on their individual treatment schedule.

Prohibited concomitant medication can be found in the exclusion criteria. We ask participants to contact us before starting (prescribed) medication, vitamins or supplements during the study to check for compatibility and to register this change in medication. We also register other events that may influence fatigability (e.g. changes in work or school schedules).

#### *Outcome measures*

This study investigates the effect and efficacy of pyridostigmine on motor function and fatigability in patients with SMA. The test battery is performed in the same order, at all 5 visits.

Primary endpoint is the change in motor function and fatigability using the following measures:

- **Motor function and fatigability**
  - *Motor Function Measure (MFM)*. The MFM is a quantitative scale allowing to measure the functional motor abilities of an individual affected by a neuromuscular

disease, regardless of the diagnosis and the extent of motor deficiencies. The MFM has been validated in patients with neuromuscular disorders, aged 6-60 years old, including patients with SMA. The MFM contains 3 domains reflecting distal motor function, axial/proximal motor function and total muscle function. Studies in patients with SMA show striking differences in sub scores and total scores between patients with different SMA types.[25, 26] We use the validated English version of the MFM.

- *Repeated Nine-Hole Peg Test (r9HPT)*. The r9HPT is a modification of the 9HPT targeting endurance instead of motor function. The 9HPT is a brief, standardized, quantitative test of upper extremity function.[27, 28] The participant is seated at a table with a plastic block containing a small, shallow container holding nine pegs and nine empty holes. On a start command when a stopwatch is started, the participant picks up the nine pegs one at a time, puts them in the nine holes as quickly as possible, and, once they are in the holes, removes them again as quickly as possible one at a time, replacing them into the shallow container. The time to complete the task is recorded. Participants will perform five consecutive rounds with the same hand of choice with the Rolyan® 9HPT (Patterson Medical, Homecraft Rolyan; Sutton-in-Ashfield, United Kingdom). The score for the 9HPT is an average of the five rounds. We will also look at the change in score per round, suspecting an increase in time needed to perform the test in consecutive rounds when participants do not use pyridostigmine, as a result of the muscle fatigability.

*Secondary endpoints:* To additionally investigate the effect of treatment on muscle strength and daily life functioning the following measures are used:

- **Motor function and fatigability**

- *Medical Research Council Scale (MRC scale)*. The MRC scale is widely accepted and frequently used in the neurology and rehabilitation practice to objectively validate and follow up on muscle strength.[29, 30] The MRC scale has successfully been used in multiple trials with SMA type 2, 3 and 4.[31-33] The participant's effort is graded on a scale of 0-5 (Grade 0= no movement observed, Grade 5= Muscle contracts normally against full resistance). MRC scores of a total of 22 different muscles of both upper and lower extremities are determined.
- *Endurance Tests*  
Recently, we developed a panel of endurance tests to assess fatigability/endurance in SMA patients with a wide range of disease severity, i.e. the Endurance Shuttle Nine-Hole peg test (ESNHPT), the Endurance Shuttle Box and Block Test (ESBBT) and a modified version of the Endurance Shuttle Walk

Test (ESWT). The methodology is based on the original ESWT in which participants have to walk on a predetermined walking speed during a maximal time period of 20 minutes.[34-36] The same methodology is applied to The Box and Block Test[37] and the Nine Hole Peg Test,[27, 28] creating two endurance tests for the upper extremity. Reliability and validity are being studied in parallel with this study (Bartels et al, in progress). Ambulatory participants perform the ESWT and the ESBBT and non-ambulatory participants perform the ESNHPT and, if possible, the ESBBT, which requires more strength of the proximal arm muscles. Primary outcome measures of these tests are time to limitation and walking distance for the ESWT or number of blocks or pegs for the ESBBT and ESNHPT. We measure maximum isometric strength of 5 arm muscles and 6 leg muscles before and directly after the test to determine exercise induced muscle weakness. Surface EMG is assessed during the endurance test to determine local fatigability response of the muscle. We use the OMNI scale[38, 39] prior and directly after completion of the test to evaluate perceived exertion.

- **Patient reported outcome measures – Quality of life**
  - *The 36-Item Short Form Health Survey (SF-36)*. The SF-36 is a standardized, generic health-related quality of life measure in motor neuron[40-42] and other diseases.[43] The SF-36 covers eight dimensions (physical functioning, role limitations due to physical health problems, bodily pain, generic health perceptions, vitality, social functioning, role limitations due to emotional problems and mental health). The validated Dutch version of the SF-36 is used.[43]
  - *Pediatric Quality of Life inventory (PedsQL)*. The PedsQL M 3.0 Neuromuscular Module has been developed in the last decade to measure quality of life dimensions specific to children aged 2-18 years with neuromuscular disorders, in particular, Duchenne and SMA.[44-46] The PedsQL encompasses three domains: items on disease process and associated symptomatology, items related to the patient's ability to communicate with health care providers and others about his/her illness and items related to family financial and social support systems.
- **Patient reported outcome measures – perceived daily functioning, fatigue and fatigability**
  - *SMA-Functional Rating Scale (SMA-FRS)*. The SMA-FRS is a functional scale modified from the ALSFRS and the WeeFim protocol.[47,48] It reflects important aspects of daily functioning.

- *Perceptions of Fatigue*. In participants aged 12-17 years, fatigue is assessed with the PedsQL Multidimensional Fatigue Scale.[49-51] In participants aged  $\geq 18$  years, fatigue is assessed with the Fatigue Severity Scale (FSS).[52]
- *Fatigability Questionnaire*. Perceived fatigability during activities of daily life is assessed with a questionnaire in all participants. We use the fatigability questionnaire developed for patients with peripheral nerve disorders by Straver et al. for adult participants and an adjusted form combined with the Child Health Assessment Questionnaire for children.[53, 54]
- ***Nerve conduction studies***
  - *Nerve conduction studies with repetitive nerve stimulation (NCS-RNS)*. Four different muscles are tested (musculus abductor digiti minimi, musculus flexor carpi radialis, musculus trapezius, and musculus nasalis) for supramaximal CMAP recording and 3Hz repetitive stimulation (train of 10) in rest and after 60 seconds of maximal voluntary muscle activation.[13]
- ***Adverse events***
  - All adverse events (AEs) that are reported spontaneously by the participant or observed by the investigator or study staff members are recorded and if necessary, appropriate measures are taken.

### **Statistical analysis**

We will analyze differences in baseline characteristics between participants for single measures (i.e. age, disease duration) using t-tests or non-parametric tests, depending on the distribution of data. We will use a linear mixed effects model to analyze differences in outcome for the different treatment arms. Treatment arms will be entered as fixed effect, while the repeated measurements on the participants will be entered as random effects. A linear mixed effects model for repeated measures allows us to additionally adjust for age, disease duration, gender, SMA type, and other possible influencing factors.

We will summarize incidence of AEs by treatment group and in all treatment groups combined in frequency tables.

### **Ethics, dissemination and safety monitoring**

The local and national medical ethical committees, *Medical Ethical Committee of the University Medical Center Utrecht* and *Central Committee on Research Involving Human Subjects* respectively, approved the study protocol (dates: 21-04-2015 and 03-11-2014). This study is registered in the Dutch registry for clinical studies and trials.(NL38048.041.14; <http://www.ccmo-online.nl>), the European registry for clinical studies and trials ( 2011-004368-34; <https://www.clinicaltrialsregister.eu>) and in the American registry for clinical



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2  
3 studies and trials (NCT02941328; <https://clinicaltrials.gov>). The investigator obtains written  
4 informed consent before study participation from participants and from parents if the  
5 participant is <18 years old.  
6

7 The trial is monitored by an external independent party (Jullius Clinical; Broederplein 41-43,  
8 3703 CD Zeist The Netherlands). Because of the short trial period, consecutive monitor  
9 visits are only separated by a few months, therefore monitoring is intense and extensive.  
10 Because of the short study period with short visit intervals, mild potential risks of the study  
11 medication and intensive monitoring, an interim analysis or safety surveillance by a data  
12 safety monitoring board is not indicated. All participants are insured by the sponsor in case of  
13 harm due to trial participation.  
14

15 The study is conducted according to the principles of the Declaration of Helsinki (latest  
16 version WMA General Assembly 2008, Seoul) and in accordance with the Medical Research  
17 Involving Human Subjects Act (WMO). Directly after study inclusion, we assign a random ID  
18 code to the participant, which will be used on all documents to ensure confidentiality.  
19

20 The results of this study will be shared with the academic and medical community, funding  
21 and patient organizations in order to contribute to optimization of medical care and quality of  
22 life for SMA patients.  
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### 28 29 30 **Strengths and limitations**

31 At the start of this trial, no treatment to cure or slow down SMA was available. Various  
32 treatment strategies had been tested to prolong survival in SMA type 1 and improve motor  
33 function and strength in SMA types 1-3, but none of them had shown efficacy.[55, 56.]

34 The discovery of structural and physiological abnormalities of the neuromuscular junction  
35 resulted in new treatment opportunities to improve motor strength, endurance and  
36 consequently quality of life. For this purpose, we decided to conduct this trial, with the well-  
37 known and safe drug pyridostigmine. It is important to note that even if pyridostigmine is  
38 capable of improving the NMJ function and shows to be effective in improving strength  
39 and/or endurance it will not resolve all symptoms of SMA, but hopefully it will improve daily  
40 functioning with minimal side effects.  
41

42 In the autumn of 2016 efficacy of the antisense oligonucleotide nusinersen, defined as  
43 improvement on the HINE and Hammersmith functional motor scales in infants and children  
44 with SMA was reported.[21, 22] The FDA and EMA have approved treatment of patients with  
45 SMA types 1-4. However, evidence for effects in patients with milder disease severity and  
46 longstanding disease course is currently still lacking and this may complicate reimbursement  
47 decisions in at least some countries. Thus, there remains a need for low cost, easy-to-  
48 administer drugs that improve motor function, fatigability and quality of life of patients with  
49 longer disease duration who can't or do not want to be treated with (repetitive) intrathecal  
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3 injections of nusinersen. More in general, expanding treatment options for all types of SMA in  
4 all ages and life stages is essential and pyridostigmine is a well-known, safe and low-cost  
5 option, stressing the importance of this trial.

6  
7 One of the major challenges in SMA research is the development and use of outcome  
8 measures that can capture the wide variability between and within SMA types and monitor  
9 (small) changes of muscle strength, function or fatigability in this slowly progressive disease.  
10 The incorrect use of instruments or measurement of irrelevant parameters could result in  
11 unnecessary type II errors in trials. Therefore, we developed various new instruments to  
12 capture fatigability and objectify endurance capacity (Bartels et al. in progress). An obvious  
13 limitation of using these tests is that they have not been validated in a large group. This is  
14 currently being done parallel to this study. Nevertheless, these tests allow us to investigate  
15 the effect of pyridostigmine on endurance in SMA patients, a dimension of SMA for which  
16 outcome measures are currently largely lacking. Similarly, the r9HPT is not a validated test,  
17 but data from our previous study shows the r9HPT to detect fatigability in patients with SMA  
18 type 2, and there was a good test-retest reliability (Stam et al, submitted data).

19  
20 The cross-over design we use in this study allows participants to act as their own control and  
21 is an ideal design for rare diseases with a wide range of disease severity including SMA,  
22 because the unsystematic variance is drastically reduced allowing systematic variance to be  
23 detected in a smaller number of participants. Although we cannot exclude external  
24 confounders completely since participants are monitored over a 4 to 5-month period, in which  
25 external factors can be introduced. To minimize the effect of confounders we ask participants  
26 extensively about possible confounding factors such as changes in work or school  
27 schedules, lack of sleep and outside temperature. The cross-over design does require  
28 specific attention to possible carry-over effect and medication-specific adjustment of the  
29 wash-out period. In our study, the short half-life of pyridostigmine results in no or minimal  
30 carry-over effect and the wash-out period could therefore be minimized to 1 week. Another  
31 strength of this study is the use of different outcome measures to evaluate fatigability,  
32 (perceived) fatigue and quality of life from several angles, allowing us to take these in  
33 consideration when analysing the effect of pyridostigmine.

34  
35 In conclusion, we believe that we can properly investigate the effect and efficacy of  
36 pyridostigmine in this double-blinded, placebo-controlled, cross-over trial and we expect the  
37 results of to confirm that pyridostigmine could be used as an (add-on) therapy to improve the  
38 function of neuromuscular junction defects in patients with SMA resulting in improved  
39 strength and/or endurance and/or fatigability.

#### 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 **Author contributions**

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3 Study concept and design were conducted by MS, RIW, CAW and WLP. Critical revision of  
4 concept and design and intellectual input in the study protocol was done by MS, RIW, CAW,  
5 BB, HSG, JFG, MAGCS, IC, LHB and WLP. Collection of data is done by MS, CAW, BB,  
6 FLA, LAMO, HSG and LEH. Technical, administrative and material support was provided by  
7 FLA and BB. Drafting of the manuscript was done by MS and RIW. Critical revision of the  
8 manuscript was performed by MS, RIW, CAW, BB, FLA, LAMO, HSG, LEH, JFG, MAGCS,  
9 IC, LHB and WLP. Study supervision is conducted by LHB and WLP.

### 14 15 **Competing interests**

16 M. Stam, R.I. Wadman, C.A. Wijngaarde, F. Asselman, L.A.M. Otto, H.S. Goedee, L.E.  
17 Habets, J.F. de Groot, M.A.G.C. Schoenmakers and I. Cuppen report no conflicts of interest.  
18 B. Bartels serves on scientific advisory board for Roche Hoffman-La Roche Ltd, Zurich  
19 L.H. van den Berg serves on scientific advisory boards for the Prinses Beatrix Spierfonds,  
20 Thierry Latran Foundation, Biogen Idec and Cytokinetics; received an educational grant from  
21 Baxter International Inc.; serves on the editorial board of Amyotrophic Lateral Sclerosis and  
22 the Journal of Neurology, Neurosurgery and Psychiatry; and receives research support from  
23 the Prinses Beatrix Fonds, Netherlands ALS Foundation, The European Community's Health  
24 Seventh Framework Programme (grant agreement n° 259867), The Netherlands  
25 Organization for Health Research and Development (Vici Scheme, JPND (SOPHIA,  
26 STRENGTH)).  
27 W.L. van der Pol serves on scientific advisory boards of Biogen and Avexis and the LMI070  
28 data monitoring committee of Novartis and receives research support from the Prinses  
29 Beatrix Spierfonds, Netherlands ALS Foundation and Stichting Spieren voor Spieren  
30  
31

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40 Funders did not have any role in the design of the study, collection, analysis, and/or  
41 interpretation of data. The investigators have full access to the data and have the right to  
42 publish this data separate and apart from any sponsor.  
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53 Medical Center (LUMC), the Netherlands, for his kind advise on pyridostigmine dosing.  
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## 19 FIGURE LEGENDS

### 20 **Figure 1. Flow chart of the study protocol**

21  
22 Visit 1 has to take place 5 to 14 days after the baseline visit. Visit 2 has to take place 7 to 9  
23 weeks after visit 1. The wash-out period consists of at least 7 days up to a maximum of 14  
24 days. Visit 3 is planned at the end of the wash-out period. Visit 4 has to take place 7 to 9  
25 weeks after visit 3. There is no physical close out visit. Participants are instructed to contact  
26 the study team if any events occur in the first week after last intake of study medication.  
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29 *Abbreviations: V=visit*  
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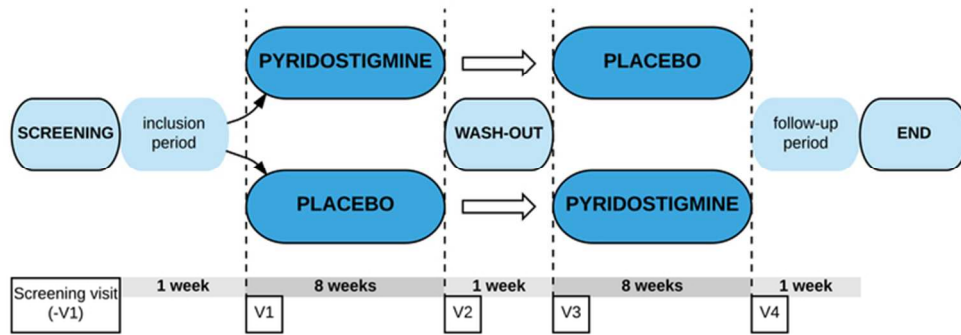


Figure 1. Flow chart of the study protocol

Visit 1 has to take place 5 to 14 days after the baseline visit. Visit 2 has to take place 7 to 9 weeks after visit 1. The wash-out period consists of at least 7 days up to a maximum of 14 days. Visit 3 is planned at the end of the wash-out period. Visit 4 has to take place 7 to 9 weeks after visit 3. There is no physical close out visit. Participants are instructed to contact the study team if any events occur in the first week after last intake of study medication.

Abbreviations: V=visit

65x24mm (300 x 300 DPI)



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	1, 2, 4-12, 16
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 15
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA

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2				
3	<b>Introduction</b>			
4				
5	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	<b>3, 4</b>
6	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
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8		6b	Explanation for choice of comparators	<b>10-12</b>
9				
10	Objectives	7	Specific objectives or hypotheses	<b>4</b>
11				
12	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	
13			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	<b>4</b>
14				
15	<b>Methods: Participants, interventions, and outcomes</b>			
16				
17	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	<b>4</b>
18			be collected. Reference to where list of study sites can be obtained	
19				
20	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	<b>5, 6</b>
21			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
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23	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	<b>7-9</b>
24			administered	
25				
26		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	<b>8</b>
27			change in response to harms, participant request, or improving/worsening disease)	
28				
29		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	<b>10</b>
30			(eg, drug tablet return, laboratory tests)	
31				
32		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<b>5, 6, 10</b>
33				
34	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	<b>10-12</b>
35			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
36			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
37			efficacy and harm outcomes is strongly recommended	
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3	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	<b>Figure 1, Table 2</b>
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6	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	<b>6, 7</b>
7				
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9	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<b>5</b>
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### 11 **Methods: Assignment of interventions (for controlled trials)**

#### 12 Allocation:

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15	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	<b>7</b>
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21	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	<b>7</b>
22				
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24				
25	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<b>7</b>
26				
27				
28	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<b>7</b>
29				
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31		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<b>8</b>
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### 35 **Methods: Data collection, management, and analysis**

36				
37	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	<b>10-12</b>
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3		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	<b>6, 12</b>
4				
5	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	<b>Not in the manuscript, Available on request</b>
6				
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11	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	<b>13</b>
12				
13				
14		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<b>13</b>
15				
16		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	<b>NA</b>
17				
18				
19	<b>Methods: Monitoring</b>			
20				
21	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	<b>13</b>
22				
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27		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	<b>NA</b>
28				
29				
30	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	<b>13</b>
31				
32				
33	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<b>13</b>
34				
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36	<b>Ethics and dissemination</b>			
37				
38	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<b>13</b>
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3	Protocol	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes,	<b>13</b>
4	amendments		analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals,	
5			regulators)	
6				
7	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	<b>13</b>
8			how (see Item 32)	
9				
10		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary	<b>NA</b>
11			studies, if applicable	
12				
13	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained	<b>13</b>
14			in order to protect confidentiality before, during, and after the trial	
15				
16	Declaration of	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<b>15, 16</b>
17	interests			
18				
19	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that	<b>16</b>
20			limit such access for investigators	
21				
22	Ancillary and post-	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial	<b>13</b>
23	trial care		participation	
24				
25	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals,	<b>13</b>
26			the public, and other relevant groups (eg, via publication, reporting in results databases, or other data	
27			sharing arrangements), including any publication restrictions	
28				
29		31b	Authorship eligibility guidelines and any intended use of professional writers	<b>15</b>
30				
31		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<b>NA/13</b>
32				
33				
34	<b>Appendices</b>			
35				
36	Informed consent	32	Model consent form and other related documentation given to participants and authorised surrogates	<b>Available on</b>
37	materials			<b>request</b>
38				
39	Biological	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular	<b>NA</b>
40	specimens		analysis in the current trial and for future use in ancillary studies, if applicable	
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\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.

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# BMJ Open

## Protocol for a phase II, monocenter, double-blind, placebo-controlled, cross-over trial to assess efficacy of pyridostigmine in patients with spinal muscular atrophy types 2, 3 and 4 (SPACE trial)

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Secondary Subject Heading:	Research methods, Neurology, Pharmacology and therapeutics
Keywords:	spinal muscular atrophy, SMA, neuromuscular junction, pyridostigmine, fatigability, cross-over

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**TITLE**

Protocol for a phase II, monocenter, double-blind, placebo-controlled, cross-over trial to assess efficacy of pyridostigmine in patients with spinal muscular atrophy types 2, 3 and 4 (SPACE trial)

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**STUDY DETAILS**

**Protocol version and date:** 6.1, September 2015

**Start date:** 24-11-2015

**Planned End date:** 31-12-2017

**MAIN TEXT WORD COUNT:** 4569

## ABSTRACT

### Introduction

Hereditary proximal spinal muscular atrophy (SMA) is caused by homozygous loss-of-function of the survival motor neuron 1 (*SMN1*) gene. The main characteristic of SMA is degeneration of alpha motor neurons in the anterior horn of the spinal cord, but recent studies in animal models and patients have shown additional anatomical abnormalities and dysfunction of the neuromuscular junction (NMJ). NMJ dysfunction could contribute to symptoms of weakness and fatigability in patients with SMA. We hypothesize that pyridostigmine, an acetylcholinesterase inhibitor that improves neuromuscular transmission, could improve neuromuscular junction function and thereby muscle strength and fatigability in patients with SMA.

### Methods and analysis

We designed a monocenter, placebo-controlled, double-blind cross-over trial with pyridostigmine and placebo to investigate the effect and efficacy of pyridostigmine on muscle strength and fatigability in patients with genetically confirmed SMA. We aim to include 45 patients with SMA types 2, 3 and 4, aged 12 years and older in the Netherlands. Participants receive 8 weeks of treatment with pyridostigmine and 8 weeks of treatment with placebo in a random order separated by a wash-out period of one week. Treatment allocation is double-blinded. Treatment dose will gradually be increased from 2 mg/kg/day to the maximum dose of 6 mg/kg/day in four daily doses, in the first week of each treatment period. The primary outcome measures are a change in the Motor Function Measure and repeated Nine-Hole Peg Test before and after treatment. Secondary outcome measures are changes in recently developed endurance tests, i.e. the Endurance Shuttle Nine Hole Peg Test, the Endurance Shuttle Box and Block Test and the Endurance Shuttle Walk test, muscle strength, level of daily functioning, quality of and activity in life, perceived fatigue and fatigability, presence of decrement upon repetitive nerve stimulation, and adverse events.

### Ethics and dissemination

The protocol is approved by the local medical ethical review committee at the University Medical Center Utrecht and by the national Central Committee on Research Involving Human Subjects. Findings will be shared with the academic and medical community, funding and patient organizations in order to contribute to optimization of medical care and quality of life for SMA patients.

### Trial registration

- US registry NCT02941328 ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) registration date: October 21, 2016
- European registry 2011-004369-34 ([www.clinicaltrialsregister.eu](http://www.clinicaltrialsregister.eu)) registration date: November 3, 2014

**Keywords** spinal muscular atrophy, SMA, neuromuscular junction, NMJ, pyridostigmine, cross-over, muscle strength, motor function, fatigability

## STRENGTHS AND LIMITATIONS

- This randomized double-blind, placebo-controlled cross-over trial will provide important information to clinicians and patients with spinal muscular atrophy about efficacy of treatment of fatigability, lack of endurance and diminished motor function with pyridostigmine.
- The cross-over design is an ideal design for this rare disease with striking variability, because participants will be their own controls, which reduces unsystematic variance, subsequently reducing the necessary sample size to detect systematic variance after treatment.
- Permuted block randomization ensures treatment group numbers are evenly balanced at the end of each block and at the end of the study with this relatively small number of participants.
- The use of tests that are still in the process of validation is a limitation of this protocol. However, these tests can capture a dimension of SMA for which validated outcome measures are largely lacking.

## MAIN TEXT

### Introduction

Hereditary proximal Spinal Muscular Atrophy (SMA) is a motor neuron disease in children and adults caused by a homozygous deletion of the survival motor neuron 1 (*SMN1*) gene or a heterozygous deletion combined with a loss-of-function mutation on the other allele, resulting in a significant reduction of full length functional SMN protein.[1, 2] The main characteristic of SMA is the degeneration of alpha motor neurons in the anterior horns of the spinal cord, resulting in progressive muscle weakness of axial muscles and muscles of the arms and legs with a mild to severely reduced life expectancy in the majority of patients.[3-5] SMN protein is ubiquitously expressed and is involved in the pre-mRNA splicing pathway, ubiquitin and cytoskeleton homeostasis, endocytosis and axonal transport.[6-10] Although motor neurons are most sensitive to the disruption of cellular pathways caused by SMN deficiency, other cell types and tissues may be affected as well.[11, 12] Histological and electrophysiological studies have shown that sufficient levels of SMN protein are essential for the development, maturation and function of the neuromuscular junction (NMJ).[13, 14] SMN-deficient mice display both presynaptic (i.e. abnormal density and distribution of synaptic vesicles and abnormal accumulation of neurofilaments at the nerve terminal of the

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3 NMJ) and postsynaptic (i.e. shrinkage of motor endplates) abnormalities.[15-18] In patients  
4 with SMA type 1, abnormal aggregation of acetylcholine receptors at the muscle endplates  
5 has been reported.[18, 19] Nerve conduction studies with repetitive nerve stimulation (NCS-  
6 RNS) in patients with SMA types 2 and 3, a specific but not very sensitive test for NMJ  
7 dysfunction, showed an abnormal decremental response in 49% of patients.[13] SMA  
8 patients frequently complain of fatigability, which is defined as a decrease in performance  
9 over a given time or sustained measure of mechanical output,[20] in addition to muscle  
10 weakness.

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14 Intrathecal administration of SMN-specific anti-sense oligonucleotides that augment cellular  
15 SMN levels improves motor development in infants and children with SMA, but efficacy has  
16 not been tested in adults.[21, 22] There is a clear need for low cost treatment that is easy to  
17 administer in patients with longer disease duration. The finding of post-synaptic dysfunction  
18 of the neuromuscular junction in SMA suggests that patients may benefit from drugs that  
19 facilitate neuromuscular transmission. Acetylcholinesterase inhibitors may represent a new  
20 category of candidate drugs for the treatment of SMA. Pyridostigmine, an  
21 acetylcholinesterase inhibitor with relatively long half-life, is an FDA and EMA approved first  
22 line treatment of disorders of the post-synaptic neuromuscular junction, i.e. Myasthenia  
23 Gravis. Pyridostigmine inhibits the natural enzymatic breakdown of acetylcholine and thereby  
24 increases its biological availability at the neuromuscular junction enhancing neuromuscular  
25 transmission.[23]

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34 Our aim in this study is to investigate the efficacy and effect of pyridostigmine on muscle  
35 strength and fatigability in SMA. We designed a placebo-controlled, cross-over trial in  
36 patients with SMA types 2, 3 or 4, with double-blind treatment allocation. The crossover  
37 design is an ideal design for this rare disease with striking variability, as using participants as  
38 their own controls will reduce the unsystematic variance (error variance). This allows for  
39 easier detection of systematic variance following the intervention using fewer study  
40 participants. The short half-life of pyridostigmine minimizes carry-over effects.

## 41 42 43 44 45 **Methods and Design**

### 46 *Study setting and design*

47  
48 We conduct this study at the neuromuscular department of the University Medical Center  
49 Utrecht, a tertiary referral center for neuromuscular diseases in The Netherlands. All  
50 members of the study team, consisting of physicians, physical therapists and nurses, have  
51 broad experience with SMA due to the national cohort study that is carried out in this center  
52 since 2010.[24]

This investigator-initiated, monocenter, placebo-controlled study has a cross-over, double-blinded design, with blinding of participants and investigators. The pharmacist is not blinded for allocation of treatment. The study protocol was designed using the recommendations of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. (See Additional file 1 for the SPIRIT checklist 2013 statement).

The study is currently ongoing; the first participant was included on November 25, 2015. We expect study completion by the end of 2017.

### Participants

The details of the inclusion and exclusion criteria are provided in Table 1. The main inclusion criteria are: a clinical diagnosis of SMA type 2, 3 or 4 and a genetically confirmed homozygous *SMN1* deletion and age 12 years or older. We recruit patients with SMA types 2-4 through the national SMA registry that contains detailed clinical information of approximately 300 patients.[24] To minimize selection bias, all eligible patients, based on known SMA type, are invited to participate.

<b>Table 1. Selection criteria</b>	
<b>Inclusion criteria</b>	
	Clinical diagnosis of SMA type 2, 3 or 4 <ul style="list-style-type: none"> <li>• type 2: age at onset &gt;6 months and ability to sit unsupported but not to walk unsupported</li> <li>• type 3: age at onset &gt;18 months and the ability to walk unsupported at any point in life</li> <li>• type 4: age at onset <math>\geq</math>30 years and the ability to walk unsupported at any point in life</li> </ul> In case of discrepancy between age at onset and highest acquired motor milestone, the latter is used to define SMA type
	Genetically confirmed homozygous <i>SMN1</i> deletion
	Given oral and written informed consent when $\geq$ 18 years old and additional informed consent by the parents or legal representative in case of participants aged $\geq$ 12 till <18 years old
	Ability to perform at least 2 subsequent rounds of the Nine Hole Peg Test
	A maximum Motor Function Measure score of 80%
<b>Exclusion criteria</b>	
	Known concomitant disorders of the NMJ (Lambert Eaton myasthenic syndrome, myasthenia gravis)
	Use of drugs that may alter NMJ function <ul style="list-style-type: none"> <li>• cholinergic medication (e.g. rivastigmine, neostigmine, galantamine, fysostigmine, succinylcholine)</li> <li>• non-depolarising musclerelaxans (e.g. (cis)atracurium, gallamine, mivacurium, pancuronium, rocurpnium, vecuronium)</li> <li>• other antagonizing medication of prydostimine (procainamide, quinidine, propranolol,</li> </ul>



	lithium, chloroquine, hydroxychloroquine, aminoglycoside antibiotics, clindomycine, polymixine)
	SMA type 1
	Apprehension for participation in nerve conduction studies
	Inability to meet study visits
	Mechanical gastro-intestinal, urinary or biliary obstruction
	Clinical significant alterations of blood tests drawn within 14 days prior to start of study entry
	Electrocardiophysiology abnormalities known as a contraindication for pyridostigmine use
	Current pregnancy or breast-feeding
	Known allergy to bromides
	Severe bronchial asthma (in case of uncertainty of diagnosis, we will contact the treating pulmonologist or physician)

We will not replace withdrawn or unblinded participants. We will not include them in the study again once dropped-out and we will not re-use their identification number and treatment.

With permission of the participant, we will plan a follow-up by phone for at least one week. If there is an adverse event that is still present after one week, follow-up will be longer.

#### *Sample size calculation*

We aim to recruit 45 participants with SMA types 2-4 based on two power calculations that we performed based on the cross-over design using pilot data on repeated measures of the total score of the MFM test (unpublished data). First, we calculated the within-participant standard deviation, and next the standard deviation of the difference between subsequent measurements of the participants.

Calculation 1: If a total of 40 participants will enter this two-treatment cross-over study, the probability is 80 % that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.093 units. This is based on the assumption that the within-participant standard deviation of the response variable is 1.7 units.

Calculation 2: If a total of 40 participants will enter this two-treatment cross-over study, the probability is 80 % that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.409 units. This is based on the assumption that the standard deviation of the difference in the response variables is 3.1 units.

Both calculations show similar results in terms of the detectable difference based on 80% power, a two-sided significance level of 0.05 and 40 participants in total in the trial. Five

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3 additional participants will be recruited to compensate for potential dropouts. The total  
4 number of included participants will therefore be 45.  
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### 6 7 *Intervention and participant timeline*

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9 Once the investigator confirms eligibility, the participant is assigned to one of the two  
10 treatment groups (pyridostigmine or placebo) and will cross-over during the trial.

11  
12 Randomization to a treatment order (i.e. to start with either pyridostigmine or placebo) is  
13 done through a permuted 4-block design by the pharmacist, who is the only one not blinded  
14 for treatment allocation. Permuted block randomization ensures treatment group numbers  
15 are evenly balanced at the end of each block and at the end of the study with this relatively  
16 small number of participants.  
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21 *Figure 1* shows the participant timeline. At the screening visit we investigate whether  
22 participants are eligible for participation in the trial concerning all in- and exclusion criteria.  
23 As a safety measure, participants are screened for clinical significant alterations in blood  
24 tests (sodium, potassium, hemoglobin, hematocrit, c-reactive protein (CRP), urea, creatinine,  
25 estimated glomerular filtration rate (eGFR), aspartate transaminase (AST), alanine  
26 transaminase (ALAT), gamma glutamyltranspeptidase (GGT), anti-acetylcholine receptor  
27 (AChR) antibodies and beta-HCG levels as a pregnancy test.), and they are screened for  
28 EKG alterations to ensure that included participants have no kidney dysfunction, liver  
29 function alterations, bradycardia or arrhythmias and/or a present pregnancy. If any of the  
30 screening tests results in a clinical significant alteration, the participant is excluded from  
31 study participation. The study schedule is presented in *Table 2*. At the start of the study,  
32 participants are randomized to one of two intervention groups (double-blinded; A or B). Each  
33 participant receives pyridostigmine (tablet) and placebo (matching tablet with no  
34 pharmacological ingredients) in consecutive periods.  
35  
36 Group A starts with 8 weeks of treatment with pyridostigmine at a final dose of 6mg/kg a day.  
37 After a one week wash-out they start an 8-week period with placebo treatment.  
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39 Group B starts with 8 weeks of treatment with placebo. After a one week wash-out they start  
40 an 8-week period with pyridostigmine at a final dose of 6mg/kg treatment a day.  
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42 Each treatment is given 4 times a day and dosage is gradually increased in the first week of  
43 each treatment period to minimize side-effects; starting at 2 mg/kg a day in the first 3 days  
44 after the first administration. When this dose is well tolerated, the dose is increased to  
45 4mg/kg a day during day 4 up till day 7. When this dose is well tolerated, the dose is  
46 increased to the maximum dose of 6mg/kg a day after one week. In case of unfavorable  
47 side-effects of the medicinal product at 6mg/kg a day, the participant continues to use the  
48 highest achievable dose (2 or 4 mg/kg/day). The investigator gives the approval for increase  
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of the dosage after the first three days and after seven days by phone, after checking for invalidating side effects. If there are side effects the investigator can decide to not increase the dosage or to (temporarily) stop the medication depending on the extent of the side effects. In case of severe side-effects, the investigator can decide to intervene.

Pyridostigmine can cause a cholinergic crisis when overdosed due to the parasympathomimetic induction. Symptoms of a cholinergic crisis are excessive salivation, urinary urgency, diarrhoea, muscle weakness, fasciculations, cramps of striated muscles and respiratory problems. In case of symptoms of diarrhoea, excessive salivation and or sweating atropinesulphate can be given orally, 0.125 mg 1-2 times per day. In case of severe symptoms, these symptoms can be treated with intravenous 1-2 mg atropinesulphate on slow infusion and supportive care of respiratory function, if needed. When it's necessary to unblind the treatment of a participant, for example in medical emergencies, this is done by the on-call pharmacist. The rest of the study team remains blinded.

**Table 2. Trial schedule of enrolment, interventions, and assessments**

TIMEPOINT	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
	$-V_1$	0	$V_1$	$V_2$	Wash-out	$V_3$	$V_4$	$V_x$
<b>ENROLMENT:</b>								
Eligibility screen	█							
Informed consent	█							
Inclusion		█						
Allocation		█						
<b>INTERVENTIONS:</b>								
[Intervention group A]			█	█		█	█	
[Intervention group B]			█	█		█	█	
<b>ASSESSMENTS:</b>								
Blood tests	█							
EKG	█							
<b>FUNCTIONAL TESTS</b>								
MFM	█		█	█		█	█	
R9HPT	█		█	█		█	█	
MRC scale	█		█	█		█	█	
ESNHPT	█		█	█		█	█	
ESBBT	█		█	█		█	█	
ESWT	█		█	█		█	█	

<b>PATIENT REPORTED OUTCOME MEASURES</b>							
SMA-FRS							
SF-36 / PedsQL							
FSS							
Fatigability questionnaire							
<b>NERVE CONDUCTION STUDIES</b>							
NCS-RNS							

**Legend Table 2.** Participants are asked to take the study medication 1-1.5 hour prior to the test battery on the day of their study visit to ensure the maximum effect of the pyridostigmine is measured. The tests are performed in the same order at each visit.

*Abbreviations: V=visit, EKG=Electrocardiography, MFM=Motor Function Measure, R9HPT=Repeated Nine-Hole Peg Test, MRC= Medical Research Council Scale, ESNHPT=Endurance Shuttle Nine-Hole Peg Test, ESBBT=Endurance Shuttle Box and Block Test, ESWT=Endurance Shuttle Walk Test, SMA-FRS= SMA-Functional Rating Scale, SF-36=36-Item Short Form Health Survey, PedsQL= Pediatric Quality of Life inventory, FSS=fatigue severity scale, NCS-RNS=nerve conduction studies with repetitive nerve stimulation*

To monitor therapy adherence, we inquire participants about any problems taking the medication and we manually count residual study medication and compare this to the expected amount based on their individual treatment schedule.

Prohibited concomitant medication can be found in the exclusion criteria. We ask participants to contact us before starting (prescribed) medication, vitamins or supplements during the study to check for compatibility and to register this change in medication. We also register other events that may influence fatigability (e.g. changes in work or school schedules).

#### *Outcome measures*

This study investigates the effect and efficacy of pyridostigmine on motor function and fatigability in patients with SMA. The test battery is performed in the same order, at all 5 visits.

Primary endpoint is the change in motor function and fatigability using the following measures:

- **Motor function and fatigability**
  - *Motor Function Measure (MFM)*. The MFM is a quantitative scale allowing to measure the functional motor abilities of an individual affected by a neuromuscular disease, regardless of the diagnosis and the extent of motor deficiencies. The

MFM has been validated in patients with neuromuscular disorders, aged 6-60 years old, including patients with SMA. The MFM contains 3 domains reflecting distal motor function, axial/proximal motor function and total muscle function. Studies in patients with SMA show striking differences in sub scores and total scores between patients with different SMA types.[25, 26] We use the validated English version of the MFM.

- *Repeated Nine-Hole Peg Test (r9HPT)*. The r9HPT is a modification of the 9HPT targeting endurance instead of motor function. The 9HPT is a brief, standardized, quantitative test of upper extremity function.[27, 28] The participant is seated at a table with a plastic block containing a small, shallow container holding nine pegs and nine empty holes. On a start command when a stopwatch is started, the participant picks up the nine pegs one at a time, puts them in the nine holes as quickly as possible, and, once they are in the holes, removes them again as quickly as possible one at a time, replacing them into the shallow container. The time to complete the task is recorded. Participants will perform five consecutive rounds with the same hand of choice with the Rolyan® 9HPT (Patterson Medical, Homecraft Rolyan; Sutton-in-Ashfield, United Kingdom). The score for the 9HPT is an average of the five rounds. We will also look at the change in score per round, suspecting an increase in time needed to perform the test in consecutive rounds when participants do not use pyridostigmine, as a result of the muscle fatigability.

*Secondary endpoints:* To additionally investigate the effect of treatment on muscle strength and daily life functioning the following measures are used:

- **Motor function and fatigability**

- *Medical Research Council Scale (MRC scale)*. The MRC scale is widely accepted and frequently used in the neurology and rehabilitation practice to objectively validate and follow up on muscle strength.[29, 30] The MRC scale has successfully been used in multiple trials with SMA type 2, 3 and 4.[31-33] The participant's effort is graded on a scale of 0-5 (Grade 0= no movement observed, Grade 5= Muscle contracts normally against full resistance). MRC scores of a total of 22 different muscles of both upper and lower extremities are determined.
- *Endurance Tests*  
Recently, we developed a panel of endurance tests to assess fatigability/endurance in SMA patients with a wide range of disease severity, i.e. the Endurance Shuttle Nine-Hole peg test (ESNHPT), the Endurance Shuttle Box and Block Test (ESBBT) and a modified version of the Endurance Shuttle Walk Test (ESWT). The methodology is based on the original ESWT in which

participants have to walk on a predetermined walking speed during a maximal time period of 20 minutes.[34-36] The same methodology is applied to The Box and Block Test [37] and the Nine Hole Peg Test,[27, 28] creating two endurance tests for the upper extremity. Reliability and validity are being studied in parallel with this study (Bartels et al, in progress). Ambulatory participants perform the ESWT and the ESBT and non-ambulatory participants perform the ESNHPT and, if possible, the ESBT, which requires more strength of the proximal arm muscles. Primary outcome measures of these tests are time to limitation and walking distance for the ESWT or number of blocks or pegs for the ESBT and ESNHPT. We measure maximum isometric strength of 5 arm muscles and 6 leg muscles before and directly after the test to determine exercise induced muscle weakness. Surface EMG is assessed during the endurance test to determine local fatigability response of the muscle. We use the OMNI scale [38, 39] prior and directly after completion of the test to evaluate perceived exertion.

- **Patient reported outcome measures – Quality of life**
  - *The 36-Item Short Form Health Survey (SF-36)*. The SF-36 is a standardized, generic health-related quality of life measure in motor neuron [40-42] and other diseases.[43] The SF-36 covers eight dimensions (physical functioning, role limitations due to physical health problems, bodily pain, generic health perceptions, vitality, social functioning, role limitations due to emotional problems and mental health). The validated Dutch version of the SF-36 is used.[43]
  - *Pediatric Quality of Life inventory (PedsQL)*. The PedsQL M 3.0 Neuromuscular Module has been developed in the last decade to measure quality of life dimensions specific to children aged 2-18 years with neuromuscular disorders, in particular, Duchenne and SMA.[44-46] The PedsQL encompasses three domains: items on disease process and associated symptomatology, items related to the patient's ability to communicate with health care providers and others about his/her illness and items related to family financial and social support systems.
- **Patient reported outcome measures – perceived daily functioning, fatigue and fatigability**
  - *SMA-Functional Rating Scale (SMA-FRS)*. The SMA-FRS is a functional scale modified from the ALSFRS and the WeeFim protocol.[47,48] It reflects important aspects of daily functioning.



- *Perceptions of Fatigue*. In participants aged 12-17 years, fatigue is assessed with the PedsQL Multidimensional Fatigue Scale.[49-51] In participants aged  $\geq 18$  years, fatigue is assessed with the Fatigue Severity Scale (FSS).[52]
- *Fatigability Questionnaire*. Perceived fatigability during activities of daily life is assessed with a questionnaire in all participants. We use the fatigability questionnaire developed for patients with peripheral nerve disorders by Straver et al. for adult participants and an adjusted form combined with the Child Health Assessment Questionnaire for children.[53, 54]
- ***Nerve conduction studies***
  - *Nerve conduction studies with repetitive nerve stimulation (NCS-RNS)*. Four different muscles are tested (musculus abductor digiti minimi, musculus flexor carpi radialis, musculus trapezius, and musculus nasalis) for supramaximal CMAP recording and 3Hz repetitive stimulation (train of 10) in rest and after 60 seconds of maximal voluntary muscle activation.[13]
- ***Adverse events***
  - All adverse events (AEs) that are reported spontaneously by the participant or observed by the investigator or study staff members are recorded and if necessary, appropriate measures are taken.

### **Statistical analysis**

We will analyze differences in baseline characteristics between participants for single measures (i.e. age, disease duration) using t-tests or non-parametric tests, depending on the distribution of data. We will use a linear mixed effects model to analyze differences in outcome for the different treatment arms. Treatment arms will be entered as fixed effect, while the repeated measurements on the participants will be entered as random effects. A linear mixed effects model for repeated measures allows us to additionally adjust for age, disease duration, gender, SMA type, and other possible influencing factors. We primarily focus on the results in the group as a whole but we will additionally stratify the participants by gender and we will divide participants into 'sitters' and 'walkers' by evaluation of interaction effects in the mixed model. To evaluate the effect of age we will calculate the difference in the outcome measures between the placebo and pyridostigmine period for each individual. Subsequently we will investigate if there is a correlation between this difference and age. We will do the same for disease duration and investigate possible covariation. We will summarize incidence of AEs by treatment group and in all treatment groups combined in frequency tables.

### **Ethics, dissemination and safety monitoring**



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3 The local and national medical ethical committees, *Medical Ethical Committee of the*  
4 *University Medical Center Utrecht* and *Central Committee on Research Involving Human*  
5 *Subjects* respectively, approved the study protocol (dates: 21-04-2015 and 03-11-2014). This  
6 study is registered in the European registry for clinical studies and trials (2011-004369-34;  
7 <https://www.clinicaltrialsregister.eu>) and in the American registry for clinical studies and trials  
8 (NCT02941328; <https://clinicaltrials.gov>). The investigator obtains written informed consent  
9 before study participation from participants and from parents if the participant is <18 years  
10 old.  
11

12 The trial is monitored by an external independent party (Jullius Clinical; Broederplein 41-43,  
13 3703 CD Zeist The Netherlands). Because of the short trial period, consecutive monitor  
14 visits are only separated by a few months, therefore monitoring is intense and extensive.  
15 Because of the short study period with short visit intervals, mild potential risks of the study  
16 medication and intensive monitoring, an interim analysis or safety surveillance by a data  
17 safety monitoring board is not indicated. All participants are insured by the sponsor in case of  
18 harm due to trial participation.  
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20 The study is conducted according to the principles of the Declaration of Helsinki (latest  
21 version WMA General Assembly 2008, Seoul) and in accordance with the Medical Research  
22 Involving Human Subjects Act (WMO). Directly after study inclusion, we assign a random ID  
23 code to the participant, which will be used on all documents to ensure confidentiality.  
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25 The results of this study will be shared with the academic and medical community, funding  
26 and patient organizations in order to contribute to optimization of medical care and quality of  
27 life for SMA patients.  
28

### 29 **Strengths and limitations**

30 At the start of this trial, no treatment to cure or slow down SMA was available. Various  
31 treatment strategies had been tested to prolong survival in SMA type 1 and improve motor  
32 function and strength in SMA types 1-3, but none of them had shown efficacy.[55, 56]  
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34 The discovery of structural and physiological abnormalities of the neuromuscular junction  
35 resulted in new treatment opportunities to improve motor strength, endurance and  
36 consequently quality of life. For this purpose, we decided to conduct this trial, with the well-  
37 known and safe drug pyridostigmine. It is important to note that even if pyridostigmine is  
38 capable of improving the NMJ function and shows to be effective in improving strength  
39 and/or endurance it will not resolve all symptoms of SMA, but hopefully it will improve daily  
40 functioning with minimal side effects.  
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42 In the autumn of 2016 efficacy of the antisense oligonucleotide nusinersen, defined as  
43 improvement on the HINE and Hammersmith functional motor scales in infants and children  
44 with SMA was reported.[21, 22] The FDA and EMA have approved treatment of patients with  
45 SMA.  
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3 SMA types 1-4. However, evidence for effects in patients with milder disease severity and  
4 longstanding disease course is currently still lacking and this may complicate reimbursement  
5 decisions in at least some countries. While therapy development, including gene therapy,[57]  
6 is ongoing and promising, there are currently no alternative therapies available. Thus, there  
7 remains a need for low cost, easy-to-administer drugs that improve motor function, fatigability  
8 and quality of life of patients with longer disease duration who can't or do not want to be  
9 treated with (repetitive) intrathecal injections of nusinersen. More in general, expanding  
10 treatment options for all types of SMA in all ages and life stages is essential and  
11 pyridostigmine is a well-known, safe and low-cost option, stressing the importance of this  
12 trial.

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17 One of the major challenges in SMA research is the development and use of outcome  
18 measures that can capture the wide variability between and within SMA types and monitor  
19 (small) changes of muscle strength, function or fatigability in this slowly progressive disease.  
20 The incorrect use of instruments or measurement of irrelevant parameters could result in  
21 unnecessary type II errors in trials. The six-minute walk test has been evaluated as an  
22 outcome measure for fatigability in SMA,[58] however there is some conflicting evidence for  
23 this test.[59] Furthermore, we needed an additional test to measure fatigability in upper limbs  
24 in SMA patients who are not able to walk, preferably based on a similar method as the test  
25 for patients who are able to walk. Therefore, we developed various new instruments to  
26 capture fatigability and objectify endurance capacity (Bartels et al. in progress). An obvious  
27 limitation of using these tests is that they have not been validated in a large group. This is  
28 currently being done parallel to this study. Nevertheless, these tests allow us to investigate  
29 the effect of pyridostigmine on endurance in SMA patients, a dimension of SMA for which  
30 outcome measures are currently largely lacking. Similarly, the r9HPT is not a validated test,  
31 but data from our previous study shows the r9HPT to detect fatigability in patients with SMA  
32 type 2, and there was a good test-retest reliability (Stam et al, submitted data). We use the  
33 MFM, which has been extensively validated in SMA patients, as primary outcome measure  
34 for motor function.[25, 26] Another widely used scale is the Hammersmith functional motor  
35 scale expanded (HFMSE).[60] To minimize the burden on patients, we selected one of the  
36 two scales. Since the MFM was used in several studies, including an international trial at the  
37 moment of trial design and start,[61] we decided to incorporate it in our trial as well.  
38 Based on previous neurophysiological and clinical studies [13, 58] we expect that fatigability  
39 is a feature of all SMA types, including milder forms. We will therefore focus on whole-group  
40 results, but we additionally plan to stratify participants based on their ability to walk.  
41 However, we offer all eligible patients the opportunity to participate and are dependent on the  
42 willingness of patients to enroll in this trial. Therefore, it is difficult to predict the number of  
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3 patients in each stratum, but based on incidence [62] and the exclusion criterion of an MFM  
4 score >80% we expect more 'sitters' than 'walkers'.

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6 The cross-over design we use in this study allows participants to act as their own control and  
7 is an ideal design for rare diseases with a wide range of disease severity including SMA,  
8 because the unsystematic variance is drastically reduced allowing systematic variance to be  
9 detected in a smaller number of participants. Although we cannot exclude external  
10 confounders completely since participants are monitored over a 4 to 5-month period, in which  
11 external factors can be introduced. To minimize the effect of confounders we ask participants  
12 extensively about possible confounding factors such as changes in work or school  
13 schedules, lack of sleep and outside temperature. The cross-over design does require  
14 specific attention to possible carry-over effect and medication-specific adjustment of the  
15 wash-out period. In our study, the short half-life of pyridostigmine (3-4 hours when kidney  
16 function is normal) results in minimal to no carry-over effect with a wash-out period of only 1  
17 week. Another strength of this study is the use of different outcome measures to evaluate  
18 fatigability, (perceived) fatigue and quality of life from several angles, allowing us to take  
19 these in consideration when analysing the effect of pyridostigmine.

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21 In conclusion, we believe that we can properly investigate the effect and efficacy of  
22 pyridostigmine in this double-blinded, placebo-controlled, cross-over trial and we expect the  
23 results of to confirm that pyridostigmine could be used as an (add-on) therapy to improve the  
24 function of neuromuscular junction defects in patients with SMA resulting in improved  
25 strength and/or endurance and/or fatigability.

### 26 27 **Author contributions**

28 Study concept and design were conducted by MS, RIW, CAW and WLP. Critical revision of  
29 concept and design and intellectual input in the study protocol was done by MS, RIW, CAW,  
30 BB, HSG, JFG, MAGCS, IC, LHB and WLP. Collection of data is done by MS, CAW, BB,  
31 FLA, LAMO, HSG and LEH. Technical, administrative and material support was provided by  
32 FLA and BB. Drafting of the manuscript was done by MS and RIW. Critical revision of the  
33 manuscript was performed by MS, RIW, CAW, BB, FLA, LAMO, HSG, LEH, JFG, MAGCS,  
34 IC, LHB and WLP. Study supervision is conducted by LHB and WLP.

### 35 36 **Competing interests**

37 M. Stam, R.I. Wadman, C.A. Wijngaarde, F. Asselman, L.A.M. Otto, H.S. Goedee, L.E.  
38 Habets, J.F. de Groot, M.A.G.C. Schoenmakers and I. Cuppen report no conflicts of interest.  
39 B. Bartels serves on scientific advisory board for Roche Hoffman-La Roche Ltd, Zurich  
40 L.H. van den Berg serves on scientific advisory boards for the Prinses Beatrix Spierfonds,  
41 Thierry Latran Foundation, Biogen Idec and Cytokinetics; received an educational grant from  
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3 Baxter International Inc.; serves on the editorial board of Amyotrophic Lateral Sclerosis and  
4 the Journal of Neurology, Neurosurgery and Psychiatry; and receives research support from  
5 the Prinses Beatrix Fonds, Netherlands ALS Foundation, The European Community's Health  
6 Seventh Framework Programme (grant agreement n° 259867), The Netherlands  
7 Organization for Health Research and Development (Vici Scheme, JPND (SOPHIA,  
8 STRENGTH)).  
9

10  
11 W.L. van der Pol serves on scientific advisory boards of Biogen and Avexis and the LMI070  
12 data monitoring committee of Novartis and receives research support from the Prinses  
13 Beatrix Spierfonds, Netherlands ALS Foundation and Stichting Spieren voor Spieren  
14  
15

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24 publish this data separate and apart from any sponsor.  
25  
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27

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## FIGURE LEGENDS

### ***Figure 1. Flow chart of the study protocol***

Visit 1 has to take place 5 to 14 days after the baseline visit. Visit 2 has to take place 7 to 9 weeks after visit 1. The wash-out period consists of at least 7 days up to a maximum of 14 days. Visit 3 is planned at the end of the wash-out period. Visit 4 has to take place 7 to 9 weeks after visit 3. There is no physical close out visit. Participants are instructed to contact the study team if any events occur in the first week after last intake of study medication.

*Abbreviations: V=visit*

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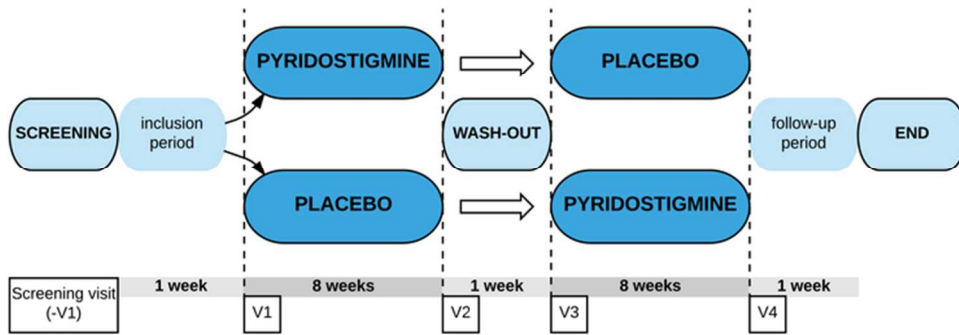


Figure 1. Flow chart of the study protocol

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Abbreviations: V=visit

65x24mm (300 x 300 DPI)

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STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

## SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description	Addressed on page number
<b>Administrative information</b>			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	1, 2, 4-13, 16
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 15
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA

## 1 Introduction

2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	<b>3, 4</b>
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	<b>9-12, 14</b>
7				
8	Objectives	7	Specific objectives or hypotheses	<b>4</b>
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial or single group),	
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	<b>4</b>
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	<b>4</b>
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	<b>5, 6</b>
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	<b>7-9</b>
23			administered	
24		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	<b>8</b>
25			change in response to harms, participant request, or improving/worsening disease)	
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	<b>9</b>
27			(eg, drug tablet return, laboratory tests)	
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<b>5, 6</b>
29				
30	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	
31			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	<b>9-12</b>
32			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
33			efficacy and harm outcomes is strongly recommended	
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1	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	<b>Figure 1, Table 2</b>
2				
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4	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	<b>6, 7</b>
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7	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<b>5</b>
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10	<b>Methods: Assignment of interventions (for controlled trials)</b>			
11				
12	Allocation:			
13				
14	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	<b>7</b>
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20	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	<b>7</b>
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24	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<b>7</b>
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28	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<b>7</b>
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31		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<b>8</b>
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35	<b>Methods: Data collection, management, and analysis</b>			
36				
37	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	<b>9-12</b>
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1		18b	Plans to promote participant retention and complete follow-up, including list of any outcomes data to be collected for participants who discontinue or deviate from intervention protocols	<b>6</b>
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4	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	<b>Not in the manuscript, Available on request</b>
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9	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	<b>12</b>
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13		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<b>12</b>
14				
15		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	<b>NA</b>
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19	<b>Methods: Monitoring</b>			
20				
21	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	<b>13</b>
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26		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	<b>NA</b>
27				
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29	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	<b>12, 13</b>
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33	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<b>13</b>
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37	<b>Ethics and dissemination</b>			
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39	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<b>13</b>
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1	Protocol	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	<b>13</b>
2	amendments			
3				
4				
5	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	<b>13</b>
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8		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	<b>NA</b>
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11	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	<b>13</b>
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14	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	<b>15, 16</b>
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18	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	<b>16</b>
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21	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	<b>13</b>
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24	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, health care professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	<b>13</b>
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29		31b	Authorship eligibility guidelines and any intended use of professional writers	<b>15</b>
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31		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	<b>NA/13</b>
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34	<b>Appendices</b>			
35				
36	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	<b>Available on request</b>
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39	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	<b>NA</b>
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1 \*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items.  
2 Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons  
3 "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.  
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