

Proton pump inhibition for secondary hemochromatosis in hereditary anemia, a phase III placebo controlled randomized cross-over clinical trial.

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Coordinating investigator/project leader	A.J. van Vuren, MSc. A.J.vanVuren@umcutrecht.nl Van Creveldkliniek UMC Utrecht Huispost C01.425 Postbus 85500 3508 GA Utrecht
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	Dr. E.J. Beers E.J.vanBeers-3@umcutrecht.nl Van Creveldkliniek UMC Utrecht Huispost C01.425 Postbus 85500 3508 GA Utrecht <i>Other sites</i>
Co- investigator	AMC: Dr. B.J. Biemond, internist-hematologist, department of hematology, Academic Medical Center, Amsterdam b.j.biemond@amc.uva.nl
Co-investigator	HAGA: Dr. J.L. Kerkhoffs, internist-hematologist, department of hematology, HAGA hospital, the Hague J.Kerkhoffs@hagaziekenhuis.nl
Sponsor (in Dutch: verrichter/opdrachtgever)	UMC Utrecht
Subsidising party	ZonMw
Independent expert (s)	Dr. D. Dekker D.Dekker@umcutrecht.nl

	Department of Internal Medicine and Dermatology 088-7550714
Laboratory sites	HAGA hospital, Den Haag. AMC, Amsterdam. UMC Utrecht, Utrecht.
Pharmacy	UMC Utrecht, KGO apotheek Dr. B. Romberg Pharmacist UMC Utrecht Huispost D.00.204 Postbus 85500 3508 GA Utrecht kgo-apotheek@umcutrecht.nl <i>Pharmaceutic company providing placebo and esomeprazole</i> BasicPharma Dhr. J. Wit BasicPharma Group IMP Department Burgemeester Lemmensstraat 352 6163 JT Geleen imp@basicpharma.nl

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Department: Prof. Dr. R.E.G. Schutgens, internist-hematologist		
Coordinating Investigator: A.J. van Vuren, MSc.		
Principle Investigator: Dr. E.J. Beers, internist-hematologist		

Steering board

Dr. E.J. van Beers. Van Creveldkliniek, UMCU, University of Utrecht, Utrecht

Mw. A.J. van Vuren, MSc. Van Creveldkliniek, UMCU, University of Utrecht, Utrecht

Dr. B.J. Biemond, Department of Hematology, AMC, Amsterdam

Dr. J.L. Kerkhoffs, Department of Hematology, HagaZiekenhuis, The Hague

Drs. D. Peereboom, foundation for Rare Blood Diseases.

G.A. de Wit, PhD, associate professor, department of PHM, Julius center, UMCU, University of Utrecht, Utrecht.

Prof. dr. T. Leiner, department of radiology, UMCU, University of Utrecht, Utrecht.

Dr. P.M.J. Welsing, epidemiologist, research office, division of internal medicine and dermatology, UMCU, University of Utrecht, Utrecht.

Co-investigators

Dr. S. Schols, Department of Hematology, Radboud MC, Nijmegen

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	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)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
LIC	Liver iron content
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
PKD	Pyruvate Kinase Deficiency
PPI	Proton pump inhibitor
(S)AE	(Serious) Adverse Event
SCD	Sickle cell disease
SmPC	Summary of Product Characteristics (in Dutch: officiële productinformatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation 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.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgegevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Rationale: The number one cause of years lived with anemia in Western Europe is hereditary anemia. The major cause of morbidity and mortality in patients with hereditary anemia not requiring chronic blood transfusion is iron overload caused by increased uptake from the gut. Iron overload and hereditary anemia are a growing, underestimated emerging health care problem. Many patients on iron chelation therapy, including deferasirox (currently the most frequently used iron chelating agent) experience side effects such as gastro-intestinal problems and less frequently renal or hepatic failure. Not including the economic costs and loss of quality of life caused by side effects of iron chelation, the cost of prescription alone amounted about 5 million euros in 2014 in the Netherlands. Dietary uptake of iron can be reduced by gastric acid reduction. Observational studies suggest that PPIs reduce iron uptake. In a recent randomized controlled trial in hereditary hemochromatosis PPIs diminished the needed number of phlebotomies. Although, results of this trial cannot be extrapolated completely to patients with hereditary anemia, this is a strong suggestion for effectiveness in patients with hereditary anemias and secondary hemosiderosis. A safer alternative for the iron chelators would make it possible to intervene earlier in these patients at lower costs. Especially in low-income regions of the world, PPIs could be a life saving and affordable alternative to prevent and treat iron loading.

Objective: to show that PPIs are an effective and safe treatment of secondary hemochromatosis in patients with hereditary anemia and mild iron overload.

Study design: randomised placebo controlled cross-over trial.

Study population: 40 non-transfusion-dependent patients (adults) with a form of hereditary anemia with mild to moderate iron overload. Mild to moderate iron overload is defined as a baseline LIC (liver iron content) between 3 and 15 mg Fe/g dry weight (dw) without iron chelation therapy or on stable chelation therapy.

Intervention: 12 months treatment with esomeprazole 40 mg twice daily and 12 months treatment with placebo twice daily.

Primary endpoint: the change in LIC measured by MRI of the liver expressed in mg Fe/g dw after one year of treatment with esomeprazole compared to treatment with placebo.

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

follow-up visits; blood sampling and MRI are all according to current clinical guidelines. Extra blood tubes (gastrin, hepcidin) will be collected. Every visit three questionnaires will be filled in to assess quality of life and cost-effectiveness of the intervention.

Esomeprazole is a registered drug with a favourable safety profile. Severe adverse events are rare. We have chosen for a relatively high dosage to achieve 24 hours inhibition of gastric acid secretion. Side effects will be registered every visit.

In our opinion the potential benefit of this drug (no or less need for iron chelation with a wide spectrum of severe side effects) outweighs the possible risks of this trial.

1. INTRODUCTION AND RATIONALE

1.1 INTRODUCTION

Relevance of iron overload

About 10% percent of all years lived with disability in the world is caused by anemia. (1) In North America and Western Europe the number one cause of years lived with this disability are the hereditary anemias. (2) The major cause of morbidity and mortality in patients with hereditary anemia not requiring chronic blood transfusion is iron overload caused by increased uptake from the gut. (3) The prevalence of hereditary anemia not requiring chronic blood transfusion in the Netherlands is unknown because there is no registry of these patients. Based on reported prevalence of most frequent forms of hereditary anemia we estimate the prevalence to be at least 1:1000. (4,5) The percentage of patients with hereditary anemia that has iron overload and needs daily treatment with iron chelation (medication to reduce iron in the body) differs per type of anemia but is generally underestimated because not all patients are under medical attention and only a minority of physicians screen for iron overload using MRI in this patient group. (6) The prevalence of iron overload in thalassemia intermedia is suggested to be about 33%, in pyruvate kinase deficiency this might even be 84%. (6,7)

In the Netherlands more than 550 patients are currently using deferasirox, the most prescribed iron chelator. (www.gipdatabank.nl) More than 33% of these prescriptions originate from the participating centers of this trial. The characteristics of a sample of these patients shows that >80% of these patients have a form of hereditary anemia (the others have acquired anemia) and about 50% of them do not require regular transfusions. Based on suggested prevalence rates in the Netherlands of hereditary anemia, the reported rates of iron overload in the literature, and our own experience this is a dramatic underestimation of the number of patients that currently should receive iron chelation. Therefore iron overload and hereditary anemia seem to be a growing emergent health care problem in the Netherlands. Many patients on deferasirox experience side effects such as gastro-intestinal problems and less frequently renal or hepatic failure. Not including the economic costs and loss of quality of life caused by side effects of deferasirox the cost of prescription alone amounted about 5 million euros in 2014. (www.gipdatabank.nl). The current knowledge on iron metabolism and experimental data in animals strongly suggest that proton pump inhibitors can significantly reduce iron uptake from the gut. The most effective proton pump inhibitor (PPI) esomeprazole is about 300 times cheaper and has a much better safety profile compared to deferasirox. Here we propose a cross-over trial comparing the effectiveness of esomeprazole compared to placebo in patients with hereditary anemia. This new indication of esomeprazole holds promise to significantly decrease the burden of iron chelation for both patients with hereditary anemia as well as health care.

1.2 RATIONALE

1.2.1 Dietary iron uptake is increased in hereditary anemia

The major cause of morbidity and mortality in patients with hereditary anemia is iron overload. (3) In patients with hereditary anemia who do not require regular blood transfusion iron overload is

characterized by excessive iron absorption from the gut. In healthy individuals, iron absorption is 1–2mg/d and is balanced by iron loss from skin, gut, menstruation or pregnancy. The body has no mechanism to regulate excretion of iron. Iron absorption is increased by anemia and ineffective erythropoiesis. (8) Dietary iron uptake in non-transfusion dependent hereditary anemia can be 3 to 10 times higher as compared to normal. This adds up to 1-3.5 g per year. (9) The iron accumulates primarily in the liver and the gold standard to estimate how much iron has been accumulated is the LIC (liver iron content) quantified by MRI. (10) Currently, management of patients with hereditary anemia that do not need regular blood transfusions is almost entirely based on clinical expertise and evidence derived from observational studies. Experts refer therefore to guidelines used for patients with the most prevalent form of hereditary anemia that need regular transfusions; beta-thalassemia major. (11) A normal LIC value is < 1.8 mg Fe/g dry weight (dw). In thalassemia major chelation therapy is started at serum ferritin levels of 1000-1500ng/ml and 12 months of regular red cell transfusions, or 20 transfusions. Target values for LIC are 2-7 mg Fe/g dw. Cardiac MRI is performed yearly to monitor cardiac iron loading. The target value is a T2* value of > 20ms. Cardiac iron loading is not a relevant problem in the non-transfusion dependent anemia thalassemia intermedia due to the other mechanism of iron loading. Liver iron overload as mentioned above however is common. Chelation therapy is started with a LIC value \geq 5 mg Fe/g dw. Target of treatment is a LIC value 3-5 mg Fe/g dw. For the other hereditary anemias there is hardly no evidence. (12,13)

1.2.2 Reducing gastric acid secretion can reduce dietary iron uptake.

Only ferrous iron or Fe²⁺ is transported through the microvillus divalent metal transporter (DMT1) into the duodenal mucosa. (14) Because a low pH is needed to reduce dietary ferric or Fe³⁺ iron to Fe²⁺, (14) in patients who do not produce gastric acid (achlorhydria) iron uptake from food is about 1% compared to 56% of controls. (15,16) The iron uptake increased when gastric pH artificially was lowered. (17) Pharmacologic inhibition of gastric acid secretion in healthy human controls by H2 antagonists (a weak anti acidic drug) reduces iron uptake by 65%. (18) Inhibition of gastric acid secretion by the much more potent PPIs should be even more effective.

1.2.3 PPIs can reduce iron uptake in hereditary anemia

PPIs are the most potent inhibitors of gastric acid secretion available and esomeprazole is viewed as the most effective PPI launched thus far. (19,20) Quantitative studies on the effect of PPI on the absorption of nonheme dietary iron are, however, not available. (17) Indirect evidence of the effect of PPI on iron absorption comes from its association with iron deficiency in various patient groups. (21,22) PPI in rats reduced iron uptake significantly. (23) In a small retrospective study in patients with hereditary hemochromatosis (iron overload without anemia) PPI reduced the need for additional treatment by about 85%. (24) In a second retrospective study the need for additional treatment was reduced to about 80%. (25) There were no side effects documented in the studied patients. Expressing the reduced need for treatment into a reduction in total body iron for a patient weighing 50-75kg, this would result in a reduction of 1.0-2.0 mg/g LIC. (26) This is comparable to what can be expected of the recommended dosage of iron chelation for patients with hereditary anemia and mild

iron overload. (27,28) Another hint of treatment effect of PPI is the recent interesting observation on iron overload in Amish patients in the Pyruvate Kinase Deficiency Natural History Study. (6) Of the 194 patients, 52 (27%) were from the Pennsylvania Amish community. These patients were managed differently from the non-Amish patients, in that only 2% of the Amish patients were on iron chelation therapy in the 12 months prior to enrollment compared with 43% among the non-Amish cohort. In addition, Dr D Holmes Morton of the Central Pennsylvania Clinic, Belleville, PA, USA, clarified that he instead puts some of his patients on a low iron diet and adds a PPI to their medication. (D Holmes Morton personal communication to study team on 1-2-2017). The Amish had a significant higher prevalence of splenectomy (96% vs 52%, $p < 0.0001$) and a higher proportion who previously received transfusion (79% vs 32%, $p < 0.0001$), both associated with iron overload in the rest of the cohort. Despite this, the Amish patients had a lower prevalence of iron overload (defined as LIC > 3 mg Fe/g dw) (34% vs. 51%) than the other PKD patients. This suggests that PPI in these severely affected patients with hereditary anemia results in lower LIC values.

1.2.4 Evidence in hereditary hemochromatosis

Recently, Vanclooster et al, published the results of a relevant double-blind randomized placebo controlled trial. (29) The trial was designed to prove that proton pump inhibitors decrease the need for phlebotomy in HFE hemochromatosis. 31 patients were enrolled to either placebo or PPI (pantoprazole 40 mg/day) for 12 months. Daily use of PPI significantly reduced the number of phlebotomies needed to keep the ferritin level < 100 ug/L. No serious adverse events were encountered during the study period. In the treatment group number of phlebotomies decreased from 5.33 in year before study to 1.27 during study time. (29)

1.2.5 Need for a randomized controlled trial in hereditary anemia

Although these results are promising for patients with hereditary anemias, they cannot be fully extrapolated due to several relevant differences between the pathophysiology of the diseases. First, the chosen endpoint (number of flebotomies) is not applicable to patients with hereditary anemia. The standard therapy to reduce iron loading in these patients is chelation therapy. The anemia precludes phlebotomy. Second, in patients with hereditary anemia the aim is to prevent or improve iron overload. In the mentioned study of Vanclooster et al. serum ferritin is the parameter used to monitor iron loading. In hereditary anemias, serum ferritin is not a reliable marker for iron overload. The LIC is the golden standard to monitor iron loading. Different serum ferritin thresholds are proposed for individual hereditary anemias. (12,30-33)

In conclusion, treatment goals are different, in hereditary hemochromatosis the goal is to achieve iron depletion (serum ferritin levels low), in hereditary anemia the goal is to prevent iron loading. Iron levels should be in the non-pathological range, but will still be high-normal. (10,34-36) The results of the trial of Vanclooster et al. suggest the effectiveness of PPIs in iron loading, although due to the differences in pathophysiology between hereditary hemochromatosis and hereditary anemia, a new trial has to prove effectiveness in hereditary anemias as well.

1.2.6 Opportunities of the trial

The European Hematology Association has recently published a roadmap for hematology research in Europe. For anemia in general one of the primary research goals is *the identification of new molecules to target iron metabolism*. (37)

In this research proposal we try to show efficacy of a re-discovered class of molecules in iron metabolism, PPIs. The study will provide a high level of evidence on the efficacy, effectiveness and safety data on a potent alternative to iron chelation for the treatment of iron overload in hereditary anemia. A safe and effective alternative is needed because current treatment options frequently have disturbing side effects. Iron chelators sometimes cause severe liver and/or renal complications and are extremely expensive. Some patients that do not tolerate even the lowest dosages of iron chelation do not have an alternative treatment option at the moment. (36) In addition, some guidelines suggest to start treatment with chelation only from a LIC 7 mg/g, due to the fear of side-effect of iron chelators at relative low levels of iron overload. (38) A safe alternative to iron chelators would allow to intervene earlier in the course of iron loading.

From an international perspective the results from this trial have major implications. In certain area's of the world, especially the Middle East and Southeast Asia, the prevalence of hereditary anemia is much higher, but the availability of expensive chelators much lower. In these regions PPIs might be the only realistic treatment option for many patients.

2. OBJECTIVES

Primary Objective to show that PPIs compared to placebo are an effective treatment of secondary hemochromatosis in a relative large number of patients with hereditary anemia and mild iron overload.

Secondary Objectives:

To assess the safety and side effects of treatment with esomeprazole.

To assess quality of life during treatment with esomeprazole compared with placebo.

To evaluate cost-effectiveness of esomeprazole in treatment of iron overload in hereditary anemia.

To assess the changes in 'iron markers' during treatment with esomeprazole compared with placebo.

To assess the need for chelation therapy after one year of treatment with esomeprazole compared with placebo.

To assess the adherence to therapy in a real life setting.

3. STUDY DESIGN

Phase III trial

This is a phase III multi-center placebo-controlled randomized cross-over trial. The trial will involve two treatment periods (placebo or PPI) of 12 months, which are consecutively administered in each patient recruited in the study. There is no washout period between the two periods of treatment. We do not expect a carry-over effect due to the short half-life time of elimination of esomeprazole. (19)

The efficacy of PPI versus placebo is assessed on the basis of the within-subject difference between the two treatment periods with regard to change in MRI quantified milligram Fe/g dw LIC.

The primary endpoint will be assessed at baseline, after 12 months and after 24 months. Additional study visits are planned every 3 months for assessment of safety parameters, side effects, secondary end-points and patient's compliance. The 12-month MRI will serve as the end-point of the first period and as the baseline measurement of the second period.

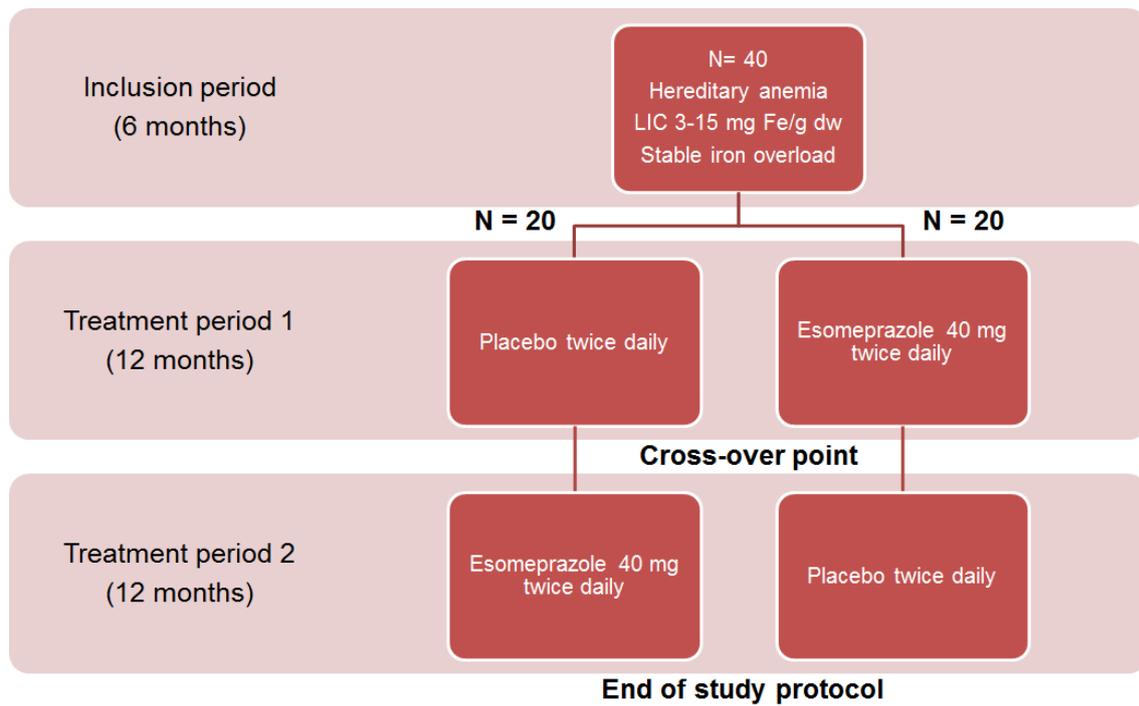
Study treatment will be started maximum 14 days after the baseline MRI. The MRI after 1 year will be planned a maximum of 1 week from the crossover point of the study. The final MRI will be planned a maximum of 1 week from the end of the study treatment.

For liver R2*, slice to slice, interobserver, intermachine, and interstudy variability estimates vary slightly among studies, but the cumulative errors are generally 5-7%. (39,40) Although there are errors related to interpatient specific differences. To overcome this last form of error, we chose a crossover design with the change in LIC as primary endpoint.

Liver iron content

MRI based LIC reproducibility within patients is very high. (39,41,42) The range of baseline LICs in our trial will be very high (inclusion of patients with LIC 3-15 mg Fe/g dw) compared to the anticipated minimal clinical relevant treatment effect of about 0.5-1.0 LIC by the study drug. Therefore a cross-over design is an efficient study design. The LIC measured by MRI before start of the treatment period of 12 months serves as the baseline value for that treatment period. (43) Besides this the accumulation of iron in these patients is a chronic stabile process that over years of positive iron balance results in high LIC values in these patients. The treatment period of 12 months is needed to observe any meaningful differences in LIC and is the most used observation period in clinical trials involving iron chelators. (27,34,36,44) The inclusion period will be 6 months. After start of study treatment the duration of the study is 2 years (12 months each esomeprazole and 12 months placebo).

FLOW CHART



4. STUDY POPULATION

4.1 Population

Description and source (base)

Recently, guidelines and clinical trials used a LIC of 3 mg Fe/g dw as threshold for iron overload. (13,34-36) Our main study population will consist of non-transfusion-dependent patients with a form of hereditary anemia with mild to moderate iron overload. Mild to moderate iron overload is determined by a LIC of 3-15 mg Fe/g dw. (11-13) We will allow patients on stable chelation therapy to enter the study. This increases the pool of eligible patients as most patients with a LIC > 7 mg Fe/g dw will be prescribed chelation therapy. Due to the difference in working mechanism of PPIs and chelators, we expect a synergistic effect of both treatments. We will include patients with all types of hereditary anemia to increase external validity of the study. The underlying pathophysiological mechanism of non-transfusion iron loading in hereditary anemia is the same in all subtypes. See Introduction (Chapter 1.1) for numbers of prevalence of hereditary anemia and iron overload. The project leaders are currently involved in an observational study describing iron overload in non-transfusion dependent patients with hereditary anemia in the Netherlands. Based on these results they confirm that including 40 patients in this study is feasible.

Reimbursement

To increase patient participation and inclusion rate the patients are fully compensated for any expense they have to make to participate in the study. The patients are also reimbursed for time spent for study visits. This approach has worked for recent studies in the Netherlands with these patient groups and is approved previously by Dutch Medical Ethics Committees (personal communication: Centre for Human Drug Research, Leiden the Netherlands). Medical care for patients with hereditary anemia is centralized in the Netherlands.

Feasibility

The participating centers are appointed as centers of expertise in hereditary anemia and are among the largest centers in the Netherlands. Based on hospital pharmacy registries for deferasirox and patient billing information (DBC/DOT) the participating centers see about 33% patients with hereditary anemia currently taking deferasirox. (www.gipdatabank.nl) In case of unanticipated low inclusion rates after two months of inclusion we will ask other academic centers to refer patients to the participating centers only for the duration of the study. We have used this approach before for clinical studies (NCT01849016, NCT02053480, NCT02476916) and it is very effective. Local hematologist can easily refer motivated patients to The Hague, Amsterdam or Utrecht for the study period. We expect no other feasibility problems because the number of visits is comparable to normal practice.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- diagnosis of hereditary anemia: hemoglobinopathy (including all sickle cell syndromes and beta-thalassemia), sideroblastic anemia, congenital dyserythropoietic anemia or an erythrocyte enzyme deficiency.
- hemoglobin level at baseline <7.0 mmol/L
- clinically stable and relevant iron overload defined as either one of:
 - a baseline LIC measurement by MRI between 3 and 15 mg Fe/g without having received iron chelation 6 months prior to entering the study.
 - OR a baseline LIC measurement by MRI between 3 and 15 mg Fe/g on stable chelation therapy (deferisirox, deferoxamine or deferiprone), with documented stable dosage the preceding 6 months and no expected dose reductions or increases the next two years.
- aged more than 18 years and able to sign informed consent.
- serum transferrin saturation higher than 0.45 once during the preceding 24 months.
- received less than 10 units of blood during the preceding 12 months.
- is expected to receive less than 4 units of blood during the following 12 months
- is not splenectomized during the preceding 24 months.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Pregnancy.
- Liver cirrhosis.
- Heart failure.
- Severe cardiac iron overload defined as MRI T2* < 20 ms.
- Severe liver iron overload defined as MRI LIC > 15 mg Fe/g dw.
- Expected poor compliance.
- Currently taking PPI and not able to stop for personal or medical reasons.
- Patients that are being phlebotomized as treatment for iron overload.
- Current peptic ulcer disease, gastro-intestinal bleeding or other causes of blood loss.
- Contra-indication for esomeprazole use.
- Concomitant use of clopidogrel.
- Contra-indication for MRI.
- Received more than 4 units blood during one of the treatment periods of 12 months.

In case one of the exclusion criteria will arise during the trial, the patient will be excluded from the study. Treatment periods of 12 months that were completed before exclusion will be included in the primary analysis.

During the six months recruitment period, patients who drop out of the study within four weeks after initiation of the treatment can be replaced by other patients at the discretion of the sponsor.

4.4 Sample size calculation

For the LIC measurement by MRI, slice-to-slice, intermachine, and interstudy variability estimates vary slightly among studies, but the cumulative errors are generally 5-7%. (39,40,45) The interobserver variability for R2* was 4.4% for the liver. (46) During serial studies, the patient specific bias observed in LIC measurements is minimized. (39,40) Inter-reader variability is low and results are highly reproducible across machine platforms. (45)

Two retrospective studies with PPIs in hereditary hemochromatosis have shown a reduced need for additional phlebotomies while on PPI treatment, the reduction varied from 80-85%. (24,25) Results were consistent in a randomized controlled trial. (29) Expressing the reduced need for treatment into a reduction in total body iron for a patient weighing 50-75kg, this would result in a reduction of the LIC of 1.0-2.0 mg Fe/g dw. (26) This is comparable to what can be expected of the recommended dosage of iron chelation for patients with hereditary anemia and mild iron overload. (27,28)

Based on current experience with PPIs in preventing and/or treatment of iron overload (paragraph 6.3.1 and 6.3.2) we suspect that PPI treatment will result in a change in LIC of at least 1.5 mg Fe/g dw in the intervention group.

We found only one study reporting the SD of MRI based LIC change over 12 months in non-transfusion dependent patients with hereditary anemia on placebo. In this group with a baseline LIC of 16 mg Fe/g dw +/- 10 mg Fe/g dw the LIC increased 0.4 mg Fe/g dw with an SD of 3.7 mg Fe/g dw. (34) This study and other studies have emphasized that treatment effect and variability are related to baseline LIC. (34,48,49) In this trial we have a relative low baseline LIC because patients with severe iron overload are excluded (which reduces both treatment effect and variability). The treatment effect of the lowest dose of the oral iron chelator, deferasirox compared to placebo in patients with non-transfusion dependent hereditary anemia is 2.3 mg Fe/g dw. (34)

Based on current literature we expect a change in LIC of at least 1.5 mg Fe/g dw plus 0.5 mg Fe/g dw (the known yearly increase in LIC in patients with hereditary anemia without intervention).

Based on the above mentioned study reporting standard deviations (34) : 29 patients are needed to detect a reduction of the LIC of 2.0 mg Fe/g dw (when assuming a SD of 0.9 mg Fe/g dw, paired T-test). (39) This group size is calculated using a significance level of 5% ($\alpha = 0.05$, $Z = 1.96$) and a power of 80% ($\beta = 0.20$).

We have increased the sample size to 40 because we expect a relatively high dropout of 15% due to unscheduled blood transfusions, surgery with blood loss or other complications, based on clinical experience with a recent previous trial performed in comparable patients in the AMC (NAC trial).

We presume that a positive trial showing efficacy in 40 patients with various forms of hereditary anemia will be enough to allow clinical guidelines to be adjusted concerning iron overload in hereditary anemia. Typical trials to proof efficacy of new chelators in hereditary anemia included 10 to 40 patients per group/treatment arm. (27,34,36,44,50) Even the most recent and largest international pharmaceutical sponsored trials published in the New England Journal of Medicine, concerning sickle

cell disease, the most prevalent form of hereditary anemia in the USA, included about 100 patients in each study arm. (51,52)

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The trial is conducted in a crossover design: 12 months 1 capsule of esomeprazole 40mg twice daily and 12 months 1 capsule of placebo twice daily. The two medicaments will be as identical in color, weight, taste, odor and package as possible.

5.2 Use of co-intervention

Not applicable

5.3 Escape medication

In case of hypomagnesemia or low plasma levels of zinc, magnesium or zinc supplementation therapy will be started.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Investigational product

Our investigational product is S-esomeprazole 40 mg twice daily, member of the group of PPIs. PPIs are frequently prescribed. In 2014 more than 1.9 million people in the Netherlands used a PPI (www.gipdatabank.nl). Registered indications are peptic ulcer disease, gastro esophageal reflux disease, Barret esophagus, Zollinger-Ellison syndrome and the prevention of gastrointestinal complications of several medicaments. (22) Besides this, PPIs are freely available over the counter in supermarkets and drugstores in the Netherlands. Because the very well known pharmacological aspects, long experience with the drug in clinical practice and the excellent safety profile of esomeprazole we included an summary of product characteristics (SmPC) that contains all necessary data to safely conduct the study instead of an investigators brochure (IB) in the trial documentation. Currently PPIs are registered for inhibition of acid secretion, the same property as we investigate in our study.

One of the most recent PPIs to come out of patent is esomeprazole, the S-enantiomer of the chiral omeprazole. Esomeprazole is viewed as the most effective PPI launched thus far. (19,20) Esomeprazole should be used between 60 and 15 minutes before the first and last meal of the day. (53)

Comparator

We have chosen placebo as comparator therapy, because this is the first phase III study evaluating the effect of PPI in patients with hereditary anemia.

An alternative strategy would be comparison to iron chelation (head-to-head trial). If a PPI has an unanticipated small but clinically relevant effect, a trial versus an iron chelator could have a negative result, hampering the introduction of PPI as an alternative to chelation therapy. However compared to placebo even a small effect would be enough to make the total iron balance negative in most patients. Guidelines could then for instance suggest starting treatment with cheap and non-toxic PPI and when treatment is not reaching a negative iron balance (LIC increases after one year of treatment), iron chelation can be added on top of the PPI treatment.

Ideally, we want to position PPI as an alternative for iron chelation. Alternatively, PPI as an additive to iron chelation may enable to lower the dose iron chelating medicaments.

So, the most important question first to be answered is the efficacy of the PPI in generating a negative iron balance.

Ongoing trials

We have performed an extensive search: www.clinicaltrialsregister.eu, <https://clinicaltrials.gov>, www.trialregister.nl, www.pubmed.com. We found one study: an open label non-randomized trial of PPI in children with a rare subgroup of hereditary anemia (Congenital Dyserythropoietic Anemia) sponsored by Sokora university with closing data 2013 which did not updated it's account since then,

extensive search on pubmed, the university website or internet didn't yield any trial results. The primary endpoint of this study is ferritin change after 6 months of treatment. This endpoint is currently regarded as inadequate for estimating liver/whole body iron content.

6.2 Summary of findings from non-clinical studies

Only ferrous iron or Fe^{2+} is transported through the microvillus divalent metal transporter (DMT1) into the duodenal mucosa. (14) Because a low pH is needed to reduce dietary ferric or Fe^{3+} iron to Fe^{2+} , (14) in patients who do not produce gastric acid (achlorhydria) iron uptake from food is about 1% compared to 56% of controls. (15,16) The iron uptake increased when gastric pH artificially was lowered. (17) Pharmacologic inhibition of gastric acid secretion in healthy human controls by H2 antagonists (a weak anti acidic drug) reduces iron uptake by 65%. (18) Inhibition of gastric acid secretion by the much more potent PPI should be even more effective.

Experiments in rats showed that pharmacologic reduction of gastric acid to pH 5 induced iron deficiency anemia and reduced LIC by 40%. (54) Another experiment with rats showed that PPI in rats reduced iron uptake significantly.(23)

After 5 days of esomeprazole 40 mg orally the pH level was during a mean of 17 of 24 hours > 4 in patients with symptomatic reflux disease. 40 mg of esomeprazole keeps pH > 4 in 97%, 92% and 56% of the patients for a minimum of 8, 12 and 16 of the 24 hours (SmPC, last update 3/2017; Esoimeprazol Sandoz 20/40 mg, www.cbg-meb.nl).

6.3 Summary of findings from clinical studies

We have outlined the findings from clinical studies in chapter 1 of this form (1.2.3 and 1.2.4). We have summarized the most important aspects in 6.3.1 and 6.3.2.

6.3.1 PPIs can reduce iron uptake in hereditary anemia

PPIs are the most potent inhibitors of gastric acid secretion available and esomeprazole is viewed as the most effective PPI launched thus far. (19,20) Quantitative studies on the effect of PPI on the absorption of nonheme dietary iron are, however, not available. (17) Indirect evidence of the effect of PPI on iron absorption comes from its association with iron deficiency in various patient groups. (21,22) In a small retrospective study in patients with hereditary hemochromatosis (iron overload without anemia) PPI (pantoprazole or esomeprazole, 40 or 20 mg daily) reduced the need for additional treatment by about 85%. (24) In a second retrospective study (lansoprazole 30 mg or omeprazole 20 mg) the need for additional treatment was reduced to about 80%. (25) There were no side effects documented in the studied patients. Expressing the reduced need for treatment into a reduction in total body iron for a patient weighing 50-75kg, this would result in a reduction of 1.0-2.0 mg/g LIC. (26) This is comparable to what can be expected of the recommended dosage of iron chelation for patients with hereditary anemia and mild iron overload. (27,28) Another hint of treatment effect of PPI is the recent interesting observation on iron overload in Amish patients in the Pyruvate Kinase Deficiency Natural History Study. (6) Of the 194 patients, 52 (27%) were from the Pennsylvania Amish community. These patients were managed differently from the non-Amish

patients, in that only 2% of the Amish patients were on iron chelation therapy in the 12 months prior to enrollment compared with 43% among the non-Amish cohort. In addition, dr D Holmes Morton of the Central Pennsylvania Clinic, Belleville, PA, USA, clarified that he instead puts some of his patient on a low iron diet and adds a PPI to their medication. (D Holmes Morton personal communication to study team on 1-2-2017). The Amish had a significant higher prevalence of splenectomy (96% vs 52%, $p < 0.0001$) and a higher proportion who previously received transfusion (79% vs 32%, $p < 0.0001$), both associated with iron overload in the rest of the cohort. Despite this, the Amish patients had a lower prevalence of iron overload (defined as LIC > 3 mg Fe/g dw) (34% vs. 51%) than the other PKD patients. This suggests that PPI in these severely affected patients with hereditary results in lower LIC values.

6.3.2 Evidence in hereditary hemochromatosis

Recently, Vanclooster et al, published the results of a relevant double-blind randomized placebo controlled trial. (29) The trial was designed to prove that proton pump inhibitors decrease the need for phlebotomy in HFE hemochromatosis. 31 patients were enrolled to either placebo or PPI (pantoprazole 40 mg/day) for 12 months. Daily use of PPI significantly reduced the number of phlebotomies needed to keep the ferritin level < 100 ug/L. No serious adverse events were encountered during the study period. In the treatment group number of phlebotomies decreased from 5.33 in year before study to 1.27 during study time. (29)

6.4 Summary of known and potential risks and benefits

PPIs are one of the most extensively prescribed drugs in daily practice. The liberal use of these drugs has been driven by their excellent safety profiles. In the Netherlands it is even available without prescription as over the counter medication. Esomeprazole is one of the most widely used PPIs. It is with rabeprazole nowadays phasing out first generation PPIs like omeprazole, lansoprazole and pantoprazole.

6.4.1 Safety profile

Esomeprazole was FDA approved in 2001. Initially, less was known about the safety profiles of the newer PPIs, as esomeprazole, compared with the older PPIs, as omeprazole. A large prescription-event monitoring study containing 11.595 patients on esomeprazole treatment was conducted in England. Diarrhea was the event with the highest incidence density in the first month (8.0 per 1.000 patient months of exposure). The conclusion of this post marketing surveillance study was that the safety profile of esomeprazole was consistent with the prescribing information and experience reported in literature. (55)

Esomeprazole provides better gastric acid control than the racemic proton pump inhibitors. In a large trial in over 5.000 patients with acid related esophagitis, esomeprazole was more effective in healing esophagitis. Esomeprazole was generally well tolerated with a tolerability profile similar to that of other PPIs. Few patients discontinued treatment due to treatment related adverse events (< 3%) with very few (<15) drug-related serious adverse events. (56)

Safety data of 2 RCTs evaluating the effect of long term PPI use (20 and 40 mg daily) versus anti-reflux surgery showed that SAEs were reported at a similar frequency in the PPI and surgery groups. Laboratory results, including routine hematology parameters and tests for liver enzymes, electrolytes, vitamin D, vitamin B12, folate and homocystein, showed no clinically relevant changes over time. The investigators concluded that there were no major safety concerns during 5-12 years of continuous PPI therapy. (57)

The most common side effect of esomeprazole are abdominal discomfort, diarrhea, constipation, flatulence, nausea, vomitus and headache (kennisbank.knmp.nl; informatorium medicamentorum esomeprazol, date 18-08-2017).

Possible, but rare side effects of PPIs are: gastroenteritis (Salmonella, Campylobacter or Clostridium), airway infections, hypomagnesaemia, acute interstitial nephritis, chronic kidney disease, vitamin B12 deficiency, iron deficiency, dementia, osteoporotic fractures. Although causality is not proven for several of these rare, but severe side effects. (58) A complete summary of the side effects of esomeprazole is provided in the Summary of Product Characteristics (SmPC chapter 4.8, last update 3/2017, Esomeprazol Sandoz 20/40 mg, www.cbg-meb.nl).

6.4.2 Pregnancy

Although esomeprazole is considered as a generally safe medicament, there is not sufficient information available over use of esomeprazole during pregnancy. Currently available data suggest that use of esomeprazole during pregnancy does not increase the incidence of congenital abnormalities. Based on the experience with omeprazole, prescription during pregnancy could be considered (kennisbank.knmp.nl; informatorium medicamentorum esomeprazol, date 18-08-2017). Based on these data we decided not to include pregnant women in our trial. Patients included in the trial will be instructed not to become pregnant.

6.5 Description and justification of route of administration and dosage

Esomeprazole is dosed orally. The peak plasma level is reached after 1-2 hours. The bioavailability is 64% after one dose 40mg esomeprazole and increases up to 89% after repeated daily ingestion. More detailed information is provided in the Summary of Product Characteristics (SmPC chapter 5.2, last update 3/2017, Esomeprazol Sandoz 20/40 mg, www.cbg-meb.nl). Concurrent intake of food delays and diminishes absorption, although this effect does not have a significant influence on the pH level in the stomach.

Besides these favorable pharmacokinetics for oral administration, oral administration of the drug is the most patient friendly way of daily treatment.

Esomeprazole will be dosed twice daily, total of 80 milligrams per day. We have chosen this dosage based on the pH lowering profile of esomeprazole, in order to elevate the pH level, and thereby diminishing iron absorption, for 24 hours a day.

Studies have shown that the secretion of acid by the stomach is effectively inhibited by esomeprazole. After 5 days of esomeprazole 40 mg orally the pH level was during a mean of 17 of 24 hours > 4 in

patients with symptomatic reflux disease. 40 mg of esomeprazole keeps pH > 4 in 97%, 92% and 56% of the patients for a minimum of 8, 12 and 16 of the 24 hours (SmPC, last update 3/2017; Esomeprazol Sandoz 20/40 mg, www.cbg-meb.nl). With once-daily administration, about 70% of the pumps are inhibited, and with twice-daily administration 80% are inhibited. It has been suggested that 30% of patients require twice-daily dosing to obtain effective control of daytime and nighttime symptoms in case of reflux disease. (53) During daytime esomeprazole 40 mg once daily results in a gastric acid pH>5 during 60% of time. (19)

These findings underline the need for a two daily regimen in order to obtain high pH levels during 24 hours. We want to test the strongest pH inhibition as possible that is safe as a continuous treatment.

6.6 Dosages, dosage modifications and method of administration

During the 12 months intervention period esomeprazole will be prescribed in a dose of 40 mg orally twice daily. Placebo will be administered orally during 12 months as 1 capsule twice daily.

No dosage modifications to either esomeprazole or placebo treatment will be made.

6.7 Preparation and labelling of Investigational Medicinal Product

Esomeprazole and placebo will be shipped to the UMC Utrecht 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 esomeprazole 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.

6.8 Drug accountability

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

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, and the unique code numbers assigned to the investigational products and trial patients. Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational products received from the sponsor.

Partially used investigational medicinal product should not be re-dispensed 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.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Primary endpoint

The main endpoint of this study, is the change in LIC from baseline (start of treatment) to 12 months measured by MRI of the liver. This endpoint will be used in the primary analysis to compare treatment with esomeprazole to treatment with placebo. The LIC will be expressed in mg Fe/g dw after data analysis of the T2* and T1 images of the MRI.

The liver is the major site of iron storage in patients with hereditary anemia, containing 70% or more of body iron stores. (26) Therefore all international guidelines agree that the LIC should be used as primary parameter to diagnose and monitor iron overload in patients with hereditary anemia. (36,59,60) MRI relaxometry is superior for serial observation of the LIC versus liver biopsy. R2* images provide the most robust values to monitor the effectiveness of iron chelation at 12 and 24 weeks, at 48 weeks R2, R2* and the perfect liver biopsy are equal. (39)

7.1.2 Secondary endpoints

1. Tolerability of esomeprazole: the incidence of side effect / adverse events will be monitored every 3 months during study visits. Measurement of vitamin B12, zinc and magnesium, T0, T12 and T24. Report of airway infections.
2. Quality of life: this will be assessed with EQ5D-forms, with time intervals of 3 months.
3. Cost-effectiveness analysis of esomeprazole in treatment of iron overload in hereditary anemia. This will be assessed by a prospective cost-effectiveness analysis. IMCQ and iPCQ questionnaires will be filled in with time intervals of 3 months.
4. Related changes in markers of iron metabolism:
 - a. Plasma hepcidin T0.
 - b. Serum ferritin T0, T12, T24.
5. Compliance to study drug
 - a. Plasma gastrin T0, T6, T12, T18, T24.
 - b. Counting of the capsules.
6. Need for chelation therapy

7.1.3 Other study parameters

Not applicable.

7.2 Randomisation, blinding and treatment allocation

The study is a randomized placebo-controlled cross-over trial. Consisting of two treatment periods (placebo and PPI) of 12 months which are consecutively administered in each patient recruited in the

study. Blinding and randomization will be performed by the pharmacy that supplies the placebo and study product. This pharmacy will also contain the key. Patients will be randomized in blocks of 4 patients stratified for the use of chelation.

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.

7.3 Study procedures

Standard blood/urine analysis:

- Every 3 months: Hb, MCV, leucocytes, thrombocytes, reticulocytes, ferritin, iron, transferrin saturation, ASAT, ALAT, AF, yGT, creatinin, ureum, bilirubin, LDH, haptoglobin, albumin, CRP.
- Every 12 months (visit 1, 5, 9): vitamin B12, folic acid, zinc, phosphate, calcium, magnesium, glucose, TSH, fT4, FSH, LH, testosterone, estradiol, IFG-1, cortisol.
- Every 12 months (visit 1, 5, 9): urine analysis: microalbumin/creatinine ratio, ureum, total protein.

Extra blood analysis

- (visit 1): serum gastrin, hepcidin.
- (visit 1, 3, 5, 7, 9): serum gastrin.

Study procedures during visits

- medication review
- check of compliance
- report of side effects
- EQ5D questionnaire (every 3 months)
- IMCQ questionnaire (every 3 months, health care use during the past 3 months)
- IPCQ questionnaire (every 3 months, productivity losses during the past 4 weeks)
- IRONIC-FFQ questionnaire (every 12 months, heme and non-heme iron intake)*

* The dietary intake of heme and non-heme iron will be assessed with the IRONIC-FFQ Intake Calculation-Food Frequency Questionnaire. (61) This questionnaire will be completed by a trialteam member based on information from the patient during a short interview.

Patients will be motivated to reduce iron intake by reducing the intake of (organ) meat.

MRI

MRI liver will be performed every year.

- MRI 1: baseline. The maximum time interval between start of study medication and the baseline MRI will be 14 days.

- MRI 2: after one year of treatment. The maximum time interval between the cross-over point and the MRI will be 7 days.
- MRI 3: after 2 years of treatment. The maximum time interval between the end of study treatment and the MRI will be 7 days.

A table of the schedule of the study is provided in appendix 1 (13.1).

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

Start of new iron chelation therapy and cessation or dose adjustment (in case of stable chelation therapy) of iron chelation therapy is not allowed during the study period.

Iron overloading in patients with non-transfusion dependent hereditary anemias is regarded as a slowly, but progressive process without intervention. The mean increase in LIC per year in non-transfusion dependent thalassemia intermedia is 0.38mg Fe/g dw. Over multiple years, without intervention, this will result in a significant degree of iron overloading. (62,63) Patients included in our trial will have mild to moderate iron overload. Based on the natural course of secondary hemochromatosis in patients with non-transfusion dependent hereditary anemias, a sudden increase in LIC until values warranting immediate start of iron chelation therapy, are not suspected unless the transfusion dependency of the patient alters during the study period. Requirement of more than 4 blood transfusions during a treatment period of 12 months is defined as an exclusion criterion. Patients will be excluded immediately and iron chelation therapy could be started as indicated according to current guidelines.

In case one of the exclusion criteria of the study will arise during the trial, the patient will be excluded from the study.

7.4.1 Specific criteria for withdrawal

Pregnancy is defined as a medical reason to be withdrawn from the study.

7.5 Replacement of individual subjects after withdrawal

If withdrawal takes place during the first six months in which the study is open for inclusion, withdrawn patients will be replaced by newly recruited subjects to reach the aimed number of participants.

7.6 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.

7.7 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. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

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

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the coordinating investigator and principal investigator without undue delay after obtaining knowledge of the events.

The coordinating investigator, or in her absence, the principal investigator, will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

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

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
 - Summary of Product Characteristics (SmPC) for an authorised medicinal product;
 - Investigator's Brochure for an unauthorised medicinal product.

The coordinating investigator or the principal investigator 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 Eudravigilance or *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.

8.2.4 Responsibilities investigators in participating centres

SAEs or SUSARs will be reported to the coordinating investigator, or in her absence the principal investigator, without delay. The coordinating investigator, or in her absence the principal investigator, will be responsible for the report through *ToetsingOnline* to the METC. The investigators in the participating centres do have to provide the information necessary to complete the report.

8.2.5 Unblinding for SUSAR report

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.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, 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.

8.4 Follow-up of adverse events

All AEs 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.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

8.5 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. (EMEA/CHMP/EWP/5872/03)

Hereditary anemia is generally not considered as a directly life-threatening disease. The study population does not contain children. Esomeprazole is a registered drug for several gastrointestinal indications and is generally well tolerated. 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.

Safety matters will be discussed in the steering board.

9. STATISTICAL ANALYSIS

9.1 Main analysis

The main analysis will consist of two separate analyses: an intention to treat analysis (ITT) and a per protocol analysis.

An intention to treat analysis (ITT) and a per protocol analysis will be performed.

ITT All included patients are used 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. 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. The consecutive change scores in LIC in patients (i.e. for the placebo and PPI treatment period; primary endpoint) will be compared between the PPI treatment period and the placebo period accounting for the stratification factor in randomization, order of treatment period and important prognostic covariates.

Per protocol A per protocol analysis of the primary endpoint will be performed using the same methodology but excluding the participants identified as not following the study protocol: subjects that were randomized into the study, but who failed to receive their allocated treatment, patients who were considered as non-compliant or patients who dropped out of the study before completing both treatment periods. *Non-compliance for the placebo period* is defined as missing more than 20% of dosages based on pill counts over the total treatment period. (64)

Non-compliance for the esomeprazole period is defined as non-adherence to treatment for more than 20% of missed dosages based on pill counts or a serum gastrin level within the reference range or less than 50% increase compared to baseline. (64)

9.2 Statistical analysis and missing data

Continuous variables will be described using means and standard deviations or median with 25th and 75th percentile. Categorical variables will be described using frequency and proportions. Primary and secondary outcomes (see study parameters) will be described and compared per treatment period. Baseline clinical and demographic variables will be described for the total trial population and for time varying variables also separately at start of both treatment periods (e.g. baseline LIC level).

For the main efficacy analysis the primary endpoint will be analyzed using linear mixed modelling with a random intercept and treatment as independent variable. Sex, iron chelator use (stratification factor for randomization) baseline LIC and order (first placebo or first active drug arm) will be used as co-variates in this analysis.

This analysis accounts for the dependency of treatment periods within patients (i.e. the cross-over design). Using this analysis all available data can be used in the analysis (including data from patients with missing information for one of the treatment periods) and formal imputation methods are not needed assuming missing data to be 'missing at random'. Therefore we will not perform formal

imputation methods for the primary analysis. Missing data will be described and differences between patients with missing data and patients without missing data will be compared.

Continuous secondary outcomes will be analyzed using the same approach. For binary secondary outcomes McNemar test will be used and mixed model logistic regression analysis will be used. For comparing count data between treatment periods Wilcoxon signed rank test will be used or Mixed-Effects Models for Count Data (Poisson regression).

9.3 Subgroup analysis

The trial population will be a relatively heterogeneous group of patients due to the inclusion of several forms of hereditary anemia. As outlined in the introduction iron uptake is increased in hereditary anemia. A subgroup in this heterogeneous group will be formed by the patients with sickle cell disease (SCD).

Current literature suggests that bone marrow derived factors such as GDF-15, TWSG-1 or erythroferone suppress hepcidin in beta-thalassemia and other hereditary anemias, leading to increased dietary iron absorption, a mechanism that is suggested to be relatively lacking in SCD. However a subgroup of our SCD patients has extreme low hepcidin/ferritin ratios (comparable to beta-thalassemia), do have iron overload and, (hence) are eligible for this study. (65) Because our observations are controversial we added a sensitivity analysis comparing the treatment effect in SCD and non-SCD patients as a subgroup.

As lined out in the previous paragraph, hepcidin is the major hormone modulating dietary iron uptake in man. Chronic suppression of this hormone cause increased dietary iron uptake and subsequent secondary hemochromatosis. Because hepcidin regulation is multifactorial we included a sensitivity analysis with hepcidin/ferritin ratio as additional co-variate in the linear mixed model ITT analysis and the per protocol analysis.

Modification of the treatment effect by SCD and hepcidin/ferritin ratio will be explored by adding interaction terms to the primary analysis model.

9.4 Data handling

All sites will be monitored by monitors of the Julius Centre, Utrecht the Netherlands. The data will be entered in the database using an eCRF (CASTOR electronic data capture).

9.5 Primary study parameter

Our primary study endpoint is the change in LIC from baseline of the treatment period. We will assess the LIC by MRI at start of the study, after the first treatment period of 12 months and after the second treatment period of 12 months. The LIC after the first treatment period is the baseline LIC for the second treatment period.

The change of LIC is expressed in milligram iron per gram dry weight.

All MRIs will be centrally reviewed at the UMC Utrecht, department of radiology.

We have not chosen for a washout period due to the following characteristics. We expect a slow rise in LIC during the placebo period (34), and a slow decrease in LIC during the treatment period with esomeprazole. The change in LIC is measured as the LIC after 12 months treatment minus the LIC at start of that treatment period (baseline). We have not chosen for a washout period, due to the short elimination half-life of esomeprazole compared to the treatment period we don't expect a carry over effect. (43)

9.6 Secondary study parameters

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

Tolerability of esomeprazole / placebo.

Incidence of hypomagnesemia, low levels of zinc and vitamin B12 requiring suppletion.

Incidence of respiratory infections.

Number of SAE reports.

Number of other AEs. The number of adverse events classified as 'definitely' or 'probably' related to trial treatment will be compared between treatment groups. Events classified as 'possibly', 'unlikely' or 'not related' will be excluded.

Quality of life

The EQ5D-5L will be used for quality of life assessment. The EQ5D-5L is a well-known health status questionnaire that consists of 5 domains of health (mobility, self-care, usual activities, pain, anxiety and depression), with 5 levels of functioning each (no problems, some problems, moderate problems, severe problems, extreme problems). The questionnaire translates patient derived scores to Quality Adjusted Life Years following a valuation study that was performed in the Dutch population (66) QALYs will be calculated following an Area under the Curve approach, using linear interpolation between the 3-monthly observations. The within-subject QALYs for both 12-months periods, with omeprazole and placebo respectively, will be compared.

Cost-effectiveness analysis and Budget Impact Analysis

Cost-effectiveness will be studied by relating cost-differences between the verum and placebo period to differences in LIC between both periods. It is anticipated that patients will both have lower LIC outcome and lower costs, because of a reduction of dosage of expensive chelation therapy (or even abstinence of using chelation therapy). Furthermore, cost-utility will be assessed by studying cost differences between the verum and placebo period in relation to quality of life changes in patients. Quality of life will be measured using the EQ5D-5L questionnaire and QALYs will be estimated following the algorithm of Versteegh et al. (66) Data on health care use (hospitalization, chelation therapy, medication, outpatient visits) will be derived from Electronic Medical Records. Patient

questionnaires will be used to observe other health care use (e.g. primary health care, homecare) and help by family and friends. Patient costs and productivity losses will be elicited through the repeated (3-monthly) administration of standard Dutch cost questionnaires (iMTA Productivity Cost Questionnaire and Medical Cost Questionnaire). Cost prices will be estimated according to recently published Dutch guidelines for economic evaluation in health care research (Zorginstituut Nederland). Questionnaires will be given at 3-monthly intervals, upon each contact moment during follow-up. Data will be presented both from a health care and a societal perspective, the latter including productivity costs and patient costs in addition to healthcare costs. Bootstrap methods will be used to present uncertainty around cost-effectiveness estimates. The economic evaluation will have a similar timeframe as the clinical study.

The design of the budget impact analysis (BIA) will be a study of different scenarios of either or not introducing PPI in the treatment of hereditary anemia. The BIA will be based on data collected alongside the clinical study. It will allow estimation of the financial consequences of introduction of PPI from the perspective of different stakeholders involved. All cost items needed for the BIA will be derived directly from our economic data collection; the valuation of those items depends on the perspective taken for the budget impact analysis. The perspectives to be included in the BIA are (1) net-BKZ or government perspective and (2) the health insurance perspective. Substitution effects, i.e. reduced need for expensive chelation therapy, will be taken into account. Different scenarios with different levels of implementation of PPI will be analysed and compared, ranging from all hereditary anemia patients using PPI to no use of PPI (i.e. current care). The time horizon for the BIA will be 10 years. BIA results will be reported separately for each year within the time horizon and indexation will be applied.

Changes in markers of iron metabolism

Serum ferritin T0, T12, T24. Change in serum ferritin level after 12 months of treatment, compared with the baseline of the treatment period.

Compliance to study drug

Compliance is based on capsule counts (for both treatment periods) and gastrin measurements (for the treatment period with esomeprazole). Gastrin measurements will be performed at regular intervals (T0, T6, T12, T18, T24). Compliance for the different treatment periods is defined in 9.3.

Need for chelation therapy

At the end of both treatment periods, we will evaluate if according to current guidelines, start of chelation therapy is indicated.

9.7 Descriptive characteristics

We will collect the following data from our study patients:

- age at inclusion

- gender
- weight and length
- blood pressure
- form of hereditary anemia
- history of splenectomy
- history of cholecystectomy
- hepatitis C status
- medication use including iron chelation therapy
- co-morbidities (e.g. diabetes, hypertension, osteoporosis)
- number of blood transfusions in last 12 months
- baseline complaints/symptoms (for AE reporting)
- heme and non-heme iron intake
- number of phlebotomies in the last 12 months
- baseline safety parameters and study parameters as listed in 7.1.2 and 7.3

These factors and LIC at baseline, serum ferritin at baseline, will be cross-tabulated against randomised treatment allocation.

9.8 Stratification factor

Stratification is performed in blocks of 4 as outlined in 7.2. We will stratify the patients by chelation therapy as we expect this to be of influence on our main outcome. As outlined in section 9.2, use of iron chelation therapy is a co-variate in our main analyses.

9.9 Interim analysis

We will not perform an interim analysis.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This protocol is in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964, revision of 2008) and amendments, last amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013, and in accordance with the Medical Research Involving Human Subjects Act (WMO).

10.2 Recruitment and 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 written information will be in Dutch.

The supervising doctor will inform the patients about the study. The doctor will ask for their consent. There is no set consideration period, although inclusion has to take place during the inclusion period. The inclusion period is 6 months from start of the study.

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.

The patient information will include the contact details of the independent expert. We have chosen for one independent expert for all centers to guarantee the expertise of this physician. The trial population consists of a patient group requiring care of a highly qualified team. Care is centralized in specialized centers. We expect our independent expert to be fully informed about the patient population and the trial. So we have chosen for one perfectly informed independent expert, instead of three experts in the different centers.

10.3 Objection by minors or incapacitated subjects

Not applicable.

10.4 Benefits and risks assessment, group relatedness

This trial aims to diminish the need for iron chelation therapy by use of esomeprazole. Potentially esomeprazole reduces the need for iron chelation therapy with its severe side effects and high costs. Esomeprazole is suspected to be a safer alternative treatment to reduce iron loading. There are potential beneficial effects on quality of life due to a decrease in iron chelation therapy and its side effects.

The risks of participation in this study are predicted to be negligible. Esomeprazole is already registered for other indications and is known to have a good safety profile with very limited side

effects. These consist mostly of gastro-intestinal complaints.

Other factors contributing to the burden to participate are related to the study procedures. Patients will have to take 1 capsule of study medication twice daily during 24 months. Patients will not have to undergo extra moments of blood sampling compared to regular follow-up (once every 3 months). Questionnaires will be performed 9 times; at baseline, and every 3 months until end of trial. The duration to fill in the 3 questionnaires will be maximum 25 minutes. Patients with hereditary anemia and iron overload are normally seen 4 times a year on the outpatient clinic. Each appointment will take about 20-30 minutes; there will be no extra appointments. Current guidelines for patients with hereditary anemia and iron overload also recommend performing a MRI once yearly.

Our research question is group related.

10.5 Compensation for injury

The sponsor has a liability insurance, which is in accordance with article 7 of the WMO. This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives

To increase patient participation and inclusion rate the patients are fully compensated for any expense they have to make to participate in the study. The patients are also reimbursed for time spent for study visits.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Personal information of the patients will be treated confidentially and anonymously. 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.

Blood tubes for gastrin and hepcidin measurements will be stored until 6 months after the end of the study. Storage of these samples on site is subject to the site's guidelines; samples may be labeled with the patients identifying information (e.g. name, hospital record number). Samples will be shipped for these analyses to the sponsor. Directly after analysis the blood samples will be destroyed.

11.2 Monitoring and Quality Assurance

Data monitoring will be performed by a certified clinical research associate of the Julius Center, UMC Utrecht. Details are provided in the monitoring plan, attached in K6.

Monitoring visits will be scheduled every year, starting with a central initiation visit and ending with a close-out visit. The following items will be monitored during the visits: rate of inclusion and dropout per centrum, presence and completeness of study files, in- and exclusion criteria, source data verification, serious adverse events, presence of instructions for study procedures, collecting and sampling of biological samples, and the certification of the laboratory and pharmacy.

11.3 Amendments

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.

11.4 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.

11.5 Temporary halt and (prematurely) end of study report

The sponsor 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.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

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.

11.6 Public disclosure and publication policy

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.

The trial will be registered in a public trial registry before the first patient is included, registration will take place in the EUDRACT and the 'Centrale Commissie Mensgebonden Onderzoek' (CCMO).

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Only ferrous iron or Fe^{2+} is transported through the microvillus divalent metal transporter (DMT1) into the duodenal mucosa. (14) Because a low pH is needed to reduce dietary ferric or Fe^{3+} iron to Fe^{2+} , (14) in patients who do not produce gastric acid (achlorhydria) iron uptake from food is about 1% compared to 56% of controls. (15,16) The iron uptake increased when gastric pH artificially was lowered. (17) Pharmacologic inhibition of gastric acid secretion in healthy human controls by H2 antagonists (a weak anti acidic drug) reduces iron uptake by 65%. (18) Inhibition of gastric acid secretion by the much more potent PPI should be even more effective.

The secretion of acid by the stomach is effectively inhibited by esomeprazole. After 5 days of esomeprazole 40 mg orally the pH level was during 17 of 24 hours > 4 in patients with symptomatic reflux disease. 40 mg of esomeprazole keeps pH > 4 in 97%, 92% and 56% of the patients for a minimum of 8, 12 and 16 of the 24 hours (SmPC, last update 3/2017; Esomeprazol Sandoz 20/40 mg, www.cbg-meb.nl). *We refer to paragraph 1.2.2.*

The working mechanism of PPIs in preventing iron uptake is plausible and proven. There are in current literature strong suggestions for the effect of PPIs in preventing iron loading in hereditary anemia. The data from a recent RCT (29) performed in patients with hereditary hemochromatosis provides us with strong arguments for the effectiveness of the PPIs in secondary hemochromatosis. Although the effectiveness in secondary hemochromatosis still has to be proven.

b. Previous exposure of human beings with the test product and/or products with a similar biological mechanism

As outlined in paragraph 1.2.3, patients with hereditary anemia already have been treated with PPIs in non-randomized trials. *We refer to the Amish patients in the Pyruvate Kinase Deficiency Natural History Study.* (6) Despite the presence of risk factors for iron loading, the Amish patients on PPI treatment had lower LIC values than the patients not on PPI treatment.

Esomeprazole was FDA approved in 2001. Initially, less was known about the safety profiles of the newer PPIs, as esomeprazole, compared with the older PPIs, as omeprazole. *We refer to paragraph 6.4.1* for an extensive description of the post marketing studies performed with esomeprazole and the safety profiles in large clinical trials.

Safety data of 2 RCTs evaluating the effect of long term PPI use versus anti-reflux surgery showed that SAEs were reported at a similar frequency in the PPI and surgery groups. The investigators concluded that there were no major safety concerns during 5-12 years of continuous PPI therapy. (57) PPIs are one of the most extensive prescribed drugs worldwide.

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

Human iron metabolism is complex. Iron loading is the consequence of years of excessive iron uptake and/ or transfusion. An ex-vivo model or animal model is not a realistic option.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Current literature underlines the selectivity for the proton/potassium ATP-ase of the parietal cells of the stomach. We did not find studies proving relevant interactions with other tissues.

e. Analysis of potential effect

In this trial we have chosen for the strongest PPI currently available, esomeprazole (*we have outlined our choice in paragraph 6.1*). We will prescribe esomeprazole in a relatively high dose (40 mg twice daily) to provide inhibition of gastric acid secretion 24 hours a day. The safety profile is considered to be favorable (*as outlined in paragraph 6.4.1*). Adverse reactions are listed *in paragraph 6.4.1*, but are rare. A summary of the side effects of esomeprazole is provided in the Summary of Product Characteristics (SmPC chapter 4.8, last update 3/2017; Esomeprazol Sandoz 20/40 mg, www.cbg-meb.nl).

A clinically relevant intoxication with PPIs does not exist. Active monitoring of side effects (low magnesium, vitamin B12, zinc) will be performed during the trial.

f. Pharmacokinetic considerations

Esomeprazole is metabolized by CYP2C19 and CYP3A4. Metabolites do not influence gastric acid secretion (metabolically inactive). Metabolites are excreted in urine (80%) and feces. Less than 1% is excreted non-metabolized. Elimination half-life time is 1.3 hours after repeated ingestion. CYP2C19 poor / intermediate metabolizers will have higher plasma concentrations, although this is not regarded as clinically relevant. Esomeprazole is an inhibitor of CYP2C19. (kennisbank.knmp.nl; informatorium medicamentorum esomeprazol, date 18-08-2017). During the trial we will repeatedly review the medication of the patients for possible relevant interactions. Patients on clopidogrel are not allowed to enter the study due to the interaction with esomeprazole.

We have outlined the most important medicaments with interacting metabolism *in paragraph 12.1.h*.

g. Study population

Our study population consists of patients with several types of hereditary anemia with mild to moderate iron overload, defined as a baseline LIC 3-15 mg Fe/g dw. Patients without or with stable iron chelation therapy will be included. Iron overload in hereditary anemia is regarded as a relatively stable condition. We will not include patients at an Intensive Care. Pregnancy is an exclusion criterion.

h. Interaction with other products

We will perform a medication review to detect possible interactions before start of the trial and repeat the review during the trial. Relevant interactions are higher levels of vitamin K antagonists, digoxin, tacrolimus and phenytoin. For these medications is dose adjustment based on INR or plasma levels possible. Co-treatment with clopidogrel might result in less effective thrombocyte aggregation inhibition. Therefore, we will exclude patients on clopidogrel. Absorption of multiple other drugs might decrease due to higher pH levels in the stomach (cefuroxime, posaconazole, ketoconazole, itraconazole, atazanavir, indinavir, dasatinib, erlotinib, cyanocobalamine). Temporarily cessation of the study drug might be necessary. If interactions are considered to be clinically relevant, exclusion from the study will be considered. (kennisbank.knmp.nl; informatorium medicamentorum esomeprazol, date 18-08-2017) The pharmacist will be informed about the study and the need to monitor for drug interactions with esomeprazole.

We do not consider interactions with medicaments that decrease the levels of esomeprazole as an exclusion criterion, although registration is important for interpretation of the results.

i. Predictability of effect

Serum ferritin will be measured every 3 months according to current guidelines in hereditary anemia. The LIC is the golden standard for iron loading. In thalassemia intermedia, a serum ferritin of 800ng/ml correlates with a LIC of 5 mg Fe/g dw. However, this cutoff point misses 50% of the patients with a LIC \geq 5 mg Fe/g dw. (10,33) . Serum ferritin is an indirect measure, an acute phase protein and has a non-linear correlation with higher degrees of iron loading. Interpretation requires knowledge of the pathophysiology of the disease. In thalassemia intermedia serum ferritin levels are relatively low. In DBA with chronic transfusion serum ferritin is low. Serum ferritin levels remain elevated after an acute crisis in sickle cell disease. (10,33) Serum ferritin may provide us with some information about the degree of iron loading during the year of treatment. The LIC measured by MRI is the golden standard due to the pitfalls in interpretation of serum ferritin.

j. Can effects be managed?

No antidote or antagonist is available, but is also not needed. Esomeprazole intoxication does not exist. Depletion of magnesium, zinc or vitamin B12 can be treated with supplements if necessary. If necessary patients will be seen during an extra visit in the outpatient clinic, or in the emergency department of the hospital when urgent medical care is needed 24 hours 7 days a week.

12.2 Synthesis

Safety of esomeprazole treatment is one of the most important outcomes of the study. We will monitor the side effects / adverse events every visit. Extra visits or contact moments will be planned if necessary in case of adverse events. Special attention should be paid to interactions with other drugs *as outlined in paragraph 12.1.h.*

In our opinion, the potential benefit of esomeprazole outweighs the possible risks of this trial. Currently the only treatment option for iron overload is iron chelation therapy with potential toxic effects and

severe side effects. Esomeprazole is generally considered as a medicament with an excellent safety profile. Therefore we consider the possible risks for the patients as acceptable.

13. Appendices

13.1 Appendix 1

Baseline visit (T0) (Visit 1)	MRI liver (standard care) Venepuncture (standard care) + urine analysis (standard care) + serum gastrin and hepcidin (extra) EQ5D, iMCQ, iPCQ, IRONIC-FFQ questionnaire Medication review
3 months (Visit 2)	Venepuncture (standard care) EQ5D, iMCQ and iPCQ questionnaire Medication review Check compliance Report of side-effects
6 months (Visit 3)	Venepuncture (standard care) + serum gastrin (extra) EQ5D, iMCQ and iPCQ questionnaire Report of side-effects Medication review Check compliance
9 months (Visit 4)	Venepuncture (standard care) EQ5D, iMCQ and iPCQ questionnaire Report of side-effects Medication review Check compliance
12 months (Visit 5)	MRI liver (standard care) Venepuncture (standard care) + urine analysis (standard care) + serum gastrin (extra) EQ5D, iMCQ, iPCQ, IRONIC-FFQ questionnaire Medication review Check compliance Report of side-effects
15 months (Visit 6)	Venepuncture (standard care) EQ5D, iMCQ and iPCQ questionnaire Medication review Check compliance Report of side-effects
18 months (Visit 7)	Venepuncture (standard care) + serum gastrin (extra) EQ5D, iMCQ and iPCQ questionnaire Report of side-effects Medication review Check compliance
21 months (Visit 8)	Venepuncture (standard care) EQ5D, iMCQ and iPCQ questionnaire Report of side-effects Medication review Check compliance
24 months (Visit 9)	MRI liver (standard care) Venepuncture (standard care) + urine analysis (standard care) + serum gastrin (extra) EQ5D, iMCQ, iPCQ, IRONIC-FFQ questionnaire Medication review Check compliance Report of side-effects

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