N-Acetylcysteine in patients with Sickle Cell Disease

Reducing the incidence of daily life pain in patients with sickle cell disease

A Multicenter Randomized Placebo Controlled Trial

Protocol version 3.0

CLINICAL STUDY PROTOCOL

Acronym: NAC Trial

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Department of Hematology
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Institute

Local Investigator Name

Local Investigator Signature

Date
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# LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ABR</td>
<td>ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)</td>
</tr>
<tr>
<td>AGE</td>
<td>Advanced Glycation End-product</td>
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<tr>
<td>AMC</td>
<td>Academic Medical Center (Amsterdam)</td>
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<tr>
<td>AR</td>
<td>Adverse Reaction</td>
</tr>
<tr>
<td>CA</td>
<td>Competent Authority</td>
</tr>
<tr>
<td>CI</td>
<td>Coordinating Investigator</td>
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<tr>
<td>CCMO</td>
<td>Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek</td>
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<tr>
<td>CML</td>
<td>Carboxymethyl lysine (Advanced glycation end product)</td>
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<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CV</td>
<td>Curriculum Vitae</td>
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<tr>
<td>DSMB</td>
<td>Data Safety Monitoring Board</td>
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<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>EudraCT</td>
<td>European drug regulatory affairs Clinical Trials</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>GSSG</td>
<td>Glutathione disulfide (oxidized form)</td>
</tr>
<tr>
<td>HR QoL</td>
<td>Health related Quality of Life</td>
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<tr>
<td>IB</td>
<td>Investigator's Brochure</td>
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<tr>
<td>IC</td>
<td>Informed Consent</td>
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<tr>
<td>ICAM</td>
<td>Intercellular adhesion molecule</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonisation</td>
</tr>
<tr>
<td>IMP</td>
<td>Investigational Medicinal Product</td>
</tr>
<tr>
<td>IMPD</td>
<td>Investigational Medicinal Product Dossier</td>
</tr>
<tr>
<td>iMTA</td>
<td>Institute for Medical Technology Assessment (Erasmus University)</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
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<tr>
<td>MCQ</td>
<td>Medical Consumption Questionnaire</td>
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<tr>
<td>METC</td>
<td>Medical research ethics committee (MREC); in Dutch: Medisch Ethische Toetsing Commissie (METC)</td>
</tr>
<tr>
<td>NAC</td>
<td>N-Acetylcysteine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>NRS</td>
<td>Numerical Rating Scale</td>
</tr>
<tr>
<td>NVK</td>
<td>Nederlandse Vereniging van Kindergeneeskunde</td>
</tr>
<tr>
<td>PCQ</td>
<td>Productivity Cost Questionnaire</td>
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<tr>
<td>PI</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>PS</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>QoL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>(S)AE</td>
<td>(Serious) Adverse Event</td>
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<tr>
<td>SCD</td>
<td>Sickle Cell Disease</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
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**Sponsor**

The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>SUSAR</td>
<td>Suspected Unexpected Serious Adverse Reaction</td>
</tr>
<tr>
<td>TAT</td>
<td>Thrombin-Antithrombin complex</td>
</tr>
<tr>
<td>UMCG</td>
<td>Universitair Medisch Centrum Groningen</td>
</tr>
<tr>
<td>VCAM</td>
<td>Vascular cell adhesion molecule</td>
</tr>
<tr>
<td>WBP</td>
<td>Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)</td>
</tr>
<tr>
<td>WGBO</td>
<td>Wet op de Geneeskundige Behandel Overeenkomst</td>
</tr>
<tr>
<td>WMO</td>
<td>Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)</td>
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SCHEME OF STUDY

SCD ≥12 years, history of frequent painful crises

Registration

Run-in period

Off study

Randomization

NAC daily

Placebo daily

SCD: Sickle Cell Disease

NAC: N-Acetylcysteine
1. SUMMARY

Rationale:
Pain is an invalidating hallmark of Sickle Cell Disease (SCD) and has a considerable impact on the Quality of Life (QoL) of patients and the medical health care system. Oxidative stress is hypothesized to play a central role in its pathophysiology. This is supported by the fact that markers of oxidative stress are associated with severity of the disease. In an open label randomized pilot study we demonstrated that administration of a scavenger of free oxygen radicals (oral N-Acetylcysteine; NAC) during 6 weeks reduced markers of oxidative stress. In another pilot study a profound effect of NAC on the hospitalization rate for painful crises was demonstrated. Our hypothesis is that NAC is able to reduce the frequency and severity of daily life pain in patients with SCD.

Objective:
In this study the effect of the administration of NAC on the frequency and severity of pain and painful crises in daily life will be assessed as well as the related frequency and length of hospital admissions and health related quality of life. In addition, several hematological markers will be monitored and societal costs will be evaluated.

Study design:
This is an international, multicenter, randomized, controlled double blind trial for a period of 6 months. A run-in period of 2 weeks will take place before randomization to evaluate compliance. Total duration of this trial including run-in and follow-up will be 6.5 months per patient.

Study population:
Patients with SCD (HbSS, HbSC, HbSβ+ or HbSβ0) 12 years of age or older with at least 3 painful crises in the last 3 years, are included in this study.

Number of patients:
140 patients

Treatment:
The patients are treated with either 1200 mg NAC per day orally (one tablet of 600 mg twice daily) or placebo (one tablet twice daily) for a period of 6 months.
Main study endpoints:
- The frequency of sickle cell related pain in daily life, evaluated with specific pain diaries.

Secondary endpoints:
- The severity of sickle cell related pain in daily life
- The frequency and severity of painful crises in daily life
- The frequency and length of hospital admission for painful crises
- Health related QoL
- Societal costs of SCD related pain care
- (Monitoring of) hematological markers of oxidative stress, hemolysis, hypercoagulability, inflammation and endothelial dysfunction.
- Tolerability of NAC
- Frequency of use of pain medication at home
- Related incidence of SCD complications (e.g. acute chest syndrome)

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

The hypothesis is that NAC will reduce the frequency of daily life pain in patients with sickle cell disease by reducing oxidative stress, with potential beneficial effects on rate of hospital admission, quality of life and societal costs.

The risks of participation in this study are predicted to be negligible. NAC is a drug that has been registered for years for different indications and has an excellent safety profile, also in children. It has proven to have only limited side effects (occasionally nausea, diarrhea or urticaria).

Patients will have to take study medication twice daily for a period of 6 months. Furthermore they will undergo blood sampling more frequently; in total 3 times, instead of 2 times with regular follow-up. They will be seen monthly in outpatient department to monitor outcomes in stead of once every 6 months. Subjects will have to fill out a daily pain diary for 2 weeks during run-in and 6 months during intervention. Moreover, they will be asked to fill out two questionnaires for a total of 3 times during the trial.
2. INTRODUCTION AND RATIONALE

Sickle Cell Disease (SCD) is an autosomal recessive hemoglobinopathy characterized by chronic anemia, recurrent painful episodes (crises) and irreversible damage to vital organs. It occurs mainly in people of African ancestry. SCD is one of the most common hereditary diseases in the world and associated with a strongly reduced life expectancy and severe morbidity including cerebral infarction, retinopathy, osteonecrosis, cardiomyopathy, pulmonary hypertension and an increased susceptibility to infections. There are approximately 1000-1200 patients with SCD in the Netherlands.\(^1,2\)

In 2003 the total number of children with SCD in this country was estimated to be 621. The annual number of children newly diagnosed as having SCD between 2003 and 2007 was 94 and this number is rising due to immigration.\(^3\)

Patients with sickle cell disease are mostly seen by pediatricians and hematologists in the urban areas around Amsterdam, Rotterdam and The Hague although a considerable number of patients live in other parts of the country. Since patients are mostly diagnosed in early childhood, SCD must be considered to be a chronic illness and strict follow up and prevention of complications are mandatory. In the present study both pediatricians and hematologist collaborate.

Despite the fact that a small subgroup of patients is largely asymptomatic, most children and adults with SCD suffer from frequent painful crises at home for which they sometimes need to be admitted. These pain-episodes account for the majority of health care utilization in this patient group. It’s even one of the top-ten reasons for hospital admission for internal medicine in Amsterdam, Rotterdam en The Hague.

Recent data from a study in the USA using pain diaries encompassing more than 31,000 patient-days revealed that SCD patients reported pain in 54% of the observed days. Reported pain was classified as a painful crisis in 17% of the observed days. In 40% of these painful crises days, health care utilization took place in this American study.\(^4\) Our research group found that in a heterogeneous group of 104 adult SCD patients in the Netherlands, 24% had at least one admission per year or more because of a pain crisis.\(^5\) In another study the median length of hospital stay for painful crisis was 7,6 days per crisis.\(^6\)

We recently demonstrated that the Health Related Quality of Life (HR QoL) of these patients is strongly affected by these frequent painful episodes.\(^7\) Consequently, reduction in pain-episodes will not only have a profound effect on the use of health care and its costs by reducing hospital admissions but will also improve the HR QoL of these patients.
Upon admission with painful crises, patients are treated with hydration therapy and intensive pain treatment consisting mostly of opioids. In case of complications, more aggressive treatment may be necessary. For example, in the case of acute chest syndrome, a sickle cell crisis of the lungs, exchange transfusion may be indicated while 15% of these patients need mechanical ventilation at the Intensive Care Unit. Other possible complications include cerebral infarction, pulmonary hypertension, infection and especially in young children acute spleen sequestration with severe anemia. These complications also partially elucidate the reduced life expectancy in SCD patients. 

Presently, the only treatment with proven efficacy in preventing painful crises is hydroxyurea (HC). This antineoplastic drug increases the synthesis of fetal hemoglobin and thereby reduces the frequency of painful episodes and the hospitalization rate for pain crises in SCD. However, HC is effective in only about 60% of the patients and is frequently not tolerated. In a study by Charache et al. in 1995 treatment with HC was stopped in almost 10% of the patients for medical reasons including myelotoxicity. Furthermore, a possible association of HC use and abnormal sperm parameters has been described recently in adult males with SCD.

Therefore, new therapeutic approaches that are less toxic are urgently needed. This project aims to prospectively evaluate the effectiveness of a new intervention for reducing pain episodes in SCD, targeting a different point of action.

Recently, it has become clear that SCD is characterized by systemically enhanced inflammation, endothelial activation and increased adhesion of both leukocytes and erythrocytes to the vessel wall leading to local ischemia and infarction resulting in further inflammatory damage. Within this complex process of inflammation, oxidative stress was demonstrated to play an important role, supported by the fact that markers of oxidative stress are strongly associated with the extent of chronic organ damage and pain. As oxidative stress also plays an important role in microvascular occlusion resulting in painful crises, the aim of the present study is to evaluate the effectiveness of the anti-oxidant N-Acetylcysteine (NAC) in reducing pain, painful crises and related hospital admission.

NAC is one of the early precursors of glutathione (GSH), a strong intracellular antioxidant. It has been utilized for the past 30 years mostly as a mucolytic drug. Possible side-effects are well described and appear to be infrequent and mild. More than 2500 patients with respiratory diseases participated in NAC trials in a wide age range where it has proven to be safe.
Furthermore, NAC is a relatively cheap drug which is important, given the restricted health care resources that are available in most areas in the world where SCD is most prevalent. In a previous clinical phase 2 trial a profound effect on sickle cell crises was found with a 70% reduction in hospital admission. In that study and a recent study by our group it was demonstrated that NAC reduces the amount of circulating dense and irreversible sickle cells and restores the glutathione levels in erythrocytes of sickle cell patients.\textsuperscript{25,26}

To prospectively evaluate whether NAC can reduce pain, painful crises and related hospitalization, a placebo controlled randomized trial will be performed with oral NAC administration. Since the frequency of sickle cell crises at home is related with the frequency of hospital admission and is responsible for the significantly reduced quality of life of SCD patients, we decided to use the frequency and intensity of SCD related pain in daily life as the primary outcome parameter. This will be evaluated by the use of personal pain diaries that subjects will have to fill out daily.

As secondary outcome parameters the frequency and severity of painful crises, the related frequency and length of hospital admission, the health related quality of life and the pain related societal costs will be evaluated. Furthermore we will monitor hematological markers of oxidative stress, hemolysis, inflammation, endothelial dysfunction and hypercoagulability. Also, we will assess the tolerability of NAC, frequency of use of pain medication at home and the incidence of SCD complications.

In our proposal we focus on patients of 12 years of age or older, that are frequently suffering from painful crises (≥1 crisis per patient year in the last 3 years). Since measurement of pain is difficult in children younger than 12 years, these patients are excluded from our study proposal.

For preventative chronic treatment, compliance is a major issue. Compliance has been demonstrated to be relatively poor in patients with SCD mostly due to the side effects of medical interventions like HC or deferasirox. NAC is well studied in trials in patients with respiratory diseases and has demonstrated to have very limited side effects which may increase compliance. Furthermore, regular outpatient visits will be performed and compliance will be checked by tablet counting. In order to further improve compliance, patients will be randomized only after a successful run-in period in which at least 80% of the days in the pain diary should have been completed by the individual patient. Also, during trial intervention patients will be reminded of their study medication and pain diary by text messages that will be sent periodically to their mobile phones (if available and consenting).
In conclusion, NAC may be a promising new alternative in the prevention of pain crises in SCD. So far, only one phase 2 clinical trial has been published, in which a profound effect on hospital admission rate for painful crises was found. Currently, no other trials with antioxidant therapy are taking place in the Netherlands or abroad.

If a similar effect as in earlier studies would be demonstrated in the present study, this may have a major impact on the prevention of painful crises in SCD with both potential improvement of quality of life of these patients, and social-economical consequences by reducing the costs for overall SCD care. Furthermore, as the intervention that is studied is relatively inexpensive itself, it may also provide a new treatment modality for patients with SCD in the developing world.
3. OBJECTIVES

Primary Objective:
To investigate the effect of the administration of NAC over a period of 6 months in reducing the frequency of self reported, SCD related pain in daily life in patients with homozygous HbSS, HbSC, HbSβ+ or HbSβ0 SCD.

Secondary Objectives:
To assess the related severity of self reported SCD related pain in daily life over a period of 6 months
To assess the related incidence and severity of painful crises in daily life over a period of 6 months
To assess the related frequency and length of hospital admissions over a period of 6 months
To assess the health related quality of life over a period of 6 months
To assess related societal costs over a period of 6 months
To assess the tolerability of NAC over a period of 6 months
To assess the frequency of use of pain medication at home over a period of 6 months
To assess the related frequency of SCD complications over a period of 6 months (e.g. acute chest syndrome)
4. STUDY DESIGN

4.1 Study design

This will be a Phase III, multicenter, double blind, randomized, placebo controlled trial. Total duration of intervention will be 6 months and a run-in period of 2 weeks will take place before randomization to evaluate compliance. Consequently, total duration of this trial including run-in and treatment will be 6.5 months.

140 patients with SCD will be included in this trial. Inclusion is targeted to occur over a 12 months period. Total trial duration is estimated to be 1.5 years.

Patients will be recruited in three Dutch university hospitals (AMC, Amsterdam, Erasmus MC, Rotterdam, and UMCG, Groningen) and one large Dutch general hospital (Haga hospital, The Hague), in which the major part of the Dutch sickle cell population is regularly treated. In the UMCG only adult patients will be included. Moreover, due to slower inclusion rates than expected in the first year of enrolment, we are working on initiation of the trial in hospitals in Belgium and London (United Kingdom) at the moment.

See also flowchart in appendix for complete trial overview.
5. STUDY POPULATION

5.1 Population (base)
Patients with HbSS, HbSC, HbSβ+ or HbSβ0, both male and female, aged ≥12 years, with a history of at least 1.0 painful crisis per year in the last 3 years

5.2 Inclusion criteria
- Age 12 years or older
- Sickle cell disease, either homozygous sickle cell disease (HbSS), HbSC sickle cell disease, HbSβ0 or HbSβ+ thalassemia.
  Genotype needs to be confirmed by high performance liquid chromatography.
- History of at least 1.0 painful crisis per year in the past 3 years.
  A crisis will be defined here as a patient defined, painful, sickle cell related episode of at least 24 hours where the subject experienced significant impediments in his/her daily activities, and pain medication had to be taken. A visit to a medical facility is not obligatory in this definition.
- Written informed consent from patient/parent/guardian is given.

5.3 Exclusion criteria
- Chronic blood transfusion or transfusion in the preceding 3 months
- Painful crisis in the last 4 weeks (with respect to the moment of inclusion).
  A crisis will be defined as above; a patient defined, painful, sickle cell related episode of at least 24 hours where the subject experienced significant impediments in his/her daily activities, and pain medication had to be taken. A visit to a medical facility is not obligatory in this definition.
- Pregnancy, breast feeding or the desire to get pregnant in the following 7 months.
  Pregnancy will be verified with a serum pregnancy test after informed consent in all female patients (determined in the first study blood sample).
- Known active gastric/duodenal ulcers
- Hydroxycarbamide (HC) treatment with unstable dose in the last 3 months or started on HC shorter than 6 months prior to study.
- Known poor compliance in earlier trials regarding the completion of pain diaries.
- Insufficient compliance in run-in period.
  Participants that do not show up for their follow-up visit without prior notice, that not bring their diary or with less than 80% of the days in the pain diary filled in, will be excluded from participation in this trial and will not be randomized.
- Known hypersensitivity to acetylcysteine or one of the other components of the study.
- Use of pain medication for sickle-cell related pains on more than 15 days per month in the past 6 months (‘chronic pain’).

**Considerations**

Chronic blood transfusion or recent transfusion alters the ratio of HbSS and HbAA in SCD patients and can therefore be a confounder in assessing the frequency of painful crises. In case of a recent painful crisis prior to this study, this same crisis can still be ongoing asymptotically with flare-ups after the trial has started, thereby confounding in assessing the frequency of crises.

The use of NAC during pregnancy or breastfeeding appears to be safe in low dosages but evidence is limited. Therefore, women who are pregnant, attempting to get pregnant or breast feeding are excluded from this study.

Because of the known possible side effects of NAC, patients with known gastric or duodenal ulcers are excluded from this study.

Patients are permitted to receive concomitant therapy with HC if they have received HC for the preceding 6 months and their dose was stabilized for at least 3 months prior to the study. Recent changes in HC dosage can have a potential confounding effect on the severity and frequency of pain crises as well.

Patients with known insufficient compliance in the run-in period or earlier studies concerning the completion of pain diaries will be excluded because of the importance of adequate compliance for this study.

As some patients are known to have complex chronic pain syndromes where it can be difficult to distinguish the acute component (potentially reversible by NAC) from other chronic pains, we will exclude these patients from participation.

**5.4 Sample size calculation**

140 sickle cell patients are needed (70 in each arm) to detect a 50% reduction in pain days. This group size is based on an expected reduction of 50% in pain days and a mean incidence of pain days of 25% in a general, adult SCD population, using a two-sided significance level of 5% and a power of 80%. This incidence percentage is based on preliminary, yet unpublished results from our research group and data that was found in a study by Smith et al in 2008.\(^4\)\(^6\)\(^27\)

In the Netherlands approximately 1000-1200 patients are known to have sickle cell disease. The large majority of these patients is treated in the above mentioned hospitals with
comprehensive care programs for sickle cell patient care. Given the fact that 24% of the patients are frequently admitted with painful crises we expect to be able to enroll adequate patient numbers and the study is feasible.
6. TREATMENT OF SUBJECTS

6.1 Investigational product/treatment

Subjects in the intervention group will receive 1 tablet of N-Acetylcysteine (NAC) 600mg twice daily for a period of 6 months. NAC is most known for its use as a mucolytic drug in bronchopulmonary disease and as an antidote for acute acetaminophen poisoning. For these and other indications it has been studied thoroughly and has proven to be safe. For more details on safety and potential side-effects of NAC see also paragraph 7.

NAC is one of the early precursors of glutathione (GSH), an intracellular antioxidant, preventing cellular damage by reactive oxygen species (ROS). It is the rate-limiting substrate for GSH generation and consequently has pleiotropic effects on inflammation and vasomotor function. NAC readily enters cells and within the cytoplasm it is converted to L-cysteine, which is a precursor to GSH.

Due to increased consumption by excessive levels of ROS, sickle cell patients have decreased levels of plasma and erythrocyte total GSH and the ratio of GSH to its oxidized form glutathione disulfide is reduced.

Subjects in the control group will receive placebo tablets in the same frequency and amount as the NAC group for the full treatment period of 6 months. The two treatments will be as identical in color, weight, taste, odour and package as possible.

6.2 Description and justification of route of administration and dosage

NAC is easiest administered and has proven to be effective orally. In a previous study, treatment of SCD patients with NAC at an oral dose of 2400 mg increased intracellular glutathione and reduced dense cell formation (a determinant in the pathophysiology of painful crises).

In a recent study using 1200 and 2400 mg doses over 6 weeks, we demonstrated that glutathione increments were comparable in both groups, with concurrent reductions in erythrocyte PS expression and plasma AGEs. Consequently, a dosage of 1200mg appeared to be equally effective as 2400mg for these outcomes. Moreover, in one patient treated with 2400 mg NAC gastro-intestinal complaints were observed which disappeared upon dose reduction to 1200 mg. Because the relatively short half life of NAC of about 6 hours, it will be administered as tablets of 600mg twice daily.

There is no clear evidence in children available on the dose-response relation of NAC for this indication but it has proven to be safe in clinical studies in different dosages. Since only children aged 12 years or older are included in this trial, the adult dosage of 1200 mg per day will also be used for the pediatric participants. This dosage is lower than the recommended dosage of 1800 mg per day for reflex sympathetic dystrophy in children, where it is also used...
as a scavenger of oxygen radicals, and it does not exceed the recommended maximum oral dosage of 1200 mg per day for coughing in children of 7 years or older, as both advised by the Dutch Knowledge Center for Pharmacotherapy in Children (kinderformularium.nl).

The clinical presentation of symptoms in sickle cell disease has great variability over time. A treatment period of 6 months is needed as this is the minimal observation time epidemiologically required for measuring a potential effect on our primary and secondary outcomes, especially frequency of painful crises, quality of life and cost effectiveness of the intervention. Furthermore, our power analysis is based on a previous study that showed potential effectiveness of NAC at an average treatment period of 5 to 6 months.26

6.3 Dosages, dosage modifications and method of administration
During the 6 months intervention period the dosage of NAC will be 1200 mg orally per day (1 tablet of 600 mg twice daily). Placebo will be administered orally as 1 tablet twice daily. No dosage modifications to either NAC or placebo treatment will be made.

6.4 Stop criteria during study protocol medication
There are no specific stop criteria defined for this study. Only if absolutely necessary for medical reasons, study medication can be stopped. If possible, study medication will also be continued during hospital admission.

6.5 Use of co-intervention
All randomized patients will be treated according to the general clinical guidelines for treatment of SCD. The use of pain medication at home (e.g. paracetamol, non-steroidal anti-inflammatory drugs, tramadol) is therefore allowed during this trial. Patients will have to record their use of pain medication in their pain diaries.

Patients are permitted to receive concomitant therapy with hydroxycarbamide (HC) if they have received HC for the preceding 6 months and their dose was stabilized for at least 3 months prior to the study (see paragraph 5.3).

Incidental blood transfusions are permitted. Any subjects with an event requiring exchange transfusion, that are started on HC or that are started on a chronic blood transfusion scheme during trial intervention will be censored from that moment on.

When combining the use of study medication with antibiotics, patients will be instructed to adopt a 2 hour interval between the ingestion of NAC and antibiotics.
7. INVESTIGATIONAL MEDICINAL PRODUCT: N-Acetylcysteine (NAC) – Placebo

7.1 Summary of findings from non-clinical studies
In vitro and animal studies have demonstrated that treatment of erythrocytes with NAC increases the intracellular concentration of the reduced form of GSH and decreases (parameters of) oxidative stress (e.g. Phosphatidylserine (PS) expression, Advanced Glycation Endproducts (AGEs)). Furthermore, in vitro studies suggest that NAC can inactivate several antibiotics. Patients will therefore be instructed to adopt a 2 hour interval between the ingestion of NAC and antibiotics.

7.2 Summary of findings from clinical studies
A wide range of clinical studies has been performed with NAC. It has been studied in more than 2500 patients with respiratory diseases in a wide age range where it has proven to be safe. Patients have been treated with NAC for meconium ileus as a complication of cystic fibrosis, to prevent hepatotoxicity in acetaminophen overdose or as a therapeutic strategy in HIV-infections. In the latter study, NAC was tested at an average dose of 6900 mg/day and no significant adverse reactions were observed. Consistent with this interpretation, in a large Cochrane meta-analysis assessing the use of NAC for respiratory tract infections in a pediatric population, the only infrequent, adverse events reported were mild gastrointestinal symptoms. Furthermore, in a meta-analysis of oral long-term NAC among >1400 adult chronic bronchopulmonary disease patients with doses varying from 300 to 1200mg per day, NAC was well tolerated and treatment interruption was never required. Severe and in some instances life-threatening anaphylactic reactions, which include urticaria, hypotension and vomiting, have only been reported after intravenous administration of NAC.

7.3 Summary of known and potential risks and benefits
Because of the broad range of studies that has been performed with NAC, the potential risks and benefits are well described. Possible, rare side-effects of NAC include hypersensitivity (e.g. urticaria, bronchospasm, pruritus), headache, tinnitus, abdominal discomfort, stomatitis, vomiting and diarrhea. No toxic maximum dose has been observed so far. As mentioned in paragraph 7.1, in vitro studies suggest that NAC can inactivate several antibiotics. Patients will therefore be instructed to adopt a 2 hour interval between the ingestion of NAC and antibiotics.
In previous trials with NAC in SCD, reduced parameters of oxidative stress, reduced dense cell formation and a reduction in hospital admission for painful crises were observed. Consequently, by reducing oxidative stress NAC may be able to reduce the frequency and intensity of pain, pain crises and hospital admission in SCD patients. Furthermore, NAC is registered for use as a mucolytic drug in respiratory disease and as an antidote in acetaminophen intoxication.

7.4 Preparation and labeling of Investigational Medicinal Product

NAC and placebo will be shipped to trial sites in containers labeled as an Investigational Medicinal Product and will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.

The sponsor will arrange delivery of NAC and placebo to trial sites. No investigational medicinal product will be shipped until the sponsor has verified that all regulatory required documents and approvals for the site are available.

6.3 Storage and handling

The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.

7.5 Drug accountability

The investigator, or a pharmacist or other appropriate individual who is designated by the investigator, should maintain records of the product’s delivery to the trial site, the inventory at the site, the use by each patient, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial patients (if applicable). Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

7.6 Study drug return and destruction

Partially used investigational medicinal product should not be redispensed to either the same or another patient after it has been returned.

The trial site should destroy used or partially used study drug containers after drug accountability records have been completed. Destruction should be documented.
8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study endpoint

The main endpoint of this study, which formed the basis for statistical power calculations, is the frequency of SCD related pain in daily life in patients with SCD. Frequency of pain will be expressed in days per patient in proportion to the total time of intervention (6 months) per treatment group. A pain day will be defined as:

- When the box “Yes, I have experienced pain” is checked in a daily diary.

8.1.2 Secondary study endpoints

- The severity of SCD related pain in daily life. This will be defined using a 0-10 numerical rating scale (NRS) in the pain diary.

- The frequency and severity of painful crises over a period of 6 months.

A painful crisis will be defined as either (overlap is possible):

- When the box “Yes, I was in a crisis” is checked in a daily diary. The number of painful crises will be defined as the number of groups of consecutive days that box is checked. The length of a painful crisis will be the number of consecutive days the box is checked. Missing diary days that are immediately preceded and followed by crisis days or by hospital admission, are considered part of the same crisis (with a maximum of 20% of missing days accepted, see paragraph 8.3).

- A visit to a hospital that lasted more than 4 hours for acute sickling-related pain, which was treated with orally or parenterally administered narcotics.

- Acute chest syndrome, defined as an episode of chest wall pain in association with findings of a new pulmonary infiltrate on chest x-ray or computerized tomography, and fever.

The rate of painful crises will be defined as the number of crises and crisis days per patient in proportion to the total time of intervention (6 months) per treatment group. Severity of pain is defined as described earlier.
- The frequency and length of hospital admissions over a period of 6 months. Hospital admission will be defined as:
  
  - When the box “Yes, I was (admitted) in the hospital the last 24 hours because of sickle cell pain” is checked in a daily diary.
  
  - Every visit to a hospital that lasted more than 4 hours for acute sickling-related disease, which was treated with orally or parenterally administered narcotics.

Admissions will be verified using hospital medical records, if available. The length of admission will be measured in days, from day of admission to day of discharge, in proportion to total time of intervention (6 months). The rate of admissions will be defined as the number of admissions per patient in proportion to total time of intervention.

- Time in days to first and second painful crisis and hospital admission (as defined above)

- The health related quality of life (HR QoL). In adults this will be assessed with SF36-forms, a short-form health survey that has been proven to be valid and reliable in the black population.\(^{46}\)

In children from 12 to 18 years old, we will use the PedsQL questionnaire, often used to assess quality of life in children, also validated in SCD patients.\(^ {47-49}\)

- The related societal costs. This will be assessed by a prospective cost-effectiveness analysis (see paragraph 8.1.4).

- The related changes in hematological markers\(^ {50}\)
  
  - Oxidative stress: Red cell glutathione (total, reduced, oxidized), Phosphatidylserine expression erythrocyte AGE’s; Pentosidine, N-carboxy-methyllysine
  
  - Hemolysis: Lactate Dehydrogenase (LDH), Reticulocytes, Free plasma Hb, Bilirubin, (Free) plasma heme
  
  - Hypercoagulability: Prothrombin fragment F1+2, Thrombin-Anti-Thrombin complex (TAT) vonWillebrand-propeptide Active vonWillebrand factor
- Inflammation: C-reactive protein (CRP)
Pentraxine-3
Nucleosomes
- Adhesion molecules: Vascular cell adhesion molecule-1 (sVCAM-1),
Intercellular adhesion molecule-1 (sICAM-1)
Soluble E-selectin and P-selectin
CD47, CD44, CD147, ICAM-4 (Sanquin)
- Endothelial dysfunction: Tissue Plasminogen Activator (tPA)
Plasminogen Activator-inhibitor-1 (PAI-1)
- Clearance of erythrocytes CD47 and conformation of this molecule
- Complement biology Complement regulatory proteins CD35, CD55,
CD59, complement iC3b, Factor H
- Percentage of dense, sickled cells

- The tolerability of NAC. This will be assessed via the monthly adverse events
reports, defined as events that are classified as ‘definitely’ or ‘probably’ related
to the use of study medication, as determined by the investigators. Events
classified as ‘possibly’, ‘unlikely’ or ‘not related’ will be excluded.

- The frequency of use of pain medication at home over a period of 6 months.
This information will be recorded by subjects in their daily pain diary, including
type and dosage of pain medication.

8.1.3 Other study parameters
- The related incidence of SCD complications
  - Acute chest syndrome, defined as an episode of chest wall pain in
association with findings of a new pulmonary infiltrate on chest x-ray or
computerized tomography, and fever.
  - Priapism, defined as spontaneous painful erection requiring hospital care.
  - Hepatic sequestration, defined as an acute, sudden increase in liver size
associated with pain in the right upper abdominal quadrant and a decrease
in the haemoglobin concentration of at least 1 mmol/liter.
  - Splenic sequestration, defined as an acute, sudden increase in spleen size
associated with pain in the left upper abdominal quadrant and a decrease in
the haemoglobin concentration of at least 1 mmol/liter.
- Cerebrovascular accident, confirmed by magnetic resonance imaging or computerized tomography.
- Leg ulcer, defined as chronic ulcers of the ankle not otherwise explained.
- Symptomatic avascular osteonecrosis, defined as local pain and reduced function with documented osteonecrosis of the femoral or humeral head (hip or shoulder X-ray)

These complications will be assessed monthly by using hospital medical records.

8.1.4 Economic evaluation

The economic evaluation in this study will be set up as a cost-effectiveness analysis. The societal costs of pain care with the use of NAC, in the intervention group, will be compared to the societal costs of current pain care in the control group. Estimates of unit costs will be based on calculation of real costs of pain care. Dutch pharmaceutical unit cost listings (www.fk.cvz.nl), guideline prices and tariffs will be used to determine cost estimates of health care resource use. Generated direct medical costs will be recorded in the case record forms and by means of the Medical Cost Questionnaire (see appendix). Indirect costs arising from losses in productivity will be assessed by means of the Productivity Cost Questionnaire (see appendix) and will be calculated by means of the friction cost method. Unit costs will be set at the 2014 price level and will be discounted by 3% annually. In a scenario-analysis the observed clinical efficacy outcome, reduction of pain and painful crises will be used as a proxy of the possible prevention of hospital admission and will be translated to long-term health economic outcomes. The extrapolation will focus on the potential benefits and possible reduction in costs and will include all factors that are a-priori believed to be predictors of hospital admission. In sensitivity analyses, the robustness of the conclusions will be evaluated.

8.2 Run-in period

Patients both eligible and consenting will consequently enter a two-week run-in period, during which only a pain diary has to be completed daily. Following inclusion, patients will be instructed and trained on how to complete a pain diary. After two weeks, compliance will be evaluated by counting the completed days in the pain diary. Participants that do not show up for their follow-up visit without prior notice, that
not bring their diary or with less than 80% of the days in the pain diary filled in, will be excluded from participation in this trial and will not be randomized.

8.3 Randomization, blinding and treatment allocation
Remaining subjects will be randomized at T0. Instruction and training on how to take the medication will be performed at this time.

Randomization will be performed according to GCP-guidelines. The process will be web-based using www.tenalea.net. By accessing the online randomization website and verifying the trial inclusion and exclusion criteria, a unique patient trial ID number can be obtained. A linked Case Report Form (CRF) will be assigned at this time. Trial enrolment is then completed. The first dose of study medication has to be administered within 24 hours after randomization.

The randomization list will be provided to the trial pharmacies of the participating centers, which will store and dispense active drugs and placebo.

This trial will be protected from selection bias by using concealed, stratified and blocked randomization. Randomization will be stratified according to centre, sickle cell genotype (Stratum A: HbSS, HbS β⁰; Stratum B: HbSC, HbS β⁺) and the use of hydroxycarbamide, in order to achieve an equal distribution in both treatment arms. The allocation ratio will be 1:1 using variable block sizes with a maximum of 4.

The trial will be performed double blind. The participants, parents and all members of the medical team, including investigators and staff, remain unaware of the individual treatment group members throughout the study. Furthermore, pain diaries, questionnaires and laboratory tests will be reviewed in a blinded fashion. The two treatments, NAC and placebo, will be produced as identical in color, weight, taste, odour and package as possible.

8.3.1 Treatment arms
Patients will be randomized to either one of two arms (see flowchart in appendix):

<table>
<thead>
<tr>
<th>Arm A:</th>
<th>N-Acetylcysteine 600mg 1 oral tablet twice daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm B:</td>
<td>Placebo 1 oral tablet twice daily</td>
</tr>
</tbody>
</table>
8.4 Study procedures

8.4.1 Medical history
At baseline prior and present other diseases/co morbidity will be registered. At all other time points a standard medical history will be documented including concomitant medications and adverse events.

8.4.2 Pain diary
Pain and painful crises will be evaluated by the use of standardized, daily pain diaries, developed according to a large study in patients with SCD in the USA, which have been implemented here recently in evaluating the burden of pain in SCD patients in Amsterdam.4,7 A 0-10 numerical rating scale will be used in our format, an established pain measure in adults and adolescents, to record intensity of pain. For children, a separate version of the diary has been made, based on the adult format and on a valid and reliable diary designed by Dampier et al. for a pediatric sickle cell population.54 For adults, an English version is available as well. Pain diaries will record presence, duration and severity of pain, whether subjects think they are in a crisis at that moment or not and the use of analgesics. Patients will be instructed to exclude non-sickle cell related pain, like a regular headache, from this diary.

8.4.3 Outpatient department (OPD) visits
Throughout the study each patient will be required to visit the hospital every month for treatment evaluation, returning pain diaries and unused medication, and receiving new supplies. Also, patients will be asked whether they have experienced any adverse effects. At the evaluation visits at baseline and after 3 and 6 months after start of treatment blood samples will be drawn, and questionnaires will have to be completed.

8.4.4 Blood sampling (by venipuncture)
Blood samples will be taken at baseline and after 3 and 6 months after start of treatment to monitor markers for oxidative stress, hemolysis, hypercoagulability, inflammation and endothelial dysfunction. For baseline characteristics, also a complete blood count and concentration of fetal haemoglobin will be determined. We will aim to combine blood drawing for routine outpatient check-ups with trial sampling, to minimize the amount of extra venipunctures. At every sampling moment an amount of ca. 20.7 cc blood will be drawn. This is ca. 10-12 cc extra than would be drawn for regular checks.
8.4.5 Physical examination

Every 3 months during evaluation visits, a complete, general physical examination will be performed, in total 3 times.

8.4.6 SF36-survey

The health related quality of life (HR QoL) in adult patients will be assessed with SF36-forms. This short-form health survey yields eight different scales (physical functioning, role limitations due to physical problems, role limitations due to emotional problems, social functioning, mental health, vitality, bodily pain, and general health perceptions) of functional health and well-being as well as psychometrically based physical and mental health summary measures. The SF-36 is a generic measure, which is not age, disease or treatment specific. Accordingly, the SF-36 has proven to be useful in surveys of general and specific populations, comparing the relative burden of diseases.\textsuperscript{55}

Duration is approximately 5-10 minutes. This survey will be performed during OPD visits, three times in total; at baseline, and after 3 and 6 months (end of the trial).

8.4.7 PedsQL inventory

The PedsQL measurement model will be used to assess health-related quality of life (HR-QOL) in pediatric patients in this trial. It is a questionnaire used in healthy children and adolescents, and those with acute and chronic health conditions, and has also been validated for sickle cell disease. It is designed to measure the core dimensions of quality of life, namely physical, emotional, social and school functioning. Duration is approximately 5-10 minutes. This survey will be performed three times in total; at baseline, and after 3 and 6 months (end of the trial).

8.4.8 Medical Consumption Questionnaire

To record direct generated medical costs, medical consumption will be assessed by means of an adapted version of the Medical Consumption Questionnaire (MCQ), also developed by the Institute for Medical Technology Assessment (iMTA) of the Erasmus University in Rotterdam. A separate, adapted format based on the Trimbos/iMTA questionnaire for Costs associated with Psychiatric Illness (TiC-P children) will be used for children, and presented to the parent that is most involved in care for the child. Duration is approximately 5-10 minutes. These surveys will be performed twice in total; at 3 and 6 months (end of the intervention).
8.4.9 Productivity Cost Questionnaire

To be able to perform a cost-effectiveness analysis, indirect costs arising from losses in productivity will be assessed by the means of the Productivity Cost Questionnaire (PCQ) developed by the Institute for Medical Technology Assessment (iMTA) of the Erasmus University in Rotterdam. This survey is designed to collect quantitative data on the relation between illness, treatment and work performance. The PCQ data permits the estimation of production losses (costs) of paid and unpaid labour.

This questionnaire will only be presented to adult patients with the assumption that subjects <18 years old are obliged to attend school by law and in general do not have a significant income yet.

Duration is approximately 5-10 minutes. This survey will be performed twice in total; at 3 and 6 months (end of the intervention).
8.5 Scheme of procedures

<table>
<thead>
<tr>
<th></th>
<th>Registration (Tpre)</th>
<th>Randomization (T0)</th>
<th>1 months</th>
<th>2 months</th>
<th>3 months</th>
<th>4 months</th>
<th>5 months</th>
<th>6 months</th>
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<tbody>
<tr>
<td></td>
<td>Start run-in period 2 weeks</td>
<td>Start treatment</td>
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<td></td>
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<tr>
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<tr>
<td>Productivity Cost Questionnaire</td>
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<td>X</td>
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8.6 Compliance

Subjects will have to take at least 80% of their medication in order to be considered compliant. Compliance will be checked by history taking and pill counts at each follow-up visit. Participants with less than 80% compliance on follow-up visits will be instructed with strategies for improvement. If consequently compliance is again less than 80% on 2 consecutive follow-up visits (over 4 weeks), these participants will be considered as lost-to-follow-up and will be excluded in our per-protocol analyses.

In case of minors, insufficient compliance will be discussed with both parents and the child.

As discussed before, a run-in period will be performed to improve compliance during intervention. To further improve compliance of study medication and completion of daily pain diaries, subjects will periodically receive text messages on their mobile phone (SMS), if available. These will be sent once or twice a week to remind participants of their study medication and pain diary throughout the intervention period. Subject (and in case of minors parental) approval to be contacted by text message will be verified on the informed consent form. Furthermore, in case of minors both one of the parents and the participant will be sent text messages, if both consenting. To secure the privacy of participants in this matter, this service will be set up in accordance with national privacy regulations according to the ‘Wet Bescherming Persoonlijke Gegevens’.

8.7 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they or their parents wish to do so without any consequences. Subjects withdrawn from the study will be treated according to the standard of care. The investigator can decide to withdraw a subject from the study for urgent medical reasons. These patients will be censored in our analyses. Specific criteria for withdrawal are:

- Death
- Insufficient compliance of the patient
- Suspected pregnancy
- Treatment with exchange transfusion
- Treatment with HC
- Treatment with a chronic blood transfusion scheme

For children, the trial will be performed according to the code of behaviour set up by the NVK “Verzet in kader van medisch-wetenschappelijk onderzoek”. This implies that research will be stopped in any case of resistance by the minor participant.
8.8 Replacement of individual subjects after withdrawal
Withdrawn patients will be replaced by newly recruited subjects to reach the aimed number of participants.

8.9 Follow-up of subjects withdrawn from treatment
No further information will be collected for patients who have withdrawn their consent. Patients who are withdrawn from protocol treatment will receive medical care according to local practice.

8.10 Premature termination of the study
The sponsor may decide to terminate the study prematurely based on the following criteria:
- There is evidence of an unacceptable risk for study patients (i.e. safety issue);
- There is reason to conclude that it will not be possible to collect the data necessary to reach the study objectives and it is therefore not ethical to continue enrolment of more patients; for example insufficient enrolment that cannot be improved.

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The sponsor will provide information regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.

8.11 Breaking the randomization code
Unblinding is only performed in emergency situations where knowledge of the identity of the study drug is considered absolutely necessary for the clinical management of the subject. The decision to unblind is made by the supervising physician on call or local investigator. If unblinding is essential in their opinion, every effort will be made to contact the PI before definitive unblinding to discuss options.
Unblinding will be possible 24 hours per day throughout the duration of the trial.

9. SAFETY REPORTING

9.1 Toxicities
The grading of toxicity and adverse events will be done using the most recent version of the NCI Common Terminology Criteria for Adverse Events, CTCAE version 4. A complete document can be downloaded from http://ctep.cancer.gov/reporting/ctc.html.
9.1 Section 10 WMO event
In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects’ health. The investigator will take care that all subjects are kept informed.

9.2 Adverse and serious adverse events
Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational drug or other study procedures. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event is any untoward medical occurrence or effect that at any dose:
- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients’ hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, lack of efficacy of an IMP used for the treatment of a life threatening disease, major safety finding from a newly completed animal study, etc.

All SAEs will be reported through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse reactions.
SAEs that result in death or are life threatening should be reported expeditiously. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.
9.2.1 Exceptions in SAE reporting: Context-specific SAEs

This study population has a risk of serious complications (so-called "context-specific SAEs") which are inherent to their disease and unrelated to the intervention which is under evaluation in this trial.

These context-specific SAEs include painful crises, SCD-related complications as mentioned in paragraph 8.1.3 and hospital admission due to one of the aforementioned events. These will be recorded in the Case Report Form. This documentation will include the date of diagnosis, classification of the complication and type of action taken if appropriate.

In the light of the above, immediate and individual reporting of all these SCD-related complications will not enhance the safety of the study. This is also in accordance with CCMO regulations.

An annual overview of these context-specific SAEs will be presented to the METC. This overview will consist of the following information: Name of the complication, date of diagnosis, classification of the complication, type of action taken, date of discharge or ongoing.

9.2.2 Suspected unexpected serious adverse reactions (SUSAR)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorised medicinal product) or the context-specific SAEs listed in paragraph 9.2.1.

The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.
- The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
- The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.
The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.2.3 Annual safety report

In addition to the expedited reporting of SUSARs, the PI will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, Medicine Evaluation Board and competent authorities of the concerned Member States.

This safety report consists of:
- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.3 Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

9.4 Data Safety Monitoring Board (DSMB)

A DSMB will not be installed for this study since the risks of participation are estimated to be negligible. According to the EMEA “Guideline on Data Monitoring Committees” the severity of the disease, the type of patient population, prior knowledge about the treatment under consideration and study design are determinants in assessing the need for a DSMB.57

Sickle Cell Disease (SCD) is not considered as a directly life-threatening disease. In daily life patients are usually able to function relatively normally. Furthermore, the study population in this study includes adults and children only of 12 years or older. More vulnerable, younger patients are excluded. NAC is a registered drug for a other indications with an excellent safety profile. In addition, no invasive procedures are involved in this trial.
except for venipuncture. Overall potential harm to patients is therefore expected to be mild and infrequent. As for the trial design, we do not plan to do any interim analyses for possible modification of the study design or early stopping. An independent physician will be appointed for assessment in case of safety matters.

10. STATISTICAL ANALYSIS

10.1 Statistical analysis

All included patients are included in the primary analysis according to their original treatment assignments, on an intention-to-treat-basis. Patients who withdraw their consent for use of their data will not be included in any analysis. Only the fact that they were enrolled into the trial and withdrew consent, the original study group to which they were allocated, and the reason for withdrawal will be reported.

Two-sided 5% significance levels will be used to identify statistically significant results. A secondary, per protocol analysis of the primary endpoint will be performed excluding the participants identified as lost to follow-up; subjects that were randomized into the study, but who failed to receive their allocated treatment, who were considered as non-compliant or who dropped out for other reasons.

The effect of NAC versus placebo on the different outcome parameters (laboratory, clinical) will be assessed by univariate analysis. The primary analysis will be the comparison of the average frequency of pain days, reported monthly, between the two intervention groups in relation to total follow-up time.

Data will be presented by means and standard deviations (SD) in case of normally distributed data. In case of skewed distributed data, they will be summarized by medians and ranges. Continuous variables will be analyzed using the Student’s t Test, Mann-Whitney U-test or Wilcoxon signed rank test as appropriate. Unpaired categorical data will be analyzed using the Chi-square test; paired categorical data will be analyzed using the MacNemar test. The effect of NAC on the primary outcome will be assessed by multi-variable logistic regression analysis.

10.1.1 Descriptive characteristics

Demographics, history of painful crises, use of HC and other descriptive characteristics will be cross-tabulated against randomised treatment allocation. If substantial imbalance exists an additional adjusted analysis will be performed.

10.1.2 Primary Efficacy Analyses

The primary analysis will be the comparison of the average frequency of daily life pain in days per patient (in relation to total follow-up time) between the two intervention groups.
10.1.3 Secondary Efficacy Analyses

The following parameters will be evaluated in our secondary analyses, and comparisons between the two intervention groups will be made.

Severity
The average intensity of pain during pain days, expressed on a numerical rating scale from 0-10, will be compared between the two groups at different points in time throughout the trial. Every 24 hours 2 ratings have to be given, one for night time and one for daytime. Intensities per day will be expressed in the average intensity of these 2 scores.

Crisis
A comparison of the average number of crises per patient in relation to total follow-up time between the two intervention groups will be made. Also the average number of crisis days per patient in relation to total follow-up time will be compared between both groups. Furthermore, the mean intensity of pain during crisis days will be evaluated and the mean duration of crises in days per patient per group.

Hospital Admission
The number of sickle cell related hospital admissions will be compared between both groups. Also the average duration of these admissions in days and the total amount of admission days will be compared between the treatment groups.

Health-related Quality of Life (HR-QoL)
QoL will be assessed for children and adults separately with age-specific questionnaires. The results will be expressed in scores from 0-100 at different points in time, comparing both treatment groups throughout the trial, with lower scores expressing lower and higher scores expressing better quality of life.

Hematological markers
Differences in the change from baseline of hematological markers will be compared between both groups (NAC versus placebo) at different points in time throughout the trial.

Tolerability of NAC
The number of adverse events classified as ‘definitely’ or ‘probably’ related to trial treatment will be compared per treatment group. Events classified as ‘possibly’, ‘unlikely’ or ‘not related’ will be excluded.

Frequency of analgesic usage at home
The measure of analgesic usage will be the proportion of days with reported analgesic use in each diary, calculated by dividing the number of days with analgesic use by the total number of days. A comparison will be made between both intervention groups.
Incidence of SCD complications
The overall, average frequency of SCD complications in relation to total follow-up time will be assessed and compared between both treatment groups.

10.2 Cost-effectiveness analysis
Total societal costs, including direct healthcare and non-healthcare costs and indirect costs from losses in productivity, will be compared between both groups.

11. ETHICAL CONSIDERATIONS

11.1 Ethical principles
This protocol is in accordance with the principles laid down by the 18th world medical assembly (Helsinki, 1964, revision of 2008)) and amendments laid down by the 29th (Tokyo, 1975), the 35th (Venice, 1983) and the 41st (Hong Kong, 1989) World Medical Assemblies.

11.2 Laws and regulations
This protocol is in accordance with laws and regulations of the country in which the study is performed.

11.3 Informed consent
The informed consent document will be used to explain in simple terms, before persons are entered into this study, the nature, scope and possible consequences of the study. This document will be made available in both Dutch and English for adult patients and parents.

The participant will give consent in writing. The signature of the physician and participant must confirm the participant’s consent. The investigator is responsible to see that informed consent is obtained from the participant and for obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedure. In patients between 12 and 18 years old, both the parents (or legally authorized representatives) and the subjects need to give written informed consent, following the “Medical Research Involving Human Subjects Act” (WMO).
11.4 Objection by minors or incapacitated subjects
The study will be conducted according to the “Code of conduct relating to expressions of objection by minors participating in medical research” approved by the Board of the Dutch Association of Pediatric Medicine (NVK) on 21 May 2001 (see Appendix).
Parents, guardians or subjects are free to decide to withdraw from the study at any stage, and for any reason, without prejudicing their child’s further treatment.

11.5 Ethical Review
Before start of the study, the study protocol and/or appropriate documents will be submitted to the ethical review committee (ERC), in accordance with local legal requirements. Only after approval will the study begin at the investigative site. The ERC will also be informed of all amendments and if necessary approval must be sought for ethical aspects.

11.6 Confidentiality
Personal information on the patients will be treated confidentially and anonymously according to the ‘Wet Bescherming Persoonlijkegegevens’. All patient names will be kept secret to anyone other than the investigator. Participants will be allotted a study number through randomization. This number will identify patients during the study throughout documentation and evaluation. The participants will be told that all study findings will be stored on computer and handled in strictest confidence formulated in the ‘Wet op de geneeskundige behandelovereenkomst’ (WGBO).

11.7 Storage of samples
Biological samples should only be stored for the purpose of additional research if the patient has given consent. If no informed consent was obtained, samples should be destroyed after the patient has completed all protocol treatment and procedures. Storage of biological samples on site is subject to the site’s guidelines; samples may be labelled with the patients identifying information (e.g. name, hospital record number). Samples that are shipped to another facility (e.g. a central laboratory) for a purpose as described in this protocol or for additional scientific research, should be stripped from any identifying information and labelled with a code (trial name or number and patient study number as assigned at enrolment).
11.8 Risks assessment, burden and benefits, group relatedness

11.8.1 Risks assessment, burden to participate versus benefits
This trial aims to assess the effects of daily use of NAC on the frequency of painful crises in SCD patients. Potentially NAC is able to reduce the incidence of daily life pain, painful crises and related hospital admission by reducing oxidative stress, with potential beneficial effects on quality of life and societal costs.

The risks of participation in this study are predicted to be negligible. NAC is already registered for other indications and is known to have an excellent safety profile with very limited side effects. These consist mostly of gastro-intestinal complaints like nausea, diarrhea and vomiting, and occasionally urticaria due to hypersensitivity.

Other factors contributing to the burden to participate are related to the study procedures. Patients will have to take 1 tablet of study medication twice daily during 6 months. They will have to fill out a pain diary daily during 6 months and 2 weeks, maximum total duration about 1 minute per day. Patients will have to undergo blood sampling 1 extra time compared to regular follow-up (once every 6 months). Questionnaires will also be performed three times; at baseline, 3 and 6 months (end of trial). All together total duration for this is approximately 45-60 minutes. Furthermore patients will have to be seen monthly to return pain diaries and unused medication, and receive new material. This will require an extra time investment of patients participating in the trial. Each appointment will take about 20-30 minutes.

Throughout the trial, from time of inclusion until the trial end, there will be 7 visits in total with an overall duration of 140 - 210 minutes. On average, two of these visits are aimed for to be part of routine check-ups.

11.8.2 Group relatedness
Our research question is group related. The risks of participation are estimated to be negligible and the burden to participate in this trial is very comparable with the burden that children with this disease experience in daily life with standard medical care. They visit the hospital frequently for check-ups where blood is regularly drawn. These patients are also used to taking daily medication such as folic acid, Broxil antibiotic prophylaxis or in some cases Hydroxycarbamide.

11.9 Compensation for injury
In accordance with Dutch law and the W.M.O., an insurance policy, covering all participating patients, has been effected as mentioned in the patient information form.
11.10 Incentives
Participants will only receive a financial compensation for their travel expenses, not for their participation as an incentive.

12. ADMINISTRATIVE ASPECTS AND PUBLICATION

12.1 Registration
Eligible patients should be registered before start of treatment. Patients need to be registered at the Trial Office of the department of Hematology of the Academic Medical Center sending the completed registration form by faxing (+31(0)206919743) or by email (hemat.trial@amc.nl) from Monday through Friday 09:00 to 17:00 CET. Patient study number will be created upon randomization.

12.2 Handling and storage of data and documents
All patient material will be anonymized. Any excess material will be stored to a maximum of 20 years. Only investigators mentioned in this protocol will have access to these samples.

12.3 Data collection
Data will be collected in the FDA proven database Oracle®. Data collected in this database are derived from the protocol and will include at least:

- inclusion and exclusion criteria;
- baseline status of patient including medical history and stage of disease;
- timing and dosage of protocol treatment;
- adverse events;
- parameters for response evaluation;
- any other parameters necessary to evaluate the study endpoints;
- survival status of patient;
- reason for end of protocol treatment;
- follow up

12.4 Data monitoring
Data monitoring will be performed by a certified clinical research associate of the participating institute. The monitor will compare the data entered into the database with the hospital or clinic records (source documents). The nature and location of all source
documents will be identified to ensure that all sources of original data required to complete the database are known to the investigational staff and are accessible for verification. At a minimum, source documentation must be available to substantiate: subject identification, eligibility and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; records of safety and efficacy parameters; adequate reporting and follow-up of adverse events; date of subject completion, discontinuation from treatment, or withdrawal from the study, and the reason if appropriate. Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the database are consistent with the original source data.

12.5 Amendments
Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.
A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:
- The safety or physical or mental integrity of the subjects of the trial;
- The scientific value of the trial;
- The conduct or management of the trial; or
- The quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.6 Annual progress report
The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.7 End of study report
The investigators will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient’s last visit. In case the study is ended prematurely, the sponsor will notify the accredited METC and
the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.8 Public disclosure and publication policy
The study will be registered in the EUDRACT, the website of the Dutch National Competent Authority, the ‘Centrale Commissie Mensgebonden Onderzoek’ (CCMO) and a public trial registry. The results from the different centers will be analyzed together and published as soon as possible in peer-reviewed international scientific journals and presented at scientific meetings. The responsibility for presentations and/or publications belongs to the investigators. No restriction regarding the public disclosure and publication of the research data have been, or will be made by the funding agency.

12.8.1 Final publication of trial results
The results will be submitted for publication in a peer reviewed scientific journal regardless of the outcome of the trial – unless the trial was terminated prematurely and did not yield sufficient data for a publication.

The final publication of the trial results will be written by the Principal Investigators and the co-investigators. A draft manuscript will be submitted for review to all co-authors. Results will also be published in a PhD-thesis.

12.8.2 Authorship
Authors of the main manuscript will include the Principal Investigator, the co-investigators and, investigators who have included evaluable patients in the trial. Others who have made a significant contribution to the trial may also be included as author, or otherwise will be included in the acknowledgement.
13. REFERENCES


10. Ware, R. E. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood 115, 5300–11 (2010).


53. Roijen, H.-V. & Tan, B. Handleiding voor kostenonderzoek; methoden en richtlijn prijzen voor economische evaluaties in de gezondheidszorg. (College voor Zorgverzekeringen, 2010).


Appendix

14. FLOWCHART TRIAL OVERVIEW

**Inclusion:**
- HbSS, HbSC, HbSβ+ or HbSβ0-thal
- Age ≥12 years
- History of ≥1 painful crisis / patient year

**Registration & Informed Consent**

**Run-in period (Tpre)**
- Daily pain diary

**Randomisation (T0)**
- Physical examination
- Blood sample
- SF-36 / PedsQL-questionnaire

**Arm A**
- N-Acetylcysteine
  - Twice daily 600mg (1 tablet)

**Arm B**
- Placebo
  - Twice daily 1 tablet

**Start treatment**
- Daily pain diary
- Daily study medication

**Off study**
If <80% of pain diary completed

1 week consideration
2 weeks
6 months
**Start intervention**
- Daily study medication (Arm A or B)
- Daily pain diary

**Treatment evaluation 1 - 2 months**
- Returning pain diary & unused medication
- Check compliance
- Administration new diary & medication
- Report adverse events

**Treatment evaluation 3 months**
- Returning pain diary & medication
- Check compliance
- Administration new diary & medication
- Report adverse events
- Physical examination
- Blood sample
- SF-36 / PedsQL
- Productivity Cost Quest.
- Medical Consumption Quest.

**Treatment evaluation 4 - 5 months**
- Returning pain diary & unused medication
- Check compliance
- Administration new diary & medication
- Report adverse events

**Treatment evaluation 6 months**
(End of trial)
- Returning pain diary & medication
- Check compliance
- Administration new diary & medication
- Report adverse events
- Physical examination
- Blood sample
- SF-36 / PedsQL
- Productivity Cost Quest.
- Medical Consumption Quest.