CLINICAL STUDY PROTOCOL

A prospective, open label, pharmacokinetic study of an oral hydroxyurea solution in children with sickle cell anemia (HUPK)

Sponsor: Nova Laboratories Ltd.

Sponsor ref: INV543

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Date: 03 Jun 2021

Version: FINAL 9.0

CLINICAL STUDY PROTOCOL

PRODUCT:	Oral hydroxyurea 100 mg/mL solution
PROTOCOL NUMBER:	INV543
EudraCT NUMBER:	2017-004568-37
VERSION NUMBER:	FINAL 9.0
DATE:	03 Jun 2021
TITLE:	**A prospective, open label, pharmacokinetic study of an oral hydroxyurea solution in children with sickle cell anemia** **Pharmacokinetics of oral hydroxyurea solution (HUPK) (SHORT TITLE)**
PHASE OF DEVELOPMENT:	Phase I/II
STUDY DESIGN:	An open label, observational, safety and pharmacokinetic study of oral hydroxyurea solution administered to children from 6 months 17.99 years over a 12- to 15-month period.
TEST PRODUCT:	1. Novel oral solution formulation of hydroxyurea – test product.
DURATION OF TREATMENT:	12 to 15 months.

Sponsor
Nova Laboratories Limited, Martin House, Gloucester Crescent, Wigston, Leicester, LE18 4YL, UK

ADMINISTRATIVE STRUCTURE

Sponsor	Nova Laboratories Limited Sponsor's Representative: Dr Hussain Mulla Martin House, Gloucester Crescent, Wigston, Leicester, LE18 4YL, UK Tel: +44 (0) 116 2230100
Sponsors Clinical Expert and Medical Advisor	Dr Russell Ware Division of Hematology, Department of Pediatrics Cincinnati Children's Hospital Medical Center [redacted]
Clinical Project Management	Dr Sarah Edwards Nova Laboratories Limited Martin House, Gloucester Crescent, Wigston, Leicester, LE18 4YL, UK Tel: +44 (0) 116 2230100
Principal Investigator	Dr Angela E Rankine-Mullings, Research Fellow, Sickle Cell Unit Caribbean Institute for Health Research, University of the West Indies, Mona, Kingston, Jamaica [redacted]
Statistics and Data Management	[redacted]
Pharmacokinetic Modelling	
Pharmacokinetic Bioanalysis	
Pharmacovigilance	

SPONSOR SIGNATURE SHEET

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this survey will be conducted according to all stipulations of the protocol including all statements regarding confidentiality and to local law and regulations.

SPONSOR
Signed: [redacted]
Title: Dr Hussain Mulla
Head of Clinical Development & Regulatory
Nova Laboratories Limited, UK

Date: 03 Jun 2021

MEDICAL ADVISOR SIGNATURE SHEET

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

MEDICAL ADVISOR

Signed: [redacted]

Title: Dr Russell Ware
Sponsor's Medical Advisor

Date: 4 June 2021

STATISTICS SIGNATURE SHEET

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

STATISTICIAN

Signed: [redacted]

Print Name: [redacted]
Title: Study Statistician

Date: 2 June 2021

PRINCIPAL INVESTIGATOR SIGNATURE SHEET

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and in accordance with the ethical principles of the Declaration of Helsinki and ICH GCP guidelines, and in compliance with the local regulatory authority requirements. I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss the material with them to ensure they are fully informed regarding the drug and the conduct of the study.

I will only use the informed consent form approved by the Sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Independent Ethics Committee (IEC) responsible for the study.

INVESTIGATOR Name: ANGELA E RANKINE-MULLINGS

Signed: [redacted]

Date: June 9, 2021.

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PROTOCOL SYNOPSIS

TITLE	A prospective, open label, pharmacokinetic study of an oral hydroxyurea solution in children with sickle cell anemia
SHORT TITLE:	Pharmacokinetics of Oral Hydroxyurea Solution (HUPK)
STUDY NUMBER:	INV543
FINAL PROTOCOL DATE:	Version 9.0: Dated 03 Jun 2021
PHASE:	I/II
SPONSOR:	Nova Laboratories Limited Martin House, Gloucester Crescent, Wigston, Leicester, LE18 4YL, United Kingdom (UK)
PRINCIPAL INVESTIGATOR(S):	Dr Angela E Rankine- Mullings
STUDY SITE LOCATIONS:	Jamaica, UK
TEST PRODUCT:	Novel oral solution formulation of hydroxyurea (proposed brand name: Xromi®). The dose administered will be variable and according to the bodyweight of the patient, and the most recent complete blood count and biochemistry (serum creatinine) results.
OBJECTIVES:	Primary objective: • To determine the pharmacokinetics (PK) of oral hydroxyurea solution (a population PK approach will be adopted to characterize the PK in this population) Secondary objectives will be to further evaluate the safety of oral hydroxyurea solution and: • To assess effects on fetal hemoglobin (HbF), hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV), white blood cell count (WBC) and differentials, absolute neutrophil count (ANC), absolute reticulocyte count, platelets, bilirubin, cystatin C and lactate dehydrogenase • To assess effects on pain, transfusions, hospitalizations, and acute chest syndrome (ACS) • To assess the acceptability and palatability of oral hydroxyurea solution • To investigate the effects of hydroxyurea dose escalation on laboratory and clinical parameters Safety Safety endpoints will also include the incidence of adverse events (AEs).
STUDY DESIGN:	An open label, observational, safety and pharmacokinetic study of oral hydroxyurea solution administered to children from 6 months to 17.99 years (i.e. to the day before 18th birthday), with a 12- to 15-month treatment period for each participant. The study treatment duration will be for 6 months at the maximum tolerated dose (MTD), which is usually reached by 6 months after initiation of treatment. For patients in whom time to MTD is longer than 6 months or not achieved at all, the maximum duration of study treatment will be 15 months.
External Safety Data Monitoring Board	An independent Data Safety Monitoring Committee will be formed to alert and/or make recommendations to the Sponsor about any existing or potential safety issues.
SAMPLE SIZE:	Up to 35 patients, both male and female, aged 6 months to 17.99 years (i.e. to the day before 18th birthday) of age will be recruited: A minimum of 6 patients need to be enrolled in the 6 months to 1.99 years age group and a minimum of 6 patients in the 2 to 5.99 years age group (where .99 represents the day before the patient's birthday). If

	required, patient withdrawal from the study before a sufficient number of PK samples are obtained may necessitate the recruitment of additional patients for that age group. If samples from a patient cannot be included in the analysis (invalid) then the study may also need to recruit additional patients for analysis, to ensure that we have up to 35 complete PK data sets.
INCLUSION CRITERIA:	1. Male or female aged from 6 months to 17.99 years of age (i.e. to the day before 18th birthday). 2. Diagnosis of sickle cell anemia (HbSS and HbSβ^0). 3. Parent(s)/legal guardian able and willing to provide written informed consent for the child to take part in the study. 4. Where applicable, the child should assent to undergo blood sampling for PK and biochemistry purposes and to allow physiological measurements to be made.
EXCLUSION CRITERIA:	1. Any clinically significant medical condition or abnormality, which, in the opinion of the investigator, might compromise the safety of the patient or which might interfere with the study. 2. Hydroxyurea use within 6 months before enrolment. 3. Renal insufficiency (known creatinine more than twice the upper limit of normal (ULN) for age and > 1.0 mg/dL [88.4 micromol/L]) 4. Clinical evidence of hepatic compromise with alanine aminotransferase (ALT) more than 3 times the ULN (a temporary swing in ALT will not result in exclusion). 5. Other significant organ system dysfunction based on the site investigator's discretion. 6. Severe active infections: fungal, viral, or bacterial (as confirmed by culture). Examples include tuberculosis, malaria, active hepatitis, osteomyelitis, or any other illness that would preclude the use of hydroxyurea in normal clinical practice. 7. Active chronic leg ulcers. 8. Known allergy to oral hydroxyurea solution or any of the excipients. 9. Positive pregnancy test for females of child-bearing potential (in post-menarcheal females) before initiation of treatment, unless patient is sexually abstinent. Note: true abstinence is considered as being in line with the preferred and usual lifestyle of the patient. Periodic abstinence (such as calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. 10. Inadequate contraception measures in sexually active females (in post-menarcheal females) and males of child-bearing age (see Section 5.13 Pregnancy Prevention). 11. Currently breastfeeding. 12. Participating in another clinical study of an investigational medicinal product (IMP). 13. Known infection with Human Immunodeficiency Virus.
WITHDRAWAL CRITERIA	1. Request of the patient/parent/legal guardian, for any reason. 2. Discretion of the investigator
TREATMENT STRATEGY:	All children with sickle cell anemia initiating oral hydroxyurea therapy will be eligible for recruitment into the study. All children will start on an oral dose of 15 mg/kg once daily. The dose will be escalated by 5 mg/kg/day every 8-12 weeks until the MTD is achieved. Give until hematological toxicity, an ANC of 1-3 × 10^9/L occurs, or a maximum dose of 35 mg/kg/day is acheived. Once the MTD is established, treatment will be maintained for a maximum of 12-15-months. Doses will be calculated based on most current bodyweight (measured at each in-clinic visit). Doses will be rounded where necessary to the nearest 10 mg. Calculations will be made using a dosing calculator or by formula. The PK of oral hydroxyurea solution will be evaluated at Day 1 and ~6 months later. Safety endpoints will mirror those of current standard care where possible, with patients evaluated at 2- to 4-week intervals for the first 2 months, then 4- to 12-week intervals thereafter (up to 12

	weeks maximal between in-clinic visits, 4 weekly safety telephone calls are mandatory between in-clinic visits), with a combination of in-clinic and telephone visits. Palatability and acceptability will be assessed once after 8 weeks.
ENDPOINTS:	**Primary Endpoints** **PK parameters** • Clearance (CL/F) • Volume of distribution (V/F) • Time to maximum concentration (T_{max}) • Maximum plasma concentration (C_{max}) • Area under plasma concentration time curve (AUC) • Half-life ($t_{½}$) **Secondary Endpoints** **Safety** • Incidence of AEs • ANC • WBC count and differentials • Platelets • Mean Corpuscular Hemoglobin • Hematocrit • Bilirubin • Elevation in liver function tests • Hb/anemia • Additional safety laboratory parameters (hematology/biochemistry) • Bacterial infections • Viral infections • Fungal infections • Leg ulcers **Biomarkers** • HbF • MCV • Cystatin C **Clinical Status** • Incidence of acute pain crises* • Number and frequency of blood transfusions • Incidence of ACS • Hospitalizations • Dose escalation i.e. time to MTD • Clinical parameters (symptoms) • Parent/caregiver acceptability/usability by questionnaire Palatability and acceptability: evaluate the taste acceptability of the new oral liquid formulation of hydroxyurea. **Exploratory Endpoints** • Transcranial doppler velocity • Urine parameters (albumin and creatinine for albumin-to-creatinine ratio) * Defined as an acute episode of pain with no known cause for pain other than a vaso-occlusive event; requiring a visit to a medical facility; and requiring treatment with a narcotic (including opiates) and/or non-steroidal anti-inflammatory drugs ; but is NOT classified as an ACS, hepatic sequestration, splenic sequestration, or priapism.
ADVERSE EVENTS	All AEs that occur during the duration of therapy will be recorded and reported per regulatory health authority requirements.
PROCEDURE	**Safety** Patients will attend in-clinic visits at 2 weekly intervals for the first 2 months from initiating treatment. At Investigator discretion the visit window may be extended to 4 weekly intervals if it is deemed safe to do so, with safety phone calls replacing the 2 weekly in-clinic visits.

<table>
<tr><td></td><td>Hydroxyurea dose adjustments will be made following in-clinic visits where applicable.

After the first 2 months (week 8), patients will have safety reviews either at in-clinic appointments or via telephone calls on a 4- to 12-weekly basis for the duration of the study (12-15 months). The frequency of in-clinic visits will be at investigator discretion to ensure patient safety, with up to a maximum of 12 weeks between in-clinic visits or in line with the current standard care.

The safety of the test product will be assessed by AEs, serious AEs and laboratory tests.

Pharmacokinetics
The PK of oral hydroxyurea solution will be evaluated at 2 study visits:
PK1 – Day 1
PK2 – Week 20-36 (~6 months)

On the day of PK sampling, hydroxyurea will be administered in the clinic. A peripheral venous catheter will be used for sampling and blood will be sampled post-dose at any of the following time points:

<table>
<tr><th>Time point (h)
< 2 years old = (Pre-dose) + any 2
≥ 2 years old = (Pre-dose) + any 3-5</th></tr>
<tr><td>*Pre-dose</td></tr>
<tr><td>0.25</td></tr>
<tr><td>0.5</td></tr>
<tr><td>1</td></tr>
<tr><td>1.5</td></tr>
<tr><td>2</td></tr>
<tr><td>3</td></tr>
<tr><td>4</td></tr>
<tr><td>5</td></tr>
<tr><td>6</td></tr>
</table>

*The pre-dose sample is mandatory in all patients. In patients < 2 years old up to 2 post-dose samples will be taken (3 samples in total), and in patients ≥ 2 years old, 3-5 additional samples will be taken (4-6 samples in total including the pre-dose sample).

A single sample for PK analysis will also be taken at interim in-clinic visits, whenever feasible (dosing will not be administered in clinic for these).

Sampling time points may be amended; however, target total blood volume and target number of samples will remain unchanged.

Palatability and Acceptability
The palatability and acceptability of the product will be assessed once after the patient has been receiving the IMP for 8 weeks, or at early withdrawal(if possible). A questionnaire-based survey will be used, probing the patient's/parent's/caregiver's views on overall acceptability (such as taste, smell, ease, and willingness to take oral hydroxyurea solution on a daily basis), and utilizing a 5-point facial hedonic scale as well as open/closed questions.</td></tr>
<tr><td>PATIENT DURATION:</td><td>Patients will be administered once daily doses of oral hydroxyurea solution for 12-15 months. The individual patient's participation will therefore be up to 15 months (i.e. 6 months at MTD which is usually reached at 6 months, but a maximum duration of participation of 15 months regardless). Irrespective of the date of exit all patients will receive the end of study visit (see week 60 visit assessments).</td></tr>
<tr><td>STATISTICAL METHODS</td><td>Safety Data Analysis:</td></tr>
</table>

	Safety analyses will include summaries of physical examinations, clinical laboratory results (where possible in-clinic), and AE reports. Adverse events will be Medical Dictionary for Regulatory Activities coded and presented by relatedness, severity, and body system. Serious AEs will be listed in detail, describing the timing of each event, the nature, severity, seriousness, and relatedness of the event. Additional analyses may be conducted if the number of events permits. **Pharmacokinetic Data Analysis:** The data from all patients taking part in the study will be pooled and analyzed simultaneously to avoid excessive blood sampling. Population PK parameters will be estimated by using a non-linear, mixed effects modeling approach. In the population approach, all data from different individuals will be fitted simultaneously using a non-linear, mixed effects modeling approach and post hoc individual kinetic parameters can be calculated with as few as 1 sample per individual. An appropriate software package such as NONMEM will be used to conduct the analysis. One interim analysis is currently planned; when all patients complete approximately 9 months of the study (i.e. when the last patient reaches their week 40 visit). The interim analyses will include all study endpoints described in the Statistical Analysis Plan.

ABBREVIATIONS

%HbF	Percentage of HbF
ACS	Acute chest syndrome
AE	Adverse event
ALT	Alanine aminotranferase
ANC	Absolute neutrophil count
ARC	Absolute reticulocyte count
AUC	Area under the plasma concentration time curve
β^S	Sickle mutation in beta globin
CBC	Complete blood count
C_{max}	Maximum plasma concentration
CL/F	Clearance
CRF	Case report form
%CV	Coefficient of variation
DSMC	Data Safety Monitoring Committee
eCRF	Electronic case report form
EDC	Electronic Data Capture
EMA	European Medicines Agency
EU	European Union
FBC	Full blood count
FDA	Food and Drug Administration
Hb	Hemoglobin
HbS	Sickle hemoglobin
HbSS	Homozygous HbS
HbSβ^0	HbS/β^0-thalassemia (compound heterozygous)
HbF	Fetal hemoglobin
HIV	Human immunodeficiency virus
IEC	Independent ethics committee
ICH-GCP	International Council for Harmonisation-Good Clinical Practice
IMP	Investigational medicinal product
LDH	Lactate dehydrogenase
LFT	Liver function test
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
MOHW	Ministry of Health and Wellness
MTD	Maximum tolerated dose
NSAID	Non-steroidal anti-inflammatory drugs
PICU	Pediatric intensive care unit
PK	Pharmacokinetics
PK1	Pharmacokinetic Day 1
PK2	Pharmacokinetic Day 2
RCT	Randomized controlled trial
RR	Ribonucleotide reductase
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCA	Sickle cell anemia
SCD	Sickle cell disease
SD	Standard deviation
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-emergent adverse event

$t_{½}$	Half-life
T_{max}	Time to maximum concentration
TCD	Transcranial doppler
UK	United Kingdom
ULN	Upper limit of normal
US	United States
V/F	Volume of distribution
VOC	Vaso-occlusive crisis
WBC	White blood cell
WHO	World Health Organization

1. INTRODUCTION

1.1. Background: Sickle Cell Anemia

Sickle cell disease (SCD) is a group of closely related inherited anemias characterized by the predominance of sickle hemoglobin (HbS) within circulating erythrocytes, which causes erythrocyte shape changes with subsequent hemolytic anemia and vascular occlusion. In most cases, the patient inherits the abnormal HbS allele from both parents and has homozygous HbS (HbSS), but compound heterozygous conditions such as HbS/β0-thalassemia and Hemoglobin SC also contribute to the clinical spectrum of sickle cell disease. HbSS and HbSβ0 thalassemia are clinically very similar and therefore are commonly referred to as sickle cell anemia (SCA). The molecular basis for erythrocyte sickling is a single nucleotide substitution in codon 6 of the β-globin gene leading to an amino acid substitution (Glu→Val) that is responsible for the (βS), which forms the abnormal HbS tetramer. Upon deoxygenation, HbS undergoes rapid intracellular polymerization with subsequent cellular dehydration, altering the shape of the erythrocyte membrane into a sickled form, and adversely affecting the usual physiologic deformability of erythrocytes that allows them to traverse the circulation freely. These rigid and deformed sickle erythrocytes have a shortened lifespan and undergo both intravascular and extravascular hemolysis, vaso-occlusion and abnormal erythrocyte-endothelial interactions, leading to acute tissue hypoxia and chronic organ damage. Clinical manifestations of SCA include acute vaso-occlusive events, chronic hemolytic anemia, and organ dysfunction due to repeated sickling episodes [1].

SCA is among the world's most common inherited hemolytic anemias. The prevalence of SCA is highest in sub-Saharan Africa. Lack of diagnostic facilities means that precise data are not available, however a recent estimate suggests that more than 230,000 affected children are born in this region every year (0.74% of the births in sub-Saharan Africa), which is about 80% of the global total. By comparison, the yearly estimate of affected births in Europe is 1300 and in North America is 2600 [2].

Despite the well-described genetics and pathophysiology of SCA, clinical care for affected individuals has been mostly supportive, typically including red blood cell transfusions, intravenous hydration, analgesics and antibiotics. Increased fetal hemoglobin (HbF) has been associated with a less severe phenotype, which has spurred interest in the identification of pharmacologic agents capable of inducing HbF expression. Hydroxyurea (International approved name; hydroxyurea, United States [US] approved name) was identified as a potent HbF inducer and was subsequently found to be both a feasible and effective treatment option for SCA. Over the last 30 years, hydroxyurea has become an established disease modifying agent in patients with SCA [1].

Hydroxyurea is a cytotoxic, antimetabolite, and antineoplastic agent used for several decades to treat a variety of medical disorders, most notably myeloproliferative neoplasms, chronic myelogenous leukemia, and human immunodeficiency virus (HIV). The first clinical application of hydroxyurea for patients with SCA was reported in 1984, when Platt and colleagues demonstrated a rapid and dramatic increase in HbF-containing reticulocytes without significant bone marrow toxicity [3]. Over the last 30 years, a series of 'proof of principle' experiments, phase 1-3 studies in adults and children with SCA treated with hydroxyurea at maximum tolerated dose (MTD) demonstrated significant dose-dependent increases in hemoglobin (Hb) and HbF, along with concurrent reduction in total white blood cells (WBC), neutrophils, and reticulocytes, and reduction in hemolysis. Hydroxyurea has become established as a disease modifying therapy [1]. Hydroxyurea was approved for the treatment of adults with SCA by the Food and Drug Administration (FDA) in 1997 and for both children and adults by the European Medicines Agency (EMA) in 2006.

In SCA, the primary effect of hydroxyurea is to increase HbF levels. HbF inhibits intracellular HbS polymerization and prevents the sickling process within erythrocytes. However, the full benefits of hydroxyurea therapy in SCA are multifactorial, extending beyond HbF induction. The principal and most well understood mechanism of action of hydroxyurea in vivo is the reversible inhibition

of ribonucleotide reductase (RR), a critical enzyme that converts ribonucleosides into deoxyribonucleosides, which are required for the synthesis and repair of deoxyribonucleic acid. Potent inhibition of RR leads to decreased intracellular pools of deoxyribonucleotide triphosphates and impedes progression of cellular division through S phase. Temporary arrest of hematopoiesis through once-daily hydroxyurea results in altered erythroid kinetics upon recovery; such 'stress erythropoiesis' features higher HbF through recruitment of early erythroid progenitors that maintain their HbF-producing capacity. Erythrocytes with more HbF are larger (higher mean corpuscular volume [MCV]) and more deformable (better rheology). Myelopoiesis is also affected, leading to dose-dependent and reversible neutropenia. Other benefits include reduced expression of surface molecules that adhere to the endothelium, decreased degree of chronic inflammation as seen by decrease in WBC and platelet counts, increased levels of nitric oxide and cyclic nucleotides that may facilitate vascular dilatation, and also induced HbF, and even direct salutary effects on the vascular endothelium [4].

1.2. Rationale: The Need for a Liquid Formulation

The optimum mg/kg dosing for a child of any given age results in a wide range of doses, with intermediate doses only achievable by the available tablets being halved or quartered. In the US only capsules are available, requiring in most cases doses to vary on a daily basis or be rounded up or down. Intermediate doses can only be delivered accurately with an oral liquid formulation. Moreover, young children especially find taking capsules and tablets very difficult.

To facilitate dose individualization and improve palatability and acceptability, children are often administered unlicensed (compounded) liquid formulations of hydroxyurea prepared either in hospital pharmacies or by commercial compounders. In response to requests by clinicians and pharmacists, Nova Laboratories have been supplying a compounded hydroxyurea oral solution to the United Kingdom (UK) market for almost 10 years. Although these unlicensed oral solutions are currently meeting a pediatric need, it potentially exposes children to medication errors and the consequential higher risk of adverse reactions or inadequate efficacy.

Although compounded oral solutions are widely used in the US and Europe, clinical data assessing systemic exposure of the oral solution, especially in infants, are limited. Nova Laboratories is therefore proposing to undertake a clinical study with their novel oral solution formulation to assess its in vivo performance. Since July 2019, the novel liquid formulation (Xromi®) has been an approved product in the UK and European Union (EU), indicated for the treatment of sickle cell disease in children over the age of 2 years.

This study aims to investigate the safety and pharmacokinetics (PK) of the new oral hydroxyurea 100 mg/mL solution formulation in children with SCA, and to extend the current licensed indication in the UK and the EU to children aged 6 months to 2 years old. The study is also required for the US FDA as a pivotal study for marketing approval in the US.

The study will be conducted in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH-GCP) and the applicable regulatory health authority requirement(s).

1.3. Justification for the Route of Administration, Dosage and Treatment Period

1.3.1. Route

Oral hydroxyurea administered to adults and children for treatment of SCA, has been shown through several randomized controlled trials (RCTs) and numerous observational studies, to improve clinical outcomes such as decreased incidence of acute pain crises and acute chest syndrome (ACS), as well as reducing the requirement for blood transfusions and hospitalizations.

Licensed oral capsule and tablet forms of hydroxyurea are available; however, many young children struggle to take solid oral dosage forms. A child-appropriate, approved oral solution formulation will permit flexible dosing, ease of use, and reduce the risk of medication errors.

All children will be dosed according to the hydroxyurea dosing guidelines recommended by the US National Heart, Lung and Blood Institute Evidence Based Guidelines[5], which includes dose titration to MTD, and overall management will be according to local clinical care guidelines.

1.3.2. Justification for Dose and Dose Escalation

Charache et al, (1992)[6] showed that the effects of hydroxyurea are dose-dependent. They studied the hydroxyurea dose-response curve in 49 patients and reported an increase in the percentage of HbF (%HbF) and fetal cells with increasing doses, approaching an asymptote at around 35 mg/kg/day. Subsequently, many clinical trials have demonstrated that the laboratory and clinical benefits of hydroxyurea are optimized when escalated to MTD, which is defined as the highest stable and tolerated dose (mg/kg/day) that achieves a target range of mild marrow suppression, most commonly determined by the absolute neutrophil count (ANC), but also by the absolute reticulocyte count (ARC).

At MTD, almost all patients with SCA have a significant increase in the total Hb concentration and the MCV, but the laboratory benefits of hydroxyurea are most often defined by the amount of HbF achieved. All children with good adherence will respond to hydroxyurea, but there is substantial interpatient variability in both the MTD itself and the %HbF levels achieved. For example, some patients tolerate an MTD as high as 35 mg/kg/day before reaching appropriate myelosuppression, while others can only tolerate a dose of 15-20 mg/kg/day. Similarly, some patients achieve HbF levels > 40%, while others are never able to reach 20%.

In a recent report from the Hydroxyurea Study of Long-Term Effects (HUSTLE), a prospective observational study (NCT00305175) with a primary goal of describing the long-term effects of hydroxyurea therapy in children with SCA, higher % HbF levels conferred greater protection against hospitalization for vaso-occlusive crisis (VOC) or ACS. In 230 children (median age 7.4 years, range 6 months to 17.9 years), providing 610 patient-years of follow up, the mean attained HbF% at MTD (median dose of 28.0 mg/kg/day, range 13.0 to 35.0 mg/kg/day) was >20% for up to 4 years of follow-up. When %HbF values were < 20%, children had twice the odds of hospitalization for any reason (P<0.0001), including VOC (P<0.01) and ACS (P<0.01), and more than four times the odds of admission for fever (P<0.001). Thirty-day readmission rates were not affected by %HbF. Neutropenia (ANC <1000 × 10^9/L) was rare (2.3% of all laboratory monitoring), transient, and benign. Therefore, attaining HbF > 20% was associated with fewer hospitalizations without significant toxicity. These data support the use of hydroxyurea in children and suggest that the preferred dosing strategy is one that targets a HbF endpoint > 20% [7].

Presently, dosing in children and adults is individualised to the MTD (to a max of 35 mg/kg/day), through careful incremental dose increases, cautious monitoring of haematology and the HbF response. The starting dose of hydroxyurea in adults and children with SCD is typically 15-20 mg/kg, and doses are escalated (titrated) to a MTD of 35 mg/kg [8,9,10,5]. Using this dose escalation strategy, MTD is reached in approximately 6-12 months. This individualized dosing approach has been reported in the pivotal RCTs and observations studies in adults and children: Multicenter Study of Hydroxyurea (pivotal RCT of hydroxyurea in adults with SCA)[11], (SWiTCH)[12], (TWiTCH)[13]. Taken together, escalation of the hydroxyurea dose to MTD is warranted for the optimal treatment response, aiming for mild marrow suppression of both neutrophils and reticulocytes as outlined above.

1.3.3. Dosage and Dose Escalation

- **Starting dosage for all patients: 15 mg/kg/day**

- Monitor complete blood count (CBC) with WBC differential and ARC about every 4 weeks when adjusting dosage
- Aim for a target absolute neutrophil count 1-3 × 10^9/L; however:
  - Maintain platelet count > 80 × 10^9/L
- If hematological toxicity occurs as defined by:
  - Neutropenia (ANC < 1.0 × 10^9/L). Additionally, if ANC < 1.5 × 10^9/L repeat CBC in a week, if there are concerns about toxicity, but maintain the same dose.
  - Thrombocytopenia (platelets < 80 × 10^9/L)
  - ARC < 80 × 10^9/L, unless Hb concentration ≥ 9.0 g/dl
  - A 20% decrease in Hb concentration, from baseline or Hb < 4.5 g/dL

  Temporarily stop hydroxyurea dosing and:
  - Monitor CBC with WBC differential weekly until toxicity resolves
  - If hematological toxicity resolves in 1 week, restart hydroxyurea at the same dose. If hematological toxicity persists for > 1 week or occurs twice in a 3-month period, the hydroxyurea dose should be withheld until recovery and then reduced by about 2.5 mg/kg/day, or at a dose of 2.5-5.0 mg/kg/day lower than the dose given before onset of cytopenias

If dose escalation is warranted based on clinical and laboratory findings, typically failure to achieve mild neutrophil and reticulocyte suppression, guidance recommends to proceed as follows:

- Increase by 5 mg/kg/day increments every 8-12 weeks (at in-clinic visits)
- Give until mild myelosuppression (ANC 1-3 × 10^9/L) is achieved. However, if ARC remains high (> 200 × 10^9 /L), dose escalation may continue if clinically indicated, to achieve the optimal dosing
- **Dose must not exceed a maximum dose of 35 mg/kg/day**
- Once a stable dose is established, laboratory safety monitoring should include CBC with WBC differential, ARC, platelet count, and blood chemistry every 4 weeks

Doses will be calculated based on most current bodyweight (measured at each in-clinic visit). Doses will be rounded where necessary to the nearest 10 mg. Calculations will be made using a dosing calculator or by formula. Dose changes will be carried out at investigator discretion following safety review and blood work at an in-clinic visit, and can be communicated to the patient via telephone (if required) following the visit. As the period between in-clinic safety review can be up to 12 weeks in investigator determined 'stable' patients, trends towards toxicity identified by the investigator can be treated proactively at the discretion of the investigator to ensure patient safety until the patients next in-clinic safety review (this may include reducing dose by 2.5-5.0 mg/kg/day, or temporary halts, as applicable).

Patients will be reminded (as per standard practice) that the effectiveness of hydroxyurea depends on their adherence to daily dosing; they should be counselled not to double up doses if a dose is missed. Hydroxyurea therapy will be continued during hospitalizations or illness unless there is hematological toxicity.

1.3.4. Treatment Period

All patients with confirmed SCA who, in the opinion of the investigator, would benefit from hydroxyurea therapy will be eligible for recruitment into the study.

Hydroxyurea will be prescribed to patients based on current guidelines[14], (see Section 1.3.2 Justification for Dose and Dose Escalation and Section 1.3.3 Dosage and Dose Escalation). Dose escalation for patients being treated with hydroxyurea will be for a period of 3 to 6 months, after which the patients will usually remain on a maintenance dose (the MTD). The duration of the study

for each patient will be for approximately 12 to 15 months (end of study is defined as 6 months after achieving MTD), after which continued treatment with hydroxyurea will be outside of this study protocol and will be managed by the clinical team according to local practice.

The PK of oral hydroxyurea 100 mg/mL solution (test product) will be evaluated at treatment initiation (Day 1 [PK2]) and at approximately 6 months (PK2) following treatment initiation. Safety endpoints will be evaluated at 2- to 4-week intervals for the first 2 months via telephone or in-clinic visits, then at 4- to 12-week intervals thereafter (up to 12 weeks maximal between in-clinic visits, 4-weekly safety telephone calls are mandatory between in-clinic visits). Palatability and acceptability of the oral solution will be assessed once after 8 weeks.

In-clinic visits will be at investigator discretion to ensure patient safety. Where an in-clinic visit is not possible, a safety phone call must be held. All measures planned for the in-clinic visit will be carried out at the next in-clinic visit. The in-clinic visits can be every 4-12 weeks, with 12 weeks as the maximum time permitted between in-clinic visits for stable patients. This is in line with current standard care practices, with safety phone calls every 4 weeks in between.

All sites are expected to ensure that they have provided patients with sufficient stock of investigational medicinal product (IMP) to ensure they can last up to a maximum of 12 weeks between in-clinic study visits, if required. This extended up to 12-week in-clinic visit window will not alter the patient's total duration on the IMP which remains a maximal 15 months (or 6 months after achieving MTD). Where necessary, at investigator or Sponsor discretion, sites may arrange for a courier to provide IMP to patients at their home address (see Section 4.5 IMP Deliveries to Patient's Home Address).

1.4. Benefits and Risks

The importance and potential benefits of this work to patients are clear, as presently in the US, hydroxyurea approved for SCA is only available as 200 mg, 300 mg, 400 mg and 500 mg capsule, and 100 mg and 1000 mg tablet formulations, respectively. However, since July 2019, a novel liquid formulation developed by Nova Laboratories (Xromi®) has been an approved product in the UK and the EU, indicated for the treatment of children over the age of 2 years only. The primary reason for conducting this study is because it has been requested by the FDA (advice received in a Type C meeting) to support a New Drug Application submission for the indication of treatment of SCD in infants (< 2 years), children and adults. It is hoped that the outcomes of this study will also support the Marketing Authorisation Application for Xromi® in the EU and UK.

US National Institute for Health, National Heart, Lung and Blood Institute guidelines (2014)[5] and evidence from the placebo-controlled infant hydroxyurea (BABY HUG) trial and recent literature [15] show that there is a strong recommendation for offering hydroxyurea to very young children with SCA within their first year, regardless of clinical severity. With the aim of reducing the severity of symptoms and on-set of serious clinical complications, with evidence of increased levels of HbF in children started on hydroxyurea before the age of 12 months.

Solid oral dosage forms such as tablets and capsules can offer advantages (over liquid formulations) of greater stability, improved palatability, and portability. However, many children under the age of 6 years have difficulty swallowing tablets or capsules. In a retrospective survey[16], it was found that children taking antiretroviral liquids preparations change to solid dose forms at approximately 7 years of age. Hence, to optimize dosing, there is a clear need for an approved child-appropriate, oral solution formulation of hydroxyurea, that can be used before the age of 12 months.

Any risks associated with taking blood samples from the peripheral venous catheter for safety assessment or PK analysis are minimized by following the established aseptic guidelines. The number of PK blood samples have been minimized by utilizing a sparse sampling population PK modelling approach.

For all patients and staff, risk assessments will be performed relating to patients in-clinic attendance by the investigator/hospital site in accordance with local policies. Induction into the study and dose escalation should adhere to the protocol wherever possible. Investigator discretion will apply when it comes to considerations for patient safety. All patient treatment should be considered in line with National and local guidelines and hospital policies [17, 18, 19, 20, 21].

2. OBJECTIVES AND PURPOSE

The aim of this study is to investigate the PK, safety, palatability and acceptability of oral hydroxyurea 100 mg/mL solution in patients with SCA aged from 6 months to 17.99 years (i.e. to the day before 18th birthday) who, in the clinical opinion of the investigator, would benefit from hydroxyurea treatment.

2.1. Primary Objective

Primary objective:

- To determine the PK of oral hydroxyurea solution (a population PK approach will be adopted to characterize the PK in this population)

2.2. Secondary Objectives

Secondary objectives will be to further evaluate the safety of oral hydroxyurea solution and:

- To assess effects on HbF, Hb, hematocrit, MCV, WBC and differentials, ANC, ARC, platelets, bilirubin, cystatin C and lactate dehydrogenase (LDH)
- To assess effects on pain, transfusions, hospitalizations, and ACS
- To assess the acceptability and palatability of oral hydroxyurea solution
- To investigate the effects of hydroxyurea dose escalation on laboratory and clinical parameters

2.3. Safety

Safety endpoints will also include the incidence of adverse events (AEs).

3. STUDY DESIGN

3.1. Description of Study Design

This is an open label, observational, safety and PK study of oral hydroxyurea solution administered to patients aged from 6 months to 17.99 years (i.e. to the day before 18th birthday). Figure 1 Study Schematic for an Individual summarizes transition through the study for an individual.

When an appointment is made for the patient to attend the hospital clinic for review or treatment of SCA, the study investigator will assess whether or not the patient could expect to benefit from treatment with hydroxyurea. If the treating physician (the study investigator) feels that the patient would benefit from treatment with hydroxyurea then the parent(s)/legal guardian of the child will be approached to discuss the study and the possibility of the child's entry into the study; information regarding the study may be sent to parents and patients prior to them attending. The parent(s)/legal guardian's consent to the patient taking part in the study will be sought. Following parental consent, the patient will be approached, and the study procedures explained to them to receive their verbal and/or written assent to participate in the study, if applicable (usually where the child is over 12 years of age). Parents and patients may still refuse to participate in the study at any stage prior to or during their treatment. Parents and patients will be given as much time as they need to consider participation in the study. Patients and parents may choose when they provide consent, and they will be given time to share information about the research with their family or their general practitioner. Investigators will ensure that families have at least 24 hours to consider involvement in the study. If the parent(s)/legal guardian decide that they would not like their child to participate in the study then this will in no way affect the onward care of the patient.

When the patient attends the research facility for initiation of hydroxyurea they will start with PK assessments on Day 1. In total, patients will attend the clinic on 2 "PK days", these will be on Day 1 (PK1) and ~6 months (or between weeks 20-36) later (PK2) . Blood samples for PK analysis will be taken over the course of up to 6 hours, after which the patients will be able to leave the clinic. At subsequent clinic visits, parents and patients will be asked if they are willing to provide an additional single sample for PK analysis, where feasible. Patients would not need to be dosed in clinic but would continue with their normal dosing schedule on these visits and a sample will be taken with other bloods to reduce burden.

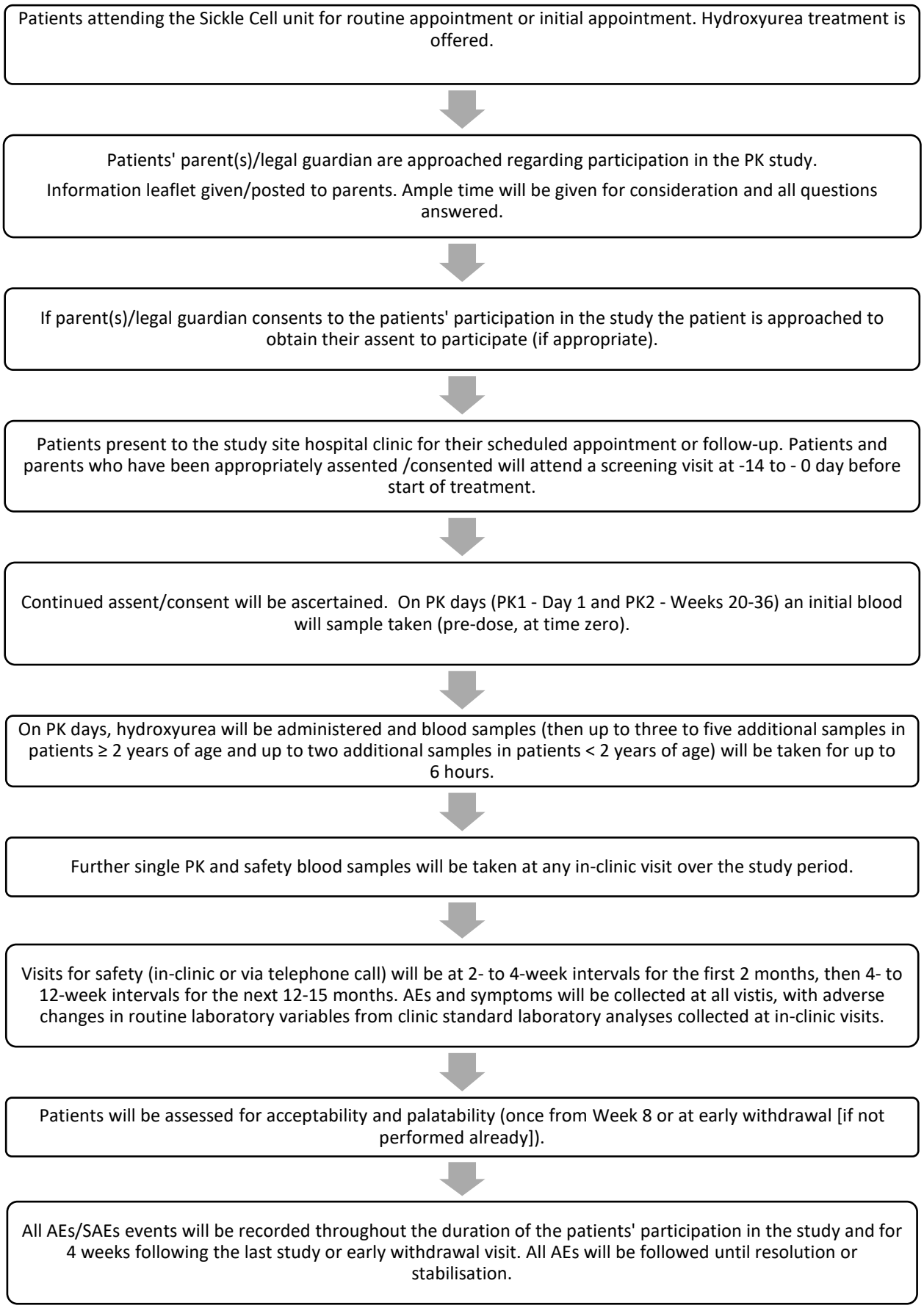
Patients will remain in the study for 12-15 months (maximum) (or 6 months after achieving MTD), whilst the dose of their hydroxyurea is escalated, and then for routine follow-up. Patients will be seen and assessed in the clinic or via telephone calls every 2 to 4 weeks for the first 2 months, and every 4 to 12 weeks for the remaining time in the study (weeks 12-60). Patients must attend either a telephone call or an in-clinic visit every 4 weeks, with an in-clinic visit required at least every 12 weeks. This may be sooner at investigator discretion for patient safety. Patients will receive a final study visit 6 months after achieving MTD/or up to 15 months since treatment initiation, whichever is sooner, and this must be completed in-clinic. Patients will be followed up via telephone call 4 weeks after study completion for a safety follow-up (week 64).

Assessments at each of the follow-up visits will be performed according to the study procedures (Section 5 STUDY PROCEDURES).

3.2. Study Schematic and Schedule

A study schematic for individual patients is presented in Figure 1 Study Schematic for an Individual and a full schedule is presented in Table 1 Schedule of Study Assessments.

Figure 1 Study Schematic for an Individual



AE = adverse event; SAE = serious adverse event.

Table 1 Schedule of Study Assessments

Visit Due	Screening Days -14 to 0 IC	PK1 Day 1 IC	Weeks 2, 4, 6, 8 IC/TC	Weeks 12, 16 IC/TC	PK2 Weeks 20, 24, 28, 32, 36 IC/TC	Weeks 40, 44, 48, 52, 56 IC/TC	Final visit Week 60 20, IC	Follow-up visit Week 64 /4 weeks post-WD
Visit Window[V] (Days)	**-14 to 0**	**± 0**	**± 3 days**	**± 7 days**	**± 14 days**	**± 14 days**	**± 14 days**	**± 14 days**
Visit Name	**0**	**PK1**	**Interim Week**	**Interim Month**	**PK2**	**Interim Month**	**Months 12-15 or early WD**	**Safety follow-up[1] IC/TC**
Informed consent	X							
Demographics[2]	X							
Anthropometric data[3, IC]	X		X	X	X	X	X	
Medical history[4]	X							
Prior SCA and other medication[5]	X	X						
Symptoms review[6]	X		X	X	X	X	X	
Leg ulcer assessment[7, IC]	X		X	X	X[IC]		X	
Physical examination[8, IC]	X		i	i	X[IC]	i	X	
TCD[9, IC]	X					X (once in-clinic weeks 40-56)		
Vital signs[10, IC]	X		i	i	X[IC]	i	X	
FBC[11*, IC]	X		X	X	X	X	X	
HbF[12, IC]	X		X (Week 4)	X (Week 12)	X[IC]		X	
Clinical chemistry[13*, IC]	X		X	X	X	X	X	
Cystatin C[14, IC]		X			X[IC]		X	
Urinalysis[15*, IC]	X				X[IC]		X	
Pregnancy test, if applicable (within 7 days of IMP)[16, IC]	X	X+						
IMP initiation IC		X						
PK profile[17, IC]		X	f	f	X[IC]/ f	f	f	
Palatability and acceptability assessment[18, IC]			X (Week 8 or next IC)				X (WD)	
AEs and concomitant medication IC/TC		X	X	X	X	X	X	X[1]
Dose escalation review[19, IC]			X	X	X	X		

AE = adverse event; FBC = full blood count; HbF = fetal hemoglobin; IC = in-clinic; IMP = investigational medicinal product; PK = pharmacokinetic; PK1 = first PK visit; PK2 = second PK visit; SCA = sickle cell anemia; TC = telephone call; TCD = transcranial doppler; WD = withdrawal.

IC/TC Visits may be conducted via in-clinic visit or telephone call for safety assessment at investigator discretion, to be indicated on the case report form and/or electronic capture datacase. Those measures unable to be conducted via telephone call will be carried out at the next in-clinic visit where applicable. At treatment initiation it is recommended that as many in-clinic visits as

possible are conducted at 2 weekly intervals until week 8 to ensure patient safety, this will be at investigator discretion. From week 8 onwards, in-clinic visits are required up to a maximum of 12 weeks apart, telephone calls should be carried out of in-clinic visits at 4 weekly intervals, where applicable.
[IC] Indicates the visit or individual measure must be conducted in-clinic.
[V] Visit windows may be flexible as indicated; however, overall schedule will be adhered to in order that the week 20-36 PK2 falls within this planned 16 week window (i.e. visits must be adjusted to follow original schedule, visit windows are available in order to accommodate unavoidable absence such as planned holidays).
X Mandatory for study protocol.
F Where a single PK sample is feasible.
^ PK2 specific visit measures (e.g. PK sampling) can be carried out once at any visit between week 20-36 (±14 days)
X[IC] can be performed once at any in-clinic visit attended during the visit windows.
i If indicated as part of clinical care.
1. For all patients the safety follow-up visit should occur 4 weeks after final visit/week 60. This may be done by telephone (if patients withdraw then the telephone follow up should occur 4 weeks following withdrawal from the study).
2. Gender, race, date of birth, and age.
3. Length (for patients < 2 years) or height (for patients ≥ 2 years) (cm) and weight (kg).
4. Medical history including other co-morbidities or diagnoses. Other acute complications in last 12 months including: pain crises, stroke, dactylitis, ACS, aplastic crisis, febrile episode requiring admission, osteomyelitis, splenic sequestration, hepatic sequestration, and priapism. Number of hospital admissions in last 12 months and number of admissions for pain crises and number of admissions to pediatric intensive care unit. Any surgical history in the last 12 months.
5. SCA medication in the last 6 months including: penicillin, folic acid, other medication (if any) with assessment of compliance (if applicable). Any vaccinations provided: Pneumovax, Hepatitis B, annual influenza vaccination. For non-SCA medication, recording will be limited to medication ongoing at time of screening. Non-SCA related medications used prior to study screening should be included in medical history.
6. Symptoms review including: painful crises, enuresis, snoring, daytime somnolence, chronic pain, shortness of breath on exertion, and stuttering priapism.
7. Patients will be examined for leg ulcers (presence/absence).
8. A physical examination will include the following: ear/nose/throat (tonsils), dermatological, cardiovascular, respiratory, gastrointestinal, lymph nodes, musculoskeletal, hepatomegaly and splenomegaly (at screening, PK2 [week 20-36], final visit/week 60 and early WD [unless otherwise indicated]), and for ophthalmological and central nervous system (at screening, final visit/week 60 and early WD [unless otherwise indicated]). Other evaluations may be performed as deemed necessary by the investigator. This will be commented upon in the clinical study report, if applicable.
9. TCD is mandated per protocol at screening (results can be used from a TCD which has been performed within 3 months of screening), and in-clinic once anywhere between week 40-56. If TCD is undertaken at other visits as part of routine care, results will be collected, if appropriate.
10. Supine systolic and diastolic blood pressure, pulse and body temperature and respiratory rate at screening (in older patients this will be following 5 mins rest in supine position). Supine blood pressure, pulse, body temperature and respiratory rate will be recorded before administration of IMP; investigators may repeat measurements as clinically indicated.
11. If a hemoglobin electrophoresis result is not already available and it is required to confirm SCD diagnosis, it is to be arranged locally as part of the patient's standard of care. At screening, weeks 2, 4, 6, 8 and then monthly thereafter (weeks 12-56) and at completion (final visit/week 60 or early WD [if possible]) samples will be taken for full blood count including: Hb, red blood cell count, hematocrit, platelets, ANC, ARC, WBC count and differentials, lymphocytes, monocytes, eosinophils, basophils, MCH, and MCV. However, the investigator may perform tests as clinically indicated. Blood sample will be taken prior to dosing on PK days.
12. HbF will be measured at screening, weeks 4, 12, at any in-clinic visit between 20-36, and at completion (final visit/week 60 or upon early WD). Blood sample will be taken prior to dosing on PK days.
13. Clinical chemistry tests will be performed at screening, weeks 2, 4, 6, 8, and then monthly thereafter (weeks 12-56) and at completion (final visit/week 60 or early WD [if possible]) samples will be taken for clinical chemistry (Serum separator tubes]): total bilirubin, alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, LDH, vitamin D, urea and creatinine. However, the investigator may perform tests as clinically indicated. Blood sample will be taken prior to dosing on PK days.
14. Cystatin C will be measured at Day 1, PK2 (any in-clinic visit between weeks 20-36) and at final vist/week 60 (ort early WD [if possible]). Blood sample for Cystatin C will be taken prior to dosing on PK days.
15. Urinalysis will be performed at screening, PK2 (any IC visit between weeks 20-36) and final visit/week 60 (or early WD [if possible]) samples will be taken for urinalysis (dipstick): protein, bilirubin, blood, and urobilinogen. Spot urine for urinary ACR ratio will also be done at the same timepoints (in-clinic). Urine sample will be taken prior to dosing on PK days. The investigator may perform tests as clinically indicated, particularly if dose escalation is indicated.
16. Urine pregnancy test to be performed no more than 7 days before the first administration of hydroxyurea in all post-menarcheal females. [+] Sample can be done at screening visit, or at PK1 if this is > 7days after initial screening sample.
17. A full PK profile. The pre-dose sample is mandatory in all patients. In patients < 2 years old, up to 2 post-dose samples will be taken (3 samples in total), and in patients ≥ 2 years old, 3-5 additional samples will be taken (4-6 samples in total including the pre-dose sample). Conducted during an in-clinic visit.
For PK2, this only needs to be performed once at an in-clinic visit between weeks 20-36.
Further single samples will be obtained at regular in-clinic visits (weeks 2-20 and 28-60/final visit) where feasible. Where a single PK sample is taken at the interim in-clinic visit, the time of the previous hydroxyurea dose must be noted.
18. Palatability and acceptability assessment will be made once at an in-clinic visit from week 8 (or early WD [if possible] and not completed previously). This will be done using a face-to-face survey.

19. Dose escalation review: See Section 1.3.3 Dosage and Dose Escalation.
20. The final study visit is to be conducted 6 months after achieving MTD or up to a maximal 15 months from treatment initiation (if MTD not achieved) whichever is sooner, this may therefore occur from 12-15 months from IMP initiation. Final visits require an in-clinic visit and cannot be conducted via telephone.
***Additional unscheduled laboratory tests (blood samples for clinical chemistry, full blood count and urinalysis) may be carried out at the discretion of the investigator in line with standard of care or for safety purposes at any point in the patients care pathway or visit schedule.**

3.3. Study Endpoints

3.3.1. Primary

PK parameters:

- Clearance (CL/F)
- Volume of distribution (V/F)
- Time to maximum concentration (T_{max})
- Maximum plasma concentration (C_{max})
- Area under plasma concentration time curve (AUC)
- Half-life ($t_{½}$)

Primary efficacy endpoints will be considered the critical data for scientific integrity of the study.

3.3.2. Secondary

Safety:

- Incidence of AEs
- ANC
- WBC count and differentials
- Platelets
- Mean corpuscular hemoglobin (MCH)
- Hematocrit
- Elevation in liver function tests (LFTs)
- Hb/anemia
- Bilirubin
- Additional safety laboratory parameters (hematology/biochemistry)
- Bacterial infections
- Viral infections
- Fungal infections
- Leg ulcers

Biomarkers:

- HbF
- MCV
- Cystatin C

Clinical Status:

- Incidence of acute pain crises*
- Number and frequency of blood transfusions
- Incidence of ACS
- Hospitalizations
- Dose escalation i.e. time to MTD
- Clinical parameters (symptoms)
- Parent/caregiver acceptability/usability by questionnaire
- Palatability and acceptability: evaluate the taste acceptability of the new oral liquid formulation of hydroxyurea

* Defined as an acute episode of pain with no known cause for pain other than a vaso-occlusive event; requiring a visit to a medical facility; and requiring treatment with a narcotic (including opiates) and/or non-steroidal anti-inflammatory drugs (NSAIDs); but is NOT classified as an ACS, hepatic sequestration, splenic sequestration, or priapism.

3.3.3. Other – Exploratory

- Transcranial doppler (TCD) velocity
- Urine parameters (albumin and creatinine for albumin-to-creatinine ratio)

4. INVESTIGATIONAL MEDICINAL PRODUCT

The term 'Investigational Medicinal Product (IMP)' will refer to the oral hydroxyurea 100 mg/mL solution administered to the patient as part of the study.

4.1. Description of Investigational Medicinal Product

Drug Substance	Hydroxyurea
Manufacturer of Drug Product	The test drug product will be manufactured and batch released by: Nova Laboratories Limited, Martin House, Gloucester Crescent, Wigston, Leicester, LE18 4YL, UK
Test Drug Product	Oral hydroxyurea 100 mg/mL solution
Daily dose	Starting dose will be 15 mg/kg/day
Frequency	Once daily
Packaging, Test Drug Product	150 mL Amber type III glass bottle with tamper evident child-resistant closure. Storage in a refrigerator (2 to 8°C)

The IMP will be prepared and dosing on the PK days will be by the site staff according to instructions provided by the Sponsor. Remaining doses will be given to the patient by their parents/guardians according to instructions provided to them by the site staff, with written instructions also provided. Parents will be trained according to these instructions and local hospital standard procedures.

Hydroxyurea will be prescribed by the investigator or delegated qualified medical personnel, per local regulations.

4.2. Hydroxyurea Dosing

The appropriate dose will be delivered to each patient according to their weight, to deliver approximately 15 mg/kg/day hydroxyurea as a starting dose.

Each patient enrolled in the study will receive hydroxyurea in accordance with the dosing requirements.

Initial doses will be administered and checked by trained medical/nursing staff and recorded accurately in medical records and on the electronic data capture (EDC) database provided by the Sponsor. Remaining doses will be recorded by the parents/guardians in the diary card.

The Sponsor's monitors will check the accuracy of the pharmacy and site for receipt of IMP, confirmation of receipt, pharmacy storage of the IMP packages, distribution records to the clinic and records of return to pharmacy and destruction of IMP. Any anomalies will be queried and clearly documented. All records will be maintained in the pharmacy files with anonymized copies retained for the Sponsor trial master file (TMF).

4.3. Packaging and Labelling

The Sponsor will provide sufficient quantities of IMP in accordance with Good Manufacturing Practice. The clinical study supplies will be properly packaged and labeled in accordance with applicable local regulatory requirements.

4.4. Supplies and Shipping

The Sponsor will provide enough IMP to sites at the start of the study for initial patient recruitment and follow-up, in accordance with sites local storage availability. IMP will be delivered directly by the Sponsor to site pharmacies for accountability and storage. Sites will request additional supplies through use of a Supplier Request Form submitted directly to the Sponsor. Patients will be dispensed IMP as per the sites local Pharmacy Manual for the study (this may be at every visit, or after several).

4.5. IMP Deliveries to Patient's Home Address

It is expected that most IMP can be provided to the patient's carer at regular site visits. However, as there is the possibility that a patient may have up to a 12 week period between in-clinic dispensing visits, or may be unable to attend the clinic (e.g. due to local or national restrictions or policies) , sites may dispense additional IMP to patients sufficient to last this 12 week period (as applicable), and to increase their local stock supply from Sponsor.

In order to reduce the need for patients to travel in for IMP dispensing outside of their scheduled safety/PK in-clinic visits, some sites may also have the capacity to utilize a home IMP delivery service which has been set up with a courier by the Sponsor to make refrigerated deliveries (2-8°C) to patients' home addresses (where applicable). A risk assessment for this home delivery of IMP has been carried out and the following practices will be implemented:

- Verbal consent to be given by the site and documented in the patient's medical file prior to sending any IMP to the patient's home address. An additional consent form explicitly describing the IMP delivery will be completed which informs the patient and/or their carers of this and asks for consent for home delivery.
- Shipments are arranged directly by the local site to ensure that patient confidentiality and safety is maintained (the Sponsor will not receive patient contact details).
- Shipments will be couriered directly to patients' home/specified recipient in refrigerated 2-8°C validated boxes.
- Couriers will observe any social distancing guidelines as stipulated by the courier company and in accordance with local/National guidelines to reduce the risk to both patient and courier employee.
- Delivery receipt will be in the form of a name given verbally or photographic evidence, or other such non-direct contact method as established by the courier company guidelines.
- Site staff will call the patient's carer to confirm that the IMP was delivered satisfactorily, and the correct amount was delivered, with no sign of damage. This call will be documented in the patient's medical file.
- Patients' dosing is based on their most current weight taken at last visit, as such changes in dosing will only be applied after safety bloods and in-clinic visits have been carried out.
- Patient's compliance and accountability will be reviewed in bottle returns and safety bloods at next in-clinic visit.

4.5.1. Risk Assessment of Sites Sending IMP to Patient's Home Address

The following risks have been identified with the amendment to the IMP supplies and shipments in response to the COVID-19 pandemic period, which have been mitigated by the following described processes above in Section 4.5 IMP Deliveries to Patient's Home Address and below:

Patient may run out of IMP prior to their next on-site clinic dispensing visit:

- The protocol has been amended to allow the sites to send IMP directly to patients/caregivers home address.

- Sites will increase the amount of IMP dispensed at on-site visits, sufficient to keep the patient supplied through an extended visit period of up to 12 weeks if required.

Patient/Caregiver may not wish to receive IMP directly:

- To mitigate this, we have created a specific additional patient information leaflet and consent form which states that they have agreed to receive IMP in this way, this has been made optional.
- If patients/caregivers have not consented to this section of the consent form and have opted out, they will not receive IMP in this way.

Patient contact details may not be up to date at hospital site:

- Patients/caregivers will be contacted by telephone by sites prior to arrangement of any shipments to confirm their postal address and arrange a shipping time/date.

Ensuring the confidentiality of patient contact details:

- Patients' identifiable data will remain with the local site team. The courier will be given a contact address and name for delivery purposes only, they will not pass this information to the Sponsor.
- The Sponsor has created a specific shipping account with the courier company that removes any 'recipient' information from the invoices.
- All shipment arrangements will be made directly with the local site and not include the Sponsor.
- Patients/caregivers have been made aware that their identifiable information will be provided to the courier company for purposes of IMP delivery only and will consent to this process.

Stability of the IMP in transit and managing delays:

- IMP is delivered same or next day by a courier specialising in delivering medicines and ensuring the product stays within 2-8°C.
- The courier service will use tracking information which can be followed up directly by the study team and they will be made aware of any failed attempts or delays.
- IMP is shipped in validated boxes (2-8°C), these do not contain temperature monitoring but are validated for up to 4 days under normal room temperature conditions.

Confirmation that patient received the IMP in satisfactory condition:

- Patients/caregivers will be asked to contact the study team when IMP has arrived to confirm. If they fail to do this within 24 hours, the site will follow this up with a telephone call..
- If the delivery is not received in satisfactory condition, the patients/caregivers will have been advised on how to handle breakages during their initial induction into the study; however, they will be reminded of this when informing the site.
- Any breakages or failed deliveries will be followed up by the study team and a new delivery arranged.
- Courier will record the delivery time, address, and the name of the person for their records. This can be supplied to the local study team for confirmation.

4.6. Preparation, Handling, and Storage

The IMP received by Pharmacy from the Sponsor will be accounted for in a drug inventory and stored in a locked area in the pharmacy or in the clinic until used in the study. Storage information

and batch characteristics will be supplied at the time of delivery. At the end of the study the site will be contacted about the handling procedures for unused IMP. Once IMP accountability is confirmed by the Sponsor, I IMP will be returned to the Sponsor or destroyed at site according to local procedures; this will be appropriately documented.

4.7. Investigational Medicinal Product Accountability

The principal investigator at each site is responsible for ensuring the IMP accountability, including reconciliation of the IMP and maintenance of IMP records.

- Upon receipt of the IMP the principal investigator (or delegated pharmacist) will check for accurate delivery and acknowledge receipt by signing (or initialing) and dating the documentation provided by the supplier and faxing/scanning a copy back to the supplier. The original will be retained in the investigator/pharmacy file.
- The dispensing of the IMP will be carefully recorded on the appropriate drug accountability forms provided by the Sponsor. An accurate account will be made available for verification by the Sponsor monitor at each monitoring visit.
- IMP accountability records will include:
    - Release of the IMP by the supplier's Qualified Person.
    - Confirmation of IMP delivery to the study site.
    - The inventory at the site of IMP provided by the supplier.
    - Details of pharmacy dispensing.
    - Administration of IMP to patients (in clinic, and on diary cards).
    - Destruction of excess IMP. All unused IMP will be destroyed by pharmacy personnel according to normal hospital policy. Destruction will be clearly recorded, documented, and checked by the Sponsor's monitors.
    - Return to the supplier or alternative disposition of unused IMP.
- The principal investigator should maintain records that adequately document that:
    - Patients were treated with the dose specified in the protocol.
    - Preparation/administration and return/destruction of IMP was properly conducted.

Unused IMP must not be discarded without prior permission from the Sponsor or used for any purpose other than the present study. IMP that has been dispensed must be accurately recorded for each patient.

The Sponsor monitor will periodically collect the IMP accountability forms and check all IMP data (both used and unused) prior to arranging for the return of unused IMP to the supplier or authorizing their destruction by the study site or, if required, return to the Sponsor.

5. STUDY PROCEDURES

5.1. Blood Sampling for Pharmacokinetic Analyses

On their PK day (PK1 – Day 1 and PK2 – ~6 months [once between weeks 20-36]) patients will be asked to come to the clinic early, in order that the site study team can implement a fast, prior to dosing in-clinic (no food for 1 hour and no water for 30 minutes before dosing). Patients should also be reminded not to take a dose at home the same day as attending the PK visit. A peripheral venous catheter will be used for blood sampling. The patient will have a baseline blood sample (1 mL) taken before they receive hydroxyurea. Patients will receive their dose according to the liquid dosing instructions, which will include a 2-hour fast after dosing, on PK days only. Dosing instructions for liquid hydroxyurea will be provided to site and detailed within the Pharmacy Manual.

All patients are required to provide a pre-dose blood sample. For patients > 2 years old, up to 5 additional blood samples (1 mL per sample) will be taken post-dose at the time-points specified in Table 2 Blood Sampling Schedule below. For patients < 2 years of age, up to 2 additional samples will be taken at an appropriate sample time. Sampling times in the patients < 2 years of age may be discussed with the Sponsor prior to the study PK sampling days, to ensure an even spread of sampling times in this younger population of patients.

Table 2 Blood Sampling Schedule

Time point (h)	Requirements
*Pre-dose	*The pre-dose sample is mandatory in all patients. In patients < 2 years old up to 2 post-dose samples will be taken (3 samples in total), and in patients ≥ 2 years old, 3-5 additional samples will be taken (4-6 samples in total including the pre-dose sample). Samples will be selected over 0.25 to 6 hours. **Sample "as able" applies to clinic visits over the duration of treatment (up to week 60). Where feasible, patients will be asked to provide a single PK sample when they return for review at the clinic. This sample should only be taken if the time and dose of the previous dose of hydroxyurea is known, and it must be noted. Sampling time points may be amended; however, target total blood volume and target number of samples will remain unchanged.
0.25	
0.5	
1	
1.5	
2	
3	
4	
5	
6	
**As able at clinic visits if feasible	

Sample handling will be according to a separate Laboratory Sample Handling Manual that will be dated and version controlled and provided to the study site.

All staff involved in the collection of the blood samples will be trained to ensure appropriate collection, processingn and storage of the PK samples.

Samples should be sent to the sample-processing laboratory following completion of PK sampling. Expenses will be paid by the Sponsor to cover all sample transport costs (see Laboratory Sample Handling Manual and laboratory kit/sending instructions).

5.2. Study Follow-up

Patients will cease taking IMP at their final visit (week 60/early withdrawal visit), which will be performed in-clinic. After this visit all patients will be followed up ~4 weeks later by telephone, whether or not the patient continues to receive licensed hydroxyurea treatment outside the study protocol. Follow-up will also be made (where possible) for patients who prematurely withdraw

from the study. This will be in the form of a telephone call (or in-clinic for patients who may be attending for routine review) to check for AEs experienced in the 4 weeks following treatment cessation.

5.3. Demography

The following demographic characteristics will be recorded during screening in the medical notes and on the study database: sex, race, and age.

5.4. Anthropometric Data

Patient's length (for patients < 2 years)/height (for patients ≥ 2 years) (cm) and weight (kg) will be checked at the time points specified in Table 1 Schedule of Study Assessments. These will be used to calculate the correct dosage and potential dose escalations to MTD, according to the protocol (See section 1.3.3 Dosage and Dose Escalation).

5.5. Medical History

SCA history and treatment will be recorded to confirm eligibility at the time points specified in Table 1 Schedule of Study Assessments. Additional relevant medical history during the 12 months prior to the study will be recorded. This will include all current conditions by diagnosis (where possible) and any concomitant medication. Other acute complications in the last 12 months will be recorded, including any episodes of: stroke, dactylitis, ACS, aplastic crisis, febrile episode requiring admission, osteomyelitis, splenic sequestration, hepatic sequestration, and priapism. The number of hospital admissions and number of admissions for pain crises in the last 12 months will be recorded, including the number of pediatric intensive care unit (PICU) admissions and attendance to the emergency facility. Any surgical history in the last 12 months will also be recorded.

5.6. Medication at Study Start

Medication received for treatment of SCA in the last 6 months will be recorded either in medical history or in current medication (depending on whether it is still on-going) including: penicillin, folic acid, and other medications (if any), with assessment of compliance (if applicable). Any vaccinations provided will be recorded including: Pneumovax, Hepatitis B, annual influenza vaccination, protein conjugated vaccines for pneumococcus, and measles. All other concomitant medication to be recorded will be limited to ongoing medication at the time of screening. Any changes in concomitant medication between the screening visit and first PK visit (PK1) will be recorded.

5.7. SCA Symptom Review

Symptoms that could be attributed to SCA will be reviewed at baseline and at the time points specified in Table 1 Schedule of Study Assessments. Clinically significant will be recorded as an AE. Symptoms to be reviewed include: painful crises*, enuresis, snoring, daytime somnolence, chronic pain, shortness of breath on exertion, and stuttering priapism. Any other symptoms of clinical significance will be recorded by the investigator. Any episodes of hospitalization, transfusion, or ACS will be recorded in the EDC database. Any unexpected deterioration or unexpected worsening of symptoms of sufficient importance such that they should be recorded in the patient's medical records/nursing notes will be recorded as AEs (See Section 9 PROCEDURE FOR RECORDING AND REPORTING ADVERSE EVENTS).

* defined as an acute episode of pain with no known cause for pain other than a vaso-occlusive event; requiring a visit to a medical facility; and requiring treatment with a narcotic (including opiates), or NSAIDs; but is NOT classified as an ACS, hepatic sequestration, splenic sequestration, or priapism.

5.8. Leg Ulcer Review

Any incidence of leg ulcers will be recorded (i.e. present or absence) at the time points specified in Table 1 Schedule of Study Assessments.

5.9. Physical Examination

A qualified medical practitioner will carry out a general physical examination at the time points specified in Table 1 Schedule of Study Assessments. Examinations can be made at other visits if clinically indicated; where this is undertaken the results will be recorded in the EDC. A general physical examination will include the following: ear/nose/throat (tonsils), dermatological, cardiovascular, respiratory, gastrointestinal, lymph nodes, musculoskeletal, hepatomegaly, and splenomegaly (at screening, PK2, final visit/week 60 and early withdrawal [unless otherwise indicated]), for ophthalmological and central nervous system (at screening, final visit/week 60 and early withdrawal [unless otherwise indicated]). Other evaluations may be performed as deemed necessary by the investigator. This will be commented upon in the clinical study report, if applicable. Physical examinations will be performed at baseline prior to treatment, to ensure no concurrent conditions exist that may exclude the patient from the study.

5.10. Transcranial Doppler

TCD assessment will be made at screening (however, results can be used from a TCD which has been performed within 3 months of screening) and once in-clinic between week 48-56. If TCD is undertaken at other visits (as part of routine standard of care for example), results will be reported in the EDC database as appropriate.

5.11. Vital Signs

Supine systolic and diastolic blood pressure, pulse and body temperature (in older patients this will be measured after 5 minutes rest in supine position) will be measured at the time points specified in Table 1 Schedule of Study Assessments. Investigators may repeat measurements as clinically indicated. Following dosing, vital signs will be monitored according to routine standard of care. Any unexpected measurements will be recorded as AEs and reported in the normal way.

5.12. Laboratory Assessments

Measurement of the hematological indices will be carried out in line with standard care. Additional unscheduled laboratory tests may be carried out at the discretion of the investigator in line with standard of care. Laboratory assessments required by the protocol will be carried out as specified in Table 1 Schedule of Study Assessments.

5.12.1. Full Blood Count

A full blood count will be performed at the timepoints specified in Table 1 Schedule of Study Assessments and will include: Hb, red blood cell count, platelets, ANC, ARC, WBC count and differentials, hematocrit, lymphocytes, monocytes, eosinophils, basophils, MCH, and MCV. HbF will also be measured at the timepoints specified in Table 1 Schedule of Study Assessments. If a Hb electrophoresis result is not already available and it is required to confirm SCD diagnosis, it is to be arranged locally as part of the patient's standard of care.

5.12.2. Clinical Chemistry

Clinical chemistry will be performed at the timepoints specified in Table 1 Schedule of Study Assessments. Samples taken for clinical chemistry (using serum separator tubes) will be analyzed for: total bilirubin, alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase (ALT), LDH, vitamin D, urea, creatinine, and cystatin C.

5.12.3. Urinalysis

Urinalysis will be performed at the timepoints specified in Table 1 Schedule of Study Assessments using a dipstick, and will be assessed for: protein, bilirubin, blood, urobilinogen. A urine sample for albumin and creatinine to determine the ACR ratio will also be taken at the same timepoints.

5.13. Pregnancy Prevention

Female patients that are of child-bearing age (and not truly sexually abstinent) receiving hydroxyurea should be advised to not become pregnant, and to inform the treating physician immediately should this occur.

An effective method of contraception is strongly recommended in women of child-bearing potential.

To prevent pregnancy female patients of child-bearing potential and male patients and their female partner must use 2 highly effective forms of contraception, i.e.:

- Condom + established use of combined hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Intravaginal
    - Transdermal
- Condom + established use of progestogen-only hormonal contraception associated with inhibition of ovulation:
    - Oral
    - Injectable
    - Implantable
- Condom + intrauterine device.
- Condom + intrauterine hormone-releasing system.
- Vasectomized partner, provided that the partner is the sole sexual partner of the female study patient.
- True abstinence, only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the IMP. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical study and the preferred and usual lifestyle of the patient. (*Periodic abstinence [e.g., calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are **not** acceptable methods of contraception).*

To prevent exposure of any partner (male or female) during non-vaginal intercourse to the semen from a male patient who has been exposed to the IMP, the following contraception must be used:

- Condom.

The chosen contraception method(s) must be followed from the first dose until at least 6 months after the last dose of IMP.

Male patients must not have unprotected sexual intercourse with a female who is pregnant or breastfeeding during the study and for 6 months after their final dose of IMP.

Patients on hydroxyurea wishing to conceive should stop treatment 6 months before pregnancy if possible. The evaluation of the risk-benefit ratio should be made on an individual basis outweighing the respective risk of hydroxyurea therapy against the switch to a blood transfusion programme.

A urine pregnancy test will be performed no more than 7 days before the first administration of hydroxyurea in all post-menarcheal females (if the PK1 visit is delayed > 7 days from screening pregnancy test, a urine pregnancy test should be repeated at the PK1 visit).

5.14. Palatability and Acceptability Assessments

When patients (together with their parents/caregivers) return to the clinic at any point after 8 weeks since starting IMP, they will be surveyed once using a standard questionnaire for their views of the palatability and acceptability of the hydroxyurea. In the case of patients < 6 years of age, the researcher will survey the view of one or both parents. For patients ≥ 6 years of age, the researcher will seek the views of one or both parents/caregivers and the patient.

The questionnaire will probe the taste and general acceptability (willingness to continue with treatment, ease of dose administration, etc.). After 6 patients from each age group have completed the survey, the use of the questionnaire will be reviewed, by both the investigator and the Sponsor to ensure that it is working satisfactorily. If the questionnaire needs to be adjusted (in terms of its administration or the ability/age groups of the patients), then this will be done. Minor amendments only will be made. All minor edits will be documented, and a copy of the amended questionnaire will be provided to the Independent Ethics Committee (IEC). If significant adjustment is required, IEC approval will be sought before administering the amended questionnaire. Data on the following will be collected using a visual (hedonic) analogue scale and verbal responses:

- Taste on first administration.
- Residual after taste.
- Smell.
- Any incidences of spitting medicine out or vomiting.
- Willingness to take hydroxyurea on a daily basis.
- Ease of dose administration using syringes.
- Preference if choosing type of hydroxyurea medicine (i.e. tablet/capsule/liquid) and reason.

The survey will be conducted by a trained research nurse. The survey will be face to face (in-clinic) with the patient and/or caregiver and will take no longer than 5 minutes. If the patient withdraws prior to week 8, then the questionnaire should be completed at the early withdrawal visit.

5.15. Tolerability Assessments

All AEs will be collected and recorded during the study and for the 4 week follow-up period. Patients and parents/caregivers will be provided with a study diary card and will be asked to record AEs. All AEs will be captured in the EDC database.

5.16. Data to be Recorded in the EDC Database

The following data will be recorded on the patient's case report form (CRF) in the EDC database:

- Unique patient identifier, to identify site and patient number in study.
- Date of informed consent (and receipt of assent if applicable).
- Demographic data: age, gender, and race.
- Genotype (HbSS and HbSβ^0 information).
- Weight and height/length.
- Vital signs: blood pressure, temperature, heart rate (supine pulse rate), and respiratory rate.
- Physical examination.
- Pregnancy test results (if applicable).
- Safety laboratory results.
- Relevant non-SCA medical history: relevant past medical history in the 4 weeks prior to the study, known allergies and relevant longstanding conditions that are ongoing.
- SCA history and treatment to include concomitant medication for SCA (i.e. confirmation of eligibility). SCA medical history including other clinically (in the opinion of the investigator) relevant co-morbidities or diagnoses. Other acute complications in last 12 months including: pain crises, stroke, dactylitis, ACS, aplastic crisis, febrile episode requiring admission, osteomyelitis, splenic sequestration, hepatic sequestration, and priapism. Number of hospital admissions in last 12 months and number of admissions for pain crises and number of admissions to PICU and number of admissions to the emergency facility in the past 12 months. Any surgical history in the last 12 months.
- Concurrent medication: any non-SCA medication ongoing at time of screening. SCA medication in the last 6 months including: penicillin, folic acid, other medication (if any) with assessment of compliance (if applicable). Any vaccinations provided: Pneumovax, Hepatitis B, annual influenza vaccination.
- Inclusion/exclusion criteria.
- Concomitant medications taken during participation in the study.
- Dose of hydroxyurea (start date, any dose changes and stopping dates).
- Confirmation of treatment and batch numbers.
- Time of treatment (PK days). Where a single PK sample is taken at an interim visit, the time of the previous dose must be recorded.
- Precise time of blood sampling.
- Palatability and acceptability.
- AEs – all events occurring during the timeframe of the study and for a period of 4 weeks afterwards (on the study diary card).
- Serious adverse events (SAEs) – all events regarded as serious during course of study and until resolution.
- Study withdrawal information, if applicable.
- Date and time of study discharge (i.e. when the patient completes the 4 week follow-up).

6. STUDY ENROLLMENT, WITHDRAWAL AND DISCONTINUATION

Up to 35 patients, both male and female, aged 6 months to 17.99 years (i.e. to the day before 18th birthday) of age will be enrolled into the study depending on the number of PK blood samples that are obtained from each patient.

To ensure the cohort is appropriately represented by age recruitment will continue until the required number is achieved in each of the following stratifications:

Age 6 months to 1.99 years: a minimum of 6 patients (i.e. to the day before 2nd birthday)
Age 2 years to 5.99 years: a minimum of 6 patients (i.e. to the day before 6th birthday)

6.1. Inclusion Criteria

To be eligible for inclusion into this study the patients must fulfil all of the following criteria:

1. Male or female aged from 6 months to 17.99 years of age (i.e. to the day before 18th birthday).
2. Diagnosis of SCA (HbSS and HbSβ^0).
3. Parent(s)/legal guardian able and willing to provide written informed consent for the child to take part in the study.
4. Where applicable, the child should assent to undergo blood sampling for PK and biochemistry purposes, and to allow physiological measurements to be made.

6.2. Exclusion Criteria

To be eligible for inclusion in this study the patients must **not** meet any of the following criteria:

1. Any clinically significant medical condition or abnormality, which, in the opinion of the investigator, might compromise the safety of the patient or which might interfere with the study.
2. Hydroxyurea use within 6 months before enrolment.
3. Renal insufficiency (known creatinine more than twice the upper limit of normal (ULN) for age and > 1.0 mg/dL [88.4 micromol/L]).
4. Clinical evidence of hepatic compromise with ALT more than 3 times the ULN (a temporary swing in ALT will not result in exclusion).
5. Other significant organ system dysfunction based on the site investigators discretion.
6. Severe active infections: fungal, viral, or bacterial (as confirmed by culture). Examples include tuberculosis, malaria, active hepatitis, osteomyelitis, or any other illness that would preclude the use of hydroxyurea in normal clinical practice.
7. Active chronic leg ulcers.
8. Known allergy to oral hydroxyurea solution or any of the excipients.
9. Positive pregnancy test for females of child-bearing potential (in post-menarcheal females) before initiation of treatment, unless patient is sexually abstinent. Note: true abstinence is considered as being in line with the preferred and usual lifestyle of the patient. Periodic abstinence (such as calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
10. Inadequate contraception measures in sexually active females (in post-menarcheal females) and males of child-bearing age (see Section 5.13 Pregnancy Prevention).
11. Currently breastfeeding.
12. Participating in another clinical study of an IMP.
13. Known infection with HIV.

6.3. Withdrawal Criteria

Parent(s)/legal guardian and patients may withdraw from the study at any time without their care being affected. Withdrawal may be:

1. On request of the patient/parent/legal guardian, for any reason.
2. At the discretion of the investigator.

6.4. Early Discontinuation of an Individual Patient

Patients and their parent(s)/legal guardian will be informed that they have the right to withdraw from the study at any time without prejudice to their medical care, and that they are not obliged to state their reasons. In addition, the investigator may remove a patient from the study if, in the investigator's opinion, it is not in the best interest of the patient to continue in the study.

Any withdrawal from the study must be fully documented in the CRF and source documents and further blood sampling will cease as part of the study.

The date of discontinuation from the study and reason for discontinuation will be recorded on the CRF and in the patient's medical records.

Patients may be discontinued from the study for the following reasons:

- Withdrawal of consent (by patient or parent/legal guardian).
- AE.
- Deviation from protocol.
- Other (to be specified on the CRF by the investigator if possible).

The data recorded until the time of withdrawal will be used as part of the clinical study unless the parent(s)/legal guardian or patient requests that it is not used.

6.5. Early Discontinuation of the Study

If, in the opinion of the investigator, the clinical observations in the study suggest that it might not be justifiable to continue, she/he may terminate the study following consultation with the Sponsor. Alternatively, the Sponsor may give written notification to the investigator, regulatory authorities, and IECs of the early discontinuation of the study, including reasons.

In case of early discontinuation of the study, safety assessments should be performed for each patient, as far as possible.

6.6. Patient and Study Completion

Patients will be considered to have completed the study when the patient has completed the safety follow-up visit, which is scheduled 4 weeks after the final study visit (month 12-15 or 6 months at MTD, whichever is sooner, or at early withdrawal). Patients who consent for the study but who do not, for whatever reason, receive IMP, will be considered screen failures, and their data will be recorded on a screening failure list. Patients who receive at least 1 dose of hydroxyurea and provide at least 1 PK blood sample will be included in the PK analyses. All patients who receive IMP will be considered for the safety analyses.

The study will be considered complete 4 weeks after the last patient has completed their safety follow-up visit.

7. TREATMENT OF PATIENTS

7.1. Dose Initiation and Escalation

Consent to take part in the study will be obtained prior to initiation of hydroxyurea.

- **Starting dosage for all patients: 15 mg/kg/day**

If dose escalation is warranted based on clinical and laboratory findings, proceed as follows:

- Increase by 5 mg/kg/day increments every 8-12 weeks (at in-clinic visits) up to a **maximum of 35 mg/kg/day**.

Doses will be calculated based on most current bodyweight (measured at each clinic visit). Doses will be rounded to the nearest 10 mg. Dose will be calculated using a dosing calculator or by formula.

Patients will be reminded (per standard practice) that the effectiveness of hydroxyurea depends on their adherence to daily dosing; they should be counselled not to double up doses if a dose is missed. Hydroxyurea therapy will be continued during hospitalizations or illness unless there is hematological toxicity.

For full details of dose escalation (see Section 1.3.3 Dosage and Dose Escalation).

Dose escalations should be carried out only after an on-site patient visit (in-clinic) in which safety bloods have been obtained, but advice on dose changes may be given to patients/caregivers by telephone.

Due to the possible extended period between clinic visits of up to 12 weeks (maximal), any indication of a trend towards toxicity identified by the investigator should be treated at the discretion of the investigator to ensure patient safety. Investigator discretion may be used to treat trends towards toxicity proactively, as though they have reached a threshold of toxicity prior to confirmation with dose reductions (at 2.5-5.0 mg/kg/day) or temporary halts, as applicable. These actions should be reported to the Sponsor and documented in the patients medical notes and EDC and would not be considered deviations.

7.2. Permitted Medications

Concomitant medication is defined as any medication (including vitamins and minerals), other than the IMP, which is taken during the study including prescription and over-the-counter medicines.

The generic names of medications should be used where possible.

Concomitant medication will be recorded in the EDC with indication, dose information, and dates of administration. Any new medications taken or any changes to the form, frequency or dose of existing medication occurring during the study will also be recorded.

7.3. Monitoring Compliance

Medication will be prescribed and then dispensed by the pharmacist. On the full PK profile days (Day 1 [PK1] and at ~6 months [PK2]), the morning doses of IMPwill be administered in-clinic. Patients will be reminded by site staff not to take a dose at home the same day as they are due to attend for a PK visit. All additional doses of IMP will be recorded fully on the patient's diary card and in the EDC database during dose escalation. In addition, the investigator will ask the patient and parents how many doses were accidently missed per week/month. Prescription, dispensing, and accountability of hydroxyurea will be accurately recorded in the drug accountability log

provided in the EDC database by the Sponsor. The study monitor will verify that the data have been accurately recorded and transferred.

7.4. Exit Points

The normal exit point from this study will be when the patient completes the 12-15-month treatment (i.e. titration to MTD and 6-month follow-up at MTD or a maximum 15 months of treatment with IMP, whichever is sooner); all AEs will be followed up to follow-up safety call at week 64.

All patients who have at least 1 blood sample taken for the PK study will be included in the population PK analysis. If the parent(s)/legal guardian or patient refuses further blood sampling, the patient may be observed over the remainder of their treatment course (safety and secondary objectives will be reviewed according to protocol).

7.5. Expected Duration of Patient Participation

Patients will be in the study over a 12-15-month period. The duration of participation will be dependent upon the time taken to titrate the patient to the MTD. Once at MTD, the patient will be followed for a period of 6 months. The maximum period of participation is the maximum period the patient can take the IMP, which is 15 months.

8. ASSESSMENT OF SAFETY

Assessments of safety will be performed throughout the study and for the safety follow-up, which is 4 weeks after study completion. Post-dose AEs are recorded in the diary card or are spontaneously reported by the patient to hospital staff, and should be recorded in the EDC database.

The ongoing safety of the IMP will be assessed through the recording, reporting, and clinical assessment of baseline medical conditions and AEs, which are recorded until discharged from the study (and for the 4-week safety follow-up period). Study site personnel will report any AE, whether observed by the research staff or reported by the patient after IMP is initiated (following PK1 visit).

8.1. Definition of Adverse Events

An AE is any untoward medical occurrence in a patient or clinical study patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not related to the IMP.

During the study after PK1 IMP initiaton, any worsening of conditions, signs or symptoms noted at the start of the visit will be recorded as an AE. In cases of surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself.

Any AE's experienced during the screening or PK1 (baseline) visit prior to treatment initiation, that are unrelated to study procedures carried out (e.g. needle stick injuries), should be recorded in medical history and would not be considered AEs.

All AEs that occur (whether treatment related or not) will be recorded in the EDC throughout the duration of the study (and during the 4 week safety follow-up period).

As far as possible, each AE must be described by its duration (start and end time and date or ongoing), its frequency (single episode, intermittent, or continuous), its severity (see Section 8.3 Adverse Events), a causality assessment (coexisting disease, concomitant medication, the IMP, or other cause), its relationship to the IMP (see Section 8.3 Adverse Events), whether this influenced the course of the IMP, whether it required specific action or therapy, and outcome.

8.2. Expected Adverse Events in Hydroxyurea Recipients

Refer to Investigator Brochure.

8.3. Adverse Events

Using the Common Terminology Criteria for Adverse Events (CTCAE) version 4, the principal investigator will assess the severity of all AEs.

The principal investigator will also assess the relationship of AEs to the IMP using the following definitions:

Definite: There is a definite clinical/biological relationship and time sequence between the onset of the AE and the administration of the IMP.

Probable: A causal relationship is clinically/biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of the IMP and there is a reasonable response on withdrawal.

Possible: A causal relationship is clinically/biologically plausible and there is a plausible time sequence between onset of the AE and administration of IMP.

Unlikely: A causal relationship is improbable and another documented cause of the AE is most plausible.

Unrelated: A causal relationship can be definitively excluded and another documented cause of the AE is most plausible.

In case of a fatality the cause of death is considered as the AE and the death is its outcome.

9. PROCEDURE FOR RECORDING AND REPORTING ADVERSE EVENTS

AE data will be recorded throughout the study.

9.1. Recording of Adverse Events in the EDC Database

As quality and precision of AE data collected is of key importance, the principal investigator or his/her designee should use the AE definitions provided in the above sections and follow the guidelines below when completing the AE log in the EDC database:

- Whenever possible recognized medical terms should be used to describe AEs rather than colloquialisms (e.g. 'influenza' rather than 'flu'). Abbreviations should be avoided. An AE term needs to be provided for each AE, preferably using the Short Name as listed in the CTCAE version 4, available online.
- AEs should be described using a specific clinical diagnosis rather than component signs and symptoms where possible (e.g. 'congestive heart failure' rather than 'dyspnoea, rales and cyanosis').
- Signs and symptoms that are considered unrelated to a specific disease or syndrome should be reported as individual AEs in the EDC database (e.g. 'nausea' and 'vomiting' should be recorded separately and not as 'nausea and vomiting').
- Provisional diagnoses (e.g. 'suspected asthma') are acceptable but should be followed up by a definite diagnosis when/if finally available.
- AEs (or other SAEs) that are secondary to other events (e.g. sequelae or complications) should be identified by the primary cause. A primary AE, if clearly identifiable, generally represents the most accurate clinical term to be recorded on the AE log in the EDC database. The principal investigator should be invited to provide his/her own opinion of which is the primary AE.

9.2. Reporting of Adverse Events

Complete and appropriate data on all AEs experienced during the clinical study will be reported in the AE forms in the EDC database on an ongoing basis for the duration of the reporting period.

It is important that each AE report includes a description of the event, its seriousness status and criteria, duration, severity, relationship to the IMP, other causality factors (if any), any concomitant medications dispensed, concomitant procedures prescribed or other actions taken, and its outcome at the end of the reporting period.

For chronic conditions such as SCA there can be worsening of initial AEs. These may be AEs that become SAEs in accordance with the definition in Section 9.3 Serious Adverse Events. To ensure that onset and end date of seriousness is captured for reconciliation purposes, these worsening AE's should be reported as 2 separate events; 1 serious and 1 not serious in the AE log in the EDC database, with the SAE onset date given as the date of seriousness and the event name changed to reflect the serious condition. The initial AE may continue to be ongoing after the SAE has resolved; therefore, the end-date for the end of the SAE and the initial AE may be different.

9.3. Serious Adverse Events

A SAE is defined as one of the following:

- An event that causes the death of the patient.
- A life-threatening* event.
- An event causing hospitalization** or prolongation of existing hospitalization.
- An event causing persistent or significant disability or incapacity.***

- An event causing a congenital anomaly or birth defect in the offspring of a woman treated before or during pregnancy.
- Important medical events (i.e. that may not result in death, be life threatening, or require hospitalization which may be considered a serious adverse drug experience when, based upon medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition).

* The term 'life-threatening' refers to an event in which the patient was at risk of death at the time of event; it does not refer to an event that hypothetically might have caused death if it were more severe.

** A hospitalization is usually defined as an overnight stay, including time spent in an emergency room, for an AE. However, for the purposes of this study, since admission for SCA associated complications is fairly common, an SAE for a hospitalization due to confirmed SCA associated complication will be defined as an AE that requires inpatient hospitalization of > 7 days or prolongation of existing hospitalization. Hospitalization for an elective procedure will not classified as an SAE, unless the hospitalization is prolonged beyond what would normally be anticipated for the elective procedure. Hospitalization or a prolongation of hospitalization for an AE other than a confirmed SCA associated complication will be considered an SAE. If the hospitalization for a confirmed SCA associated complication is longer than usually anticipated for the type of event/diagnosis on admission, then this will be defined as an SAE.

*** The term 'persistent or significant disability or incapacity' refers to an event that results in a substantial or permanent disruption of patient's ability to carry out normal life functions.

Pregnancy is not considered to be an AE. However, if any female becomes pregnant during the course of the study, she will be followed up to determine the outcome of both the mother and fetus. Also, if the partner of a male patient enrolled in the study becomes pregnant, the mother and fetus will also be followed-up to determine the outcome of both.

9.4. Reporting Serious Adverse Events

All SAEs occurring during the study must be reported to the Sponsor by email within 24 hours of the investigator becoming aware of the SAE.

SAE EMERGENCY CONTACT DETAILS
Fax: +44 (0)1753 695101
Email: pharmacovigilance@simbecorion.com

Investigators should not wait to receive additional information to fully document the event before notifying the Sponsor of a SAE. A full written summary, detailing relevant aspects of the SAE, should follow the initial fax report. Where applicable, information from relevant hospital case records and post-mortem reports should be obtained.

Any SAE that is both unexpected and suspected to be related to treatment (SUSAR) must be reported by the Sponsor to the regulatory authorities and the IEC (via the investigator) within the following time limits:

- A SUSAR that is fatal or life-threatening is to be reported to the regulatory authorities and the IEC as soon as possible, and in any case no later than 7 calendar days after the Sponsor first becomes aware of it. Relevant follow-up information must be reported within an additional 8 calendar days.
- All other SUSARs are to be reported to the regulatory authorities and the IEC as soon as possible, but within a maximum of 15 calendar days of the Sponsor first becoming aware of it.

9.4.1. Reporting Procedures

The investigator must complete the Serious Adverse Event Report Form, assess the relationship to IMP and send the completed form by fax within 24 hours to the Sponsor. The Sponsor will in any case report any death or life-threatening event to the authorities (Ministry of Health and Wellness [MOHW], Medicines and Healthcare products Regulatory Authority [MHRA], the Data Safety Monitoring Committee [DSMC], and IEC) within 7 calendar days, if it is both unexpected and considered to be drug-related, with any follow up reports within a further 8 calendar days. Any other SAE that is both unexpected and considered drug-related will be reported within 15 calendar days. The original and the duplicate copies of the Serious Adverse Event Report Form, and the email confirmation must be kept with the source data at the study site. The monitor will collect a copy of the Serious Adverse Event Report Form and deliver it to the Sponsor. Any further follow-up information that the investigator becomes aware of is to be sent to the Sponsor, re-stating the date of the original report. Either a new Serious Adverse Event Report Form is sent (stating that this is a follow-up), or the original one is re-sent (with the new information highlighted and a new date provided). The follow-up should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation. The form and fax confirmation sheet must be retained. The Sponsor may also request further follow-up information following review of the Serious Adverse Event Report Form. The investigator will make all reasonable attempts to obtain the follow-up information requested and forward it to the Sponsor as detailed above. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects. The investigator will provide these details via completion of a Pregnancy Follow-up Form.

10. EVALUATION OF RESULTS

Changes in the conduct of the study or planned analyses will be reported in the corresponding section of the clinical study report.

A Statistical Analysis Plan (SAP) will be developed by a qualified statistician together with a pharmacometrician. PK and statistical analysis will be performed according to this document. The SAP will specifically include details of how all: analysis populations, demographic and baseline characteristics, completion and discontinuation information, medication details, PK analyses, safety analyses, and palatability and acceptability will be classified and described. Palatability and acceptability data may be collected and summarized prior to the completion of the safety follow-up, and this will be documented in the SAP. The SAP will be reviewed and approved prior to any interim analysis.

One interim analysis is currently planned, when all patients have completed approximately 9 months of the study (i.e. when the last patient reaches their week 40 visit). The interim analysis will include all study endpoints described in the SAP.

The sample size chosen for the study is considered appropriate based on previous experience. No formal sample size calculations have been performed. In order to develop a robust population PK model (i.e. precisely estimated parameters), data from a minimum of 150 blood samples will be required. It is hoped that the required number of samples may be obtained from approximately 25 patients. However, if problems are encountered in obtaining samples, further patients may be included in the study to ensure sufficient samples are collected, especially for the age groups detailed below. This will not go over 35 patients in total without ensuring prior approval from both the relevant IEC and competent authority.

A minimum of 6 patients need to be enrolled in the 6 months to < 1.99 years age group and a minimum of 6 patients in the ≥ 2 to < 5.99 years age group (where .99 represents the day before the patient's birthday).

10.1. Pharmacokinetic Analysis

Since a sparse sampling approach will be employed in the present study as it is not possible to use a classical analysis to obtain PK parameter estimates. Therefore, a population PK approach will be applied. In the population approach all data from different individuals will be pooled and fitted simultaneously using a non-linear, mixed effects modelling approach and post hoc individual kinetic parameters can be calculated with as few as 1 sample per individual.

Data will be analyzed using the mixed effects non-linear regression modelling programme, NONMEM (most recent version; ICON). Post processing of NONMEM output will be undertaken with an appropriate statistical package such as R (current version).

Preliminary analyses will focus on the structural and variance models. One- and 2-compartment models with first and zero order inputs will be implemented. Between patient variability will be assumed to be log-normally distributed, and residual error will be modeled using additive, proportional and combined error structures.

Biologically plausible covariates will be included in the model for analysis e.g. age, weight. A multivariate analysis will be performed, details of this analysis will be included in the SAP. Several criteria can be used to evaluate the improvement in the model performance and thus select the final model. Comparisons of hierarchical models are based on the objective function value, i.e. 2-times the negative log likelihood value. Changes in the objective function value > 7.88 ($p<0.005$) are accepted as statistically significant. The other selection criteria used include improvement in the goodness of fit and residual plots, increased precision in parameter estimation, and reduced variance of between patient and residual errors. An assessment of model appropriateness will be

undertaken using visual predictive checks and posterior predictive checks. This will be based on the final covariate model.

The absorption function, CL/F, V/F will be considered as primary PK parameters; secondary PK parameters (such as T_{max}, C_{max}, AUC, and $t_{½}$) will be calculated from the primary model parameters.

10.2. Safety and Secondary Analyses

Reporting of safety data will be of a descriptive nature and presented using appropriate summary statistics (e.g. n, mean, standard deviation [SD], coefficient of variation [%CV], median, minimum, maximum, quartiles [if appropriate]) or frequency distributions (n%) by age group (age 6 months to 1.99 years, age 2 years to 5.99 years, and age 6 years to 17.99 years). Unless otherwise stated these tabulations will be supported by data listings.

Safety summaries will include all patients irrespective of whether or not they completed the study.

AEs will be coded according to the current version of Medical Dictionary for Regulatory Activities (MedDRA, version to be identified in the clinical study report).

Treatment-emergent adverse events (TEAEs) (AEs related to the IMP) will be determined and only these will be included in the SAP. Details of the criteria of classifying an AE as a TEAE will be provided in the SAP.

The incidence of AEs will be summarized by system organ class, preferred term, and maximum severity. AEs will also be summarized by strongest relationship to IMP by event and system organ class. If a patient experiences an AE more than once, the event with the worst severity or at the most related to IMP occurrence will be considered. Patients will be included only once at each level where they experienced 1 or more events.

A summary of the incidence of SAEs will be presented by event and system organ class.

AEs relating to hematological toxicities (cytopenias) and severe infections will be additionally listed separately and appropriately summarized.

The following hematological safety endpoints will be specifically and appropriately summarized:

- ANC
- WBC count and differentials
- Platelets
- Hematocrit
- MCH
- Hb/anemia
- Elevation in LFTs
- ARC
- Bilirubin
- LDH

The following safety endpoints relating to severe infection will be specifically summarized:

- Bacterial infections
- Viral infections
- Fungal infections
- Leg ulcers

The following biomarker endpoints will be specifically summarized:

- HbF
- MCV
- Cystatin C

The following clinical status endpoints will be appropriately summarized:

- Incidence of acute pain crises
- Number and frequency of blood transfusions
- Hospitalizations
- Incidence of ACS
- Dose escalation i.e. time to MTD
- Clinical parameters (symptoms)
- Parent/caregiver acceptability/usability by questionnaire
- Palatability and acceptability: evaluate the taste acceptability of the new oral liquid formulation of hydroxyurea

The following exploratory endpoints will be appropriately summarized:

- TCD velocity
- Urine parameters (albumin and creatinine for ACR ratio)

10.3. Palatability and Acceptability Analyses

Palatability data will be appropriately summarized.

10.4. Dose Escalation Analyses

Dose escalation data will be appropriately summarized.

10.5. Data Safety Monitoring Committee

An independent DSMC will be formed to alert and/or make recommendations to the Sponsor about any existing or potential safety issues.

10.6. Reporting Urgent Safety Measures

If any urgent safety measures are taken the investigator/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the MOHW (Jamaica), MHRA (UK), and the relevant IEC of the measures taken and the circumstances giving rise to those measures.

Please refer to the appropriate local regulations (i.e. MOHW and MHRA) for details on clinical trials safety reporting.

10.7. Statistical Analysis

10.7.1. Analysis Populations

The primary study population will be the PK population. The PK population will include those patients who have at least 1 successful blood sample taken and complete the study without significant protocol deviations/violations which are likely to affect the determination of the PK parameters. Further details on what is deemed a significant protocol deviation/violation will be documented in the SAP. This population will be used for the analysis of the primary variable.

Safety data will be presented for the Safety population, that is, it will include all patients who received IMP.

10.7.2. Sample Size

Up to 35 patients, both male and female, aged 6 months to 17.99 years of age will be recruited. This 'up to' recruitment figure will allow additional patients to be recruited to account for those patients from whom we are unable to obtain sufficient valid PK samples, for the age groups set out in the protocol.

There are no power calculations as such in this study. The objective of this study is to estimate the population PK of hydroxyurea following administration. From a statistical point of view, the collected plasma concentration data are expected to relate to a non-linear, mixed effects model involving repeated measures. The developed PK model will estimate the parameters of the model and their associated within- and between-patient variability.

10.7.3. Data Analysis

Statistical analyses will be performed after all patients have ended their participation in the study, protocol have been deviations reviewed, populations have been agreed, and the database has been locked.

Details of this analysis will be included in the SAP.

Patients withdrawn before treatment administration will not be assessed for outcome variables or safety.

Continuous variables will be summarized using descriptive statistics; n, mean, SD, %CV, median, minimum and maximum, quartiles (if appropriate), while categorical variables will be summarized as the number (and percentage) of patients in each category.

Baseline and demographic data will be summarized overall and by age group (age 6 months to 1.99 years, age 2 years to 5.99 years, and age 6 years to 17.99 years) as appropriate.

Concomitant medications will be tabulated by age group along with the current available World Health Organization (WHO) drug dictionary coding by primary term and generic drug name.

As part of the PK analysis, statistical significance will be declared at the 5% level (2-sided). All other safety analyses will be summarized using descriptive statistics.

10.7.4. Missing, Unused or Spurious Data

The handling of missing, unused, and spurious data will be described in the SAP.

10.7.5. Deviations from the Planned Statistical Analyses

Any changes to the planned analysis (as described in the protocol and SAP) will be documented in the statistical and clinical study reports.

11. REGULATORY AND ETHICAL CONSIDERATIONS

11.1. Good Clinical Practice

The investigator and Sponsor will ensure that this study will be performed in accordance with the protocol, the Declaration of Helsinki, and all applicable regulatory requirements. The study will be conducted according to the protocol and to the Sponsor's Standard Operating Procedures (SOPs) or any delegated service provider's SOPs, that meet the guidelines laid down by the ICH-GCP in clinical trials. The Sponsor has implemented a quality management system to manage quality throughout all stages of the study process, using a risk-based approach. A Study Management Plan will be developed and will include a risk analysis for identification, evaluation, control, communication, review, and reporting of the risks to the safety and integrity of the patients and the scientific integrity of the study. This risk analysis will be reviewed as appropriate during the study.

All principles enunciated in Jamaican MOHW Guidelines for the Conduct of Research on Human Subjects have been complied with.

11.2. Informed Consent

Before a patient can participate in the study, their parent(s)/legal guardian must give written informed consent to participation. Where applicable, the patient themselves will provide assent for the study. If the assent is not used, then the investigator taking consent should document the reason why in the medical notes. The informed consent process will be in accordance with ICH-GCP, the Declaration of Helsinki, and local regulatory requirements.

Patient information leaflets/informed consent forms will be based on master documents provided by the Sponsor and submitted and approved by the IEC. Patient information leaflets will be designed to target specific age groups and levels of understanding in order to obtain patient assent for the study as well as parental/legal guardian consent. The Sponsor must approve and retain copies of any changes requested by the IEC before the documents are used.

11.3. Regulatory Authority Approval

Before the study is initiated at a site the Sponsor will obtain approval to conduct the study from the appropriate regulatory authority in accordance with any applicable country specific requirements. Regulatory authority approval for the clinical study will be obtained prior to recruitment of patients into the study and shipment of IMP.

11.4. Independent Ethics Committee Requirements

Before initiation of the study at a given site written approval of the protocol, informed consent forms and patient information leaflets will be obtained from the appropriate IEC. If any amendments to any of these documents occur during the study, written approval must be obtained prior to their implementation. The site will also apply to their Local Authority and IEC as appropriate for approval to participate in the study. The principal investigator at each site is responsible for ensuring that these actions occur.

11.5. Data Safety Monitoring Committee

Before initiation of the study at a given site, the final study protocol will be supplied to the independent DMSC who will make recommendations to the Sponsor about any existing or potential safety issues.

11.6. Indemnity and Insurance

With respect to any liability directly or indirectly caused by the IMP in connection with this clinical study, the Sponsor assumes liability on behalf of the investigator for possible injury to the patient, provided the investigator has followed the instructions of the Sponsor in accordance with this protocol and any amendments thereto, that the IMP has been supplied by the Sponsor, and that the investigator has performed the clinical study in accordance with scientific practice and currently acceptable techniques and knowledge. The Sponsor's liability is covered by liability insurance.

11.7. Patient Confidentiality

The principal investigator must ensure that the patient's anonymity is maintained. On the EDC/eCRFs or other documents submitted to the Sponsor, patients should not be identified by their names, but by their assigned identification number and initials only. If patient names are included on copies of documents submitted to the Sponsor, the names (except for the initials) must be obliterated and replaced with the assigned study patient numbers.

The principal investigator should keep a separate log of patient identification numbers, names, addresses, telephone numbers, and hospital numbers (if applicable). Documents not for submission to the Sponsor, such as signed informed consent forms, should be maintained in strict confidence by the principal investigator in the study site file.

A screening failure log will be maintained for patients who have consented to participate in the study but who, for whatever reason, are not eligible, withdrawn or decide to withdraw prior to taking part. This log will contain the following information:

- Date of screening visit
- Patient study number
- Patient initials
- Reason for study withdrawal (if available)

An EDC entry will be created for these patients containing only the above data for monitoring purposes.

The investigator shall permit authorized representatives of the Sponsor, regulatory authorities and IECs to review that portion of the patient's medical record that is directly related to the study. As part of the required content of informed consent, the patient must be informed that his/her records will be reviewed in this manner.

11.8. Study Documentation and Storage

The investigator/institution should maintain the study documents in a comprehensive and centralized filing system that is suitable for inspection by representatives of the Sponsor and regulatory authorities. The investigator/institution should take measures to prevent accidental or premature destruction of these documents.

Essential documents, as defined by ICH-GCP, include the signed protocol and any amendment(s), copies of any completed paper CRFs, signed informed consent forms from the parent(s)/legal guardians of all patients for whom consent was obtained, hospital records, other source documents (including the EDC database), IEC approvals, and all related correspondence including approved documents, drug accountability records, site delegation lists and curriculum vitae, study correspondence, and a list of the patients' names and addresses.

The principal investigator must retain copies of all essential documents for the period specified by ICH-GCP and by applicable regulatory requirements.

The principal investigator will inform the Sponsor of the storage location of the essential documents and must contact the Sponsor for approval before disposing of any of these documents.

It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained. The Sponsor should be informed immediately by the investigator/institution of any change concerning archiving facilities.

11.9. End of Study

For administrative and safety reporting purposes the end of the study will be defined as 4 weeks after the last patient completes treatment (i.e. at the end of the safety follow-up period for the last patient). This provides for a single and conservative definition.

11.10. Publication Policy

The Sponsor will prepare a written clinical study report according to ICH-GCP guidelines to summarize the study following completion of the analysis.

Investigators may not submit study information for publication without prior consultation and written approval from the Sponsor. However, such approval should not be unreasonably withheld.

12. ADMINISTRATIVE OBLIGATIONS

12.1. Source Data

All relevant study data will be recorded in paper CRF's prior to the use of the EDC database. Where relevant data already exists on other source documents, such as laboratory or TCD reports, , the information required will be transcribed into the paper CRF/EDC database. All other data will be directly written into the CRF/EDC database. When the EDC database is live, the original paper CRFs will be retained with the other study documents as source data and should not be disposed of. These should be made available for monitoring.

12.2. Monitoring

Routine monitoring visits (where applicable) will be made by the monitors designated by the Sponsor to check compliance with the protocol, the completeness, accuracy and consistency of the data, and adherence to ICH-GCP.

The principal investigator must ensure that the EDC database is completed in a timely manner (~48 hours after visit completion a visit date must be created) and must allow periodical access to any previous paper CRFs, patient records, drug logs, and all other study-related documents and materials. The frequency of monitoring visits will be determined by factors such as study design and the site enrolment requirements.

The investigator will agree to provide the monitor direct access to the patients' source data, which may exist in the form of hospital records, patient files and notes, and laboratory assessment reports and results.

A risk adapted monitoring and source data verification strategy will be used for this study which will involve both on-site, central (database), and remote (off-site) monitoring. If access to a site is restricted (for example due to global pandemic or change in local or national guidelines) this may involve the use of remote monitoring tools and methods to perform off-site remote source data verification from an off-site location as supported by the FDA and EMA (see Section 13.1 Guidance Documents).

The method of remote monitoring may vary between sites or countries and there are several options available. For example, sharing/viewing of information via live screen captures or video conferencing (e.g., the virtual operating system, where no data is transferred but is instead viewed securely, on an end-to-end encrypted and isolated system, using screen captures to the monitor for review). Another method would be sharing of redacted or anonymized study-related source documents with the monitor electronically or uploading scanned PDFs. Patient confidentiality will always be maintained with whichever system is used.

12.3. Quality Control and Quality Assurance

Appropriately qualified and trained staff will be involved in this study. Staff at the investigational site will be instructed in the conduct of the study according to this protocol.

In order to check the compliance of the study regarding ICH-GCP, audits may be carried out by a quality assurance representative. The investigator will provide access to authorized persons during regulatory authority inspections or Sponsor audits.

12.4. Data Collection and Management

All CRF data will be entered into a validated EDC database provided by TrialStat and managed by McDougall Scientific Ltd. Prior to the EDC database, sites will complete paper CRFs for each patient who enters the study.

Electronic CRFs (eCRF) will be completed promptly on the EDC database (within 48 hours of the visit). When changes to CRF/eCRF data are necessary, these changes will be documented in the EDC database change report, and updated in the guidance documentation provided to the site.

Data items from the superseded paper CRFs will be entered retrospectively into the EDC database by Data Management with review and verification, prior to the EDC database going live. Following this, sites will enter ongoing data directly into the EDC database and retire the paper CRFs which will be retained to cross-check with as source data for monitoring purposes. Concomitant medication entered into the EDC database will be coded using the WHO drug dictionary. Coexisting diseases and AEs will be coded using the MedDRA. Laboratory samples will be processed, and results sent to Data Management for importing into the EDC database and to the sites directly.

Data Management will be responsible for data processing, in accordance with the Sponsor or Sponsor's delegated service provider's data management procedures. Database lock will occur once quality assurance procedures have been completed.

12.5. Adherence to the Protocol

Protocol deviations are any deviations from the procedures outlined in this document, for example, missed evaluations, incorrect timing of evaluations, non-compliance with IMP, and intake of prohibited medications. It is the investigator's responsibility to make all reasonable efforts to avoid protocol deviations in order to avoid possible exclusion of the patient from the study and/or analyses.

All protocol deviations will be reported immediately to the Sponsor and any action required, for example, discontinuation of the patient will be discussed. Evaluability of the patient(s) concerned will be performed by the Sponsor prior to the statistical analysis.

Significant deviations from the protocol or a significant number of protocol deviations could result in a discontinuation from the study of the site involved.

12.6. Study or Site Discontinuation

The Sponsor may temporarily or permanently discontinue the study at a single site or at all sites for safety, ethical, compliance or other reasons. If this is necessary, the Sponsor will endeavor to provide advance notification to the site(s) involved. If the site or study is suspended or discontinued, the principal investigator will be responsible for promptly informing the IEC. Where required by local regulations, the Sponsor will be responsible for informing the IEC of study or site discontinuation. In such cases, all study data and unused IMP must be returned to the Sponsor.

12.7. Personnel Responsibilities

The study will be conducted in accordance with the protocol, ICH-GCP, and applicable regulatory requirements.

12.7.1. Investigator

The investigator's responsibilities shall include but not be limited to:

- Adhering to the conduct of the study as described in this protocol
- Ensuring the accuracy and legibility of the CRFs and their security
- Immediately reporting any SAEs to the Sponsor and, if appropriate, the IEC

- Adhering to the guidelines described in the Declaration of Helsinki, ICH-GCP, and local regulatory requirements
- Informing the patient's general practitioner that the patient is taking part in the study, provided that the patient agrees to this contact. A copy of the correspondence should be filed in the Investigator Site File
- Provide expert research input and advice relating to study design and execution
- Be responsible for the review and sign-off of the final report

12.7.2. Clinical Monitor

The clinical monitor's responsibilities will be defined in the Monitoring Plan and shall include but aer not be limited to:

- Verifying protocol adherence
- Verifying the data on the CRFs/EDC with information in the patient's clinic notes and other source documents
- Ensuring the study documents such as the paper CRFs, protocol and any correspondence are maintained in a secure area
- Reporting and discussing any problems with the investigators and reporting them to the Sponsor

12.7.3. Sponsor

The study Sponsor's responsibilities shall include but not be limited to:

- Providing the principal investigator with all the necessary study documents and study supplies prior to study initiation
- Providing the investigator with updates on new developments regarding the IMP
- Sending the principal investigator financial reimbursement during the conduct of the study according to a schedule agreed upon in the budget

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APPENDICES

Appendix I	Individual Principal Investigator Signature Sheet
Appendix II	Protocol Amendments

13.1. APPENDIX I - INDIVIDUAL PRINCIPAL INVESTIGATOR SIGNATURE SHEETS

PRINCIPAL INVESTIGATOR SIGNATURE SHEET

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the study as outlined herein and in accordance with the ethical principles of the Declaration of Helsinki and ICH GCP guidelines, and in compliance with the local regulatory authority requirements. I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss the material with them to ensure they are fully informed regarding the drug and the conduct of the study.
I will only use the informed consent form approved by the Sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Independent Ethics Committee (IEC) responsible for the study.

INVESTIGATOR
(Principal Investigator)

Name: _____

Title: _____

Signed: _____

Date: _____

13.2. APPENDIX II - PROTOCOL AMENDMENTS

PROTOCOL AMENDMENT NUMBER 1 AND 2 (COMBINED):

Reason for Amendment - 1: Change in Service Provider from Rocky Mountain to NERI, and change in scope of activities to include Management of the DSMC. Clarification that Simbec-Orion Clinical Services will be working on the PK LABORATORY Analysis (not Hussain Mulla who is doing the PK analysis not the Laboratory Analysis)

The study will now additionally be run in up to four UK clinical sites in addition to the Jamaican Site. This has been added to the synopsis, and each UK site PI will be asked to sign a separate protocol signature sheet (see Appendix I).

The diagnosis of sickle cell disease has been amended to include clarification of the genotype (HbSS and HbSβ^0)

Introduction was changed to be explicit about availability of exposure data in infants and to delete reference to a bioequivalence study that is now complete.

The PK laboratory has reduced the volume of blood needed for PK analysis, so the volume will be reduced from 2mL to 1mL.

In Section 9.4.1 Reporting Procedures, the MHRA has been added to the SAE reporting requirements for the UK sites.

A UK PI signature sheet has been added in order that the PI's in the UK sites can sign an agreement to work to the protocol.

Minor typographical errors have been corrected.

Further consistency changes were made to the protocol Version 2 prior to full sign off following review by NERI, in order to ensure that there was no confusion over a partially signed off version of Protocol Version 2, the protocol will be signed off as Version 3.

In addition to consistency and typographical errors amended in Version 2 to Version 3. A request to reduce the minimum age of participants to 6 months (from FDA) was incorporated.

FOR FULL EDIT DETAILS PLEASE SEE TRACKED CHANGES DOCUMENT (PROTOCOL VERSION 3.0- 20180328_TRACKED CHANGES file name HU Protocol FINAL 3.0_20180328_tracked)

PROTOCOL AMENDMENT NUMBER 3:

Reason for Amendment: MHRA request for additions and clarifications

Synopsis:
Exclusion Criteria added: 13. Known infection with Human Immunodeficiency Virus (HIV).

Section 1.3.2
A section “Justification for dose and dose-escalation” was added.
Table 3.2 Schedule of Study Assessments
Urinalysis was amended to include pregnancy test if applicable, including a footnote “To include a urine pregnancy test to be performed no more than seven days before the first administration of hydroxyurea in all post-menarcheal females”
Section 5.12
The following was added: A urine pregnancy test will be performed no more than seven days before the first administration of hydroxyurea in all post-menarcheal females.
Section 6.2
Exclusion Criteria added: 13. Known infection with Human Immunodeficiency Virus (HIV).
Section 9.3
SAE definition was clarified: “SAE for a hospitalization due to pain or fever will be defined as an AE that requires inpatient hospitalization of > 7 days or prolongation of existing hospitalization. Hospitalization for an elective procedure will not classified as an SAE, unless the hospitalization is prolonged beyond what would normally be anticipated for the elective procedure. Hospitalization or prolongation of a hospitalization for an adverse event other than pain or fever will be considered an SAE”

References were added.

FOR FULL EDIT DETAILS PLEASE SEE TRACKED CHANGES DOCUMENT (PROTOCOL VERSION 4.0- 20181024_TRACKED CHANGES file name HU Protocol FINAL 4.0_20181024_tracked)

PROTOCOL AMENDMENT NUMBER 4:

Reason for Amendment:

1. Jamaican Ministry of Health's Ethics Committee request for clarification of name of IMP being hydroxyurea rather than hydroxycarbamide.
2. Proposed Brand name changed from Reostor to Xromi.
3. Laboratory discrepancies resolved.
4. Change to screening period window, so patients can start treatment as soon as screening confirms eligibility.
5. Blood sample for PK analysis at 6 hours has been made mandatory in older children in order that there is sufficient spread of sample time points across 0 to 6 hours.
6. Exclusion criteria 3 was amended, as mild proteinuria is commonly seen in SCA patients, this criterion was therefore changed to reflect renal insufficiency as evidenced by creatinine.
7. Exclusion criteria 6 was clarified to include “severe active infections” fungal, viral or bacterial and the phrase “that would preclude the use of hydroxyurea in normal clinical practice”
8. Child bearing potential was clarified as “post-menarcheal females”
9. The removal of “parenteral/oral” descriptor was removed from the definition of treatments for painful crises, as practice may vary, and it is not important.
10. Visit windows were clarified.
11. A reference to the sickle cell guidelines for Jamaica was added to section 1.3.1 and the reference section.
12. A statement that in the UK all children will be dosed according to this protocol and standard clinical practice in the UK was added to section 1.3.2
13. The ophthalmological and neurological component of the physical examination was made mandatory only at screening and exit, unless otherwise indicated. Physical examination requirements were clarified in the footnote to the schedule.
14. Minor typographical errors were corrected.
15. Page numbering was updated in the table of contents.
16. It was confirmed that there will be up to five clinical trial sites in the UK.
17. An interim analysis was added as a possibility at the point where all patients complete approximately 9 months of the study (i.e. when the last patient reaches their week 40 visit).
18. IMP was corrected, syringes and bottle stoppers will be sourced locally (although provided to Jamaican site, practice in the UK varies)
19. The SAE criteria regarding hospitalization was amended since hospitalization for fever or pain may not necessarily be for an SCA associated pain or fever.

Throughout Protocol:
Version and Date of the protocol was amended.
Hydroxycarbamide was changed to hydroxyurea

Synopsis:
Proposed Brand name changed from Reostor to Xromi.
Exclusion criterion 3. was amended to renal insufficiency as evidenced by creatinine levels: the text now reads “known creatinine more than twice the upper limit for age and > 1.0mg/dL [88.4 micromol/L].”
Exclusion criterion 6 was clarified to include “severe active infections” fungal, viral or bacterial and the phrase “that would preclude the use of hydroxyurea in normal clinical practice”
Exclusion criteria 9 and 10 females of child bearing potential was clarified by addition of “post-menarcheal females”
The wording relating to sample timing for the PK was clarified, plus the 6-hour sample was made mandatory in older children in order to ensure that samples out to six hours are obtained in a proportion of the participants.

The definition of painful crises was amended so that it was clearer, plus “parenteral or oral” was removed as descriptor from the definition of treatments for painful crises, as practice may vary.
In addition to the pre-dose sample the PK sample at 6 hours was made mandatory in children over 2. The total number of samples was made clearer. The following text is now included:
“The pre-dose sample is mandatory in all children, and 6-hour sample is mandatory in children over two years old. In children under two years old up to two post dose samples will be taken (three samples in total), and in children over two years old up to up to five additional samples will be taken (six samples in total including the pre-dose and mandatory 6-hour sample).
The removal of “parenteral/oral”
The following test was added to the synopsis:
An interim analysis may be planned when all patients complete approximately 9 months of the study (i.e. when the last patient reaches their week 40 visit). The interim analysis will include all study endpoints described in the SAP.

Section 1.3.1, Route:
A statement that the “overall management of children will be according to local clinical care guidelines”, was added rather than reference to Jamaican specific guidelines, to cover sites working in the UK.

Section 1.3.2 Dosage
Paragraph 7 “dose escalation” is from appropriate clinical guidance and should remain, however the following additional text was added to ensure that monitoring is however managed according to protocol: *“(however for the purposes of this study protocol the monitoring will be monthly)”*

Schematic and Schedule of Assessments:
The Screening Visit was amended from “-14 to -7” to “-14 to 0” so that children who are found to be eligible can start treatment immediately rather than wait for seven days, visit windows were also clarified and a footnote added.
The total number of PK samples was clarified as up to six in total in older children and up to three in total for children under 2 years.
An f was added to PK profile for Week 60, to align with protocol text that allows for a single sample at this visit if feasible.
Physical Examination was changed to: A general physical examination will include the following: ear/nose/throat (tonsils), dermatological, cardiovascular, respiratory, gastrointestinal, lymph nodes and musculoskeletal (at screening and week 24 and week 60 and WD unless otherwise indicated), for ophthalmological and central nervous system (at screening and week 60 and WD unless otherwise indicated). Directed Physical examination will include: Hepatomegaly, Splenomegaly (at screening and week 24 and week 60 and WD unless otherwise indicated). Other evaluations may be performed as deemed necessary by the investigator. This will be commented upon in the clinical study report, if applicable.
Footnote 9 was amended to include that a TCD performed within 3 months of the start of the study would be acceptable as screening TCD.
Changes were made to the footnotes to the schedule of assessments to correct discrepancies and omissions in the safety laboratory measures as follows:
Footnote 11: To standardize Full Blood Count additional measures were added “including” for the additional parameters, red blood cells (RBC), hematocrit, Lymphocytes, Monocytes, Eosinophils, Basophils, Mean Corpuscular Hemoglobin (MCH).
The requirement for hemoglobin electrophoresis was clarified, since this will not be a protocol scheduled assessment but may be done as standard of care for the patient if required to confirm diagnosis, the following text was added to footnote 11:
If a hemoglobin electrophoresis result is not already available and it is required to confirm SCD diagnosis, it is to be arranged locally as part of the patient’s standard of care).
The following text was added to footnote 15:
“and spot urine for urinary albumin/creatinine ratio (ACR).

Details regarding Cystatin C testing were moved to the appropriate footnote 14.
Footnote 15 changed to: At Screening, at Day 1, Month 6 (Week 24) and at Month 15 (Week 60), (or Withdrawal [if possible]) samples will be taken for Urinalysis (dipstick): protein, bilirubin, blood and urobilinogen. Spot urine for urinary albumin/creatinine ratio (ACR) will be done at Day 1, Month 6 (Week 24) and at Month 15 (Week 60), or if a patient withdraws, at the time of withdrawal. To include a urine pregnancy test to be performed no more than seven days before the first administration of hydroxyurea in all post-menarcheal females. Blood sample will be taken prior to dosing on PK days. The investigator may perform tests as clinically indicated, particularly if dose escalation is indicated.
Footnote 16 was changed to:
A full PK profile. The pre-dose sample is mandatory in all children, and 6-hour sample is mandatory in children over two years old. The pre-dose sample is mandatory in all children, and 6-hour sample is mandatory in children over two years old. In children under two years old up to two post dose samples will be taken (three samples in total), and in children over two years old up to up to five additional samples will be taken (six samples in total including the pre-dose and mandatory 6-hour sample). Further single samples will be obtained at regular clinic visits (Week 2-8, and Month 7-15) where feasible (f). Where a single PK sample is taken at the interim visit, the time of the previous hydroxyurea dose must be noted (where possible this sample will be taken at 12 hours post dose).

Section 4.0
The packaging was changed to show that the IMP is not packaged with oral dosing syringes as hospitals preferred to use their own in the UK. Syringes will be provided separately if required.

Section 5.1
The table number was amended from 4.6 to 5.1 (error). In addition to the pre-dose sample the PK sample at 6 hours was made mandatory in older children, and the number of additional samples amended to reflect the appropriate total number of samples (5 in older children and 3 in children under 2). The following text has been included:
"*The pre-dose sample is mandatory in all children, and 6-hour sample is mandatory in children over two years old. In children under two years old up to two post dose samples will be taken (three samples in total), and in children over two years old up to up to five additional samples will be taken (six samples in total including the pre-dose and mandatory 6-hour sample)."
The following text was added regarding the single sample at clinic "This sample should only be taken if the time and dose of the previous dose of Hydroxyurea is known, and it must be noted".

Section 5.7
The wording "uncomplicated sickle cell crisis" was removed as definition refers to definition of painful crisis, "parenteral/oral" descriptor was removed as practice may vary.

Section 5.9
The physical examination was amended to the following:
A general physical examination will include the following: ear/nose/throat (tonsils), dermatological, cardiovascular, respiratory, gastrointestinal, lymph nodes and musculoskeletal (at screening and week 24 and week 60 and WD unless otherwise indicated), for ophthalmological and central nervous system (at screening and week 60 and WD unless otherwise indicated). Directed Physical examination will include: Hepatomegaly, Splenomegaly (at screening and week 24 and week 60 and WD unless otherwise indicated). Other evaluations may be performed as deemed necessary by the investigator. This will be commented upon in the clinical study report, if applicable.

(however, results can be used from a TCD which has been performed within three months of screening), was added to the TCD

Section 5.11

The amendments to Full Blood Count detailed above for the schedule of events were also made to section 5.11.1.

Section 5.11.2
The amendments to the hemoglobin electrophoresis detailed above were also made to section 5.11.2

Section 5.15
Genotype (HbSS and HvSb0 information) was added to the data collected on the CRF. Where a single sample is collected at an interim visit the wording "must" was added to ensure time and dose of previous dose is recorded.

Section 6.2
Exclusion Criteria 3. Amendment of renal insufficiency to "known creatinine more than twice the upper limit for age and > 1.0mg/dL [88.4 micromol/L]))".
Clarification of exclusion Criteria 6 to include Severe infection- defined as one that would preclude the use of hydroxyurea in normal clinical practice.

Clarification of exclusion criteria 9 and 10 females of child bearing potential by addition of "post-menarcheal females"

Section 9.3
The SAE criteria regarding hospitalization was amended since hospitalization for fever or pain may not necessarily be for an SCA associated pain or fever.

The term "pain or fever" was replaced with "confirmed SCA complication" in order to ensure that a hospitalization for pain and/or fever that is not associated with SCA are reported as SAEs. Hospitalizations for known and "confirmed SCA associated complications" will therefore not be reported as SAE unless the hospitalization is greater than 7 days. In addition this was clarified since if the hospitalization is longer than usually anticipated for the type of event it should be reported as an SAE, the following text was added: "If the hospitalization for a confirmed SCA associated complication is longer than usually anticipated for the type of event/diagnosis on admission, then this will be defined as an SAE."

Section 10.0
The following text was added regarding the interim analysis:
An interim analysis may be planned when all patients complete approximately 9 months of the study (i.e. when the last patient reaches their week 40 visit). The interim analysis will include all study endpoints described in the SAP.

10.2 Safety and Secondary Analyses
The summary statistics for infections was amended to state "severe" rather than "serious" infections to be consistent with the definition of infections now used for infection in the exclusion criterion 3.
Adverse events relating to hematological toxicities (cytopenias) and severe infections will be additionally listed separately and appropriately summarized.

FOR FULL EDIT DETAILS PLEASE SEE TRACKED CHANGES DOCUMENT (PROTOCOL VERSION 5.0- 20190510_TRACKED CHANGES) file name HU Protocol FINAL V5.0_20190510_TRACKED CHANGES)

PROTOCOL AMENDMENT NUMBER 5:

Reason for amendment

1. Dose escalation parameters were amended to enable achievement of optimal dosing.
2. Total number of study participants was amended to allow recruitment of up to 35 patients. This will allow collection of sufficient number of samples especially in the <2 years old age groups.
3. Recruitment into the age groups has been clarified to permit more than 6 patients to be recruited into each group.
4. Remove the need for site staff to calculate BMI and z score as this will be done centrally by data management. This will ensure consistency across all sites.
5. The number of visits or attendance to the emergency room/facility will now be logged for each patient and recorded in the case report form.
6. Minor changes involving grammar, wordsmithing, punctuation, and other editorial changes have been made throughout the protocol. All are clearly identified in the track-changes version of the amendment.
7. Additional possible interim analysis point added when at least 12 patients have completed both PK visits at Day 1 and Week 24 for ongoing PK model development.
8. Clarification of urine pregnancy testing at Screening or PK Day1 (if screening visit was >7days) in Schedule of Assessments table.
9. Clarification that unscheduled lab assessments may be carried out at investigator discretion for standard of care and safety purposes.

Changes to dose escalation

Old text (in protocol version 5.0)
Section 1.3.2 Dosage

- **Starting dosage for all children: 15 mg/kg/day**

• Monitor CBC count with WBC differential and reticulocyte count at least every 4 weeks when adjusting dosage.
• Aim for a target absolute neutrophil count 2000-4000/µL; however:
  o Maintain platelet count >80 000/µL
• If hematological toxicity as defined by:
  o Neutropenia (ANC <1.0 x10^9/L). Additionally, if ANC < 1.5 10^9/L repeat CBC in a week, if there are concerns about toxicity, but maintain the same dose.
  o Thrombocytopenia (platelets <80 x10^9/L)
  o Absolute reticulocyte count (ARC <80 x10^9/L), unless Hb concentration > 9.0g/dl,
  o A 20% decrease in Hb concentration, from baseline or Hb <4.5g/dL.
Temporarily stop hydroxyurea dosing and:
  o Monitor CBC count with WBC differential weekly until toxicity resolves
  o If hematological toxicity resolves in 1 week reinstitute hydroxyurea at the same dose. If hematological toxicity persists for >1 week or occurs twice in a 3 month period, the hydroxyurea dose must be withheld until recovery and then reduced by 2.5 mg/kg/day, or at a dose of 2.5-5.0 mg/kg/d lower than the dose given before onset of cytopenias.

If dose escalation is warranted based on clinical and laboratory findings, guidance recommends to proceed as follows:
  • Increase by 5-mg/kg/day increments every 8 weeks
  • Give until mild myelosuppression (absolute neutrophil count of 2000-4000/µL) is achieved, up to a **maximum of 35mg/kg/day**
  • Once a stable dose is established, laboratory safety monitoring should include CBC count with WBC differential, reticulocyte count, and platelet count every 3 months and blood

chemistry every 3 months. *(however for the purposes of this study protocol the monitoring will be monthly)*

Doses will be calculated based on most current bodyweight (measured at each clinic visit). Doses
will be adjusted where necessary in minimum increments/decrements of 10mg. Calculations will be made using dosing calculator or by formula.

New text (in protocol version 6.0)

1.3.3 Dosage and dose escalation

- **Starting dosage for all patients: 15mg/kg/day**
- Monitor complete blood count (CBC) with WBC differential and reticulocyte count about every 4 weeks when adjusting dosage.
- Aim for a target absolute neutrophil count 1-3 $x10^9$ /L; however:
  - Maintain platelet count >80 $x10^9$ /L
- If hematological toxicity occurs as defined by:
  - Neutropenia (ANC <1.0 $x10^9$ /L). Additionally, if ANC < 1.5 $x10^9$ /L repeat CBC in a week, if there are concerns about toxicity, but maintain the same dose.
  - Thrombocytopenia (platelets <80 $x10^9$ /L)
  - Absolute reticulocyte count (ARC <80 $x10^9$ /L), unless Hb concentration ≥ 9.0g/dl,
  - A 20% decrease in Hb concentration, from baseline or Hb <4.5g/dL.

  Temporarily stop hydroxyurea dosing and:
  - Monitor CBC count with WBC differential weekly until toxicity resolves
  - If hematological toxicity resolves in 1 week restart hydroxyurea at the same dose. If hematological toxicity persists for >1 week or occurs twice in a 3-month period, the hydroxyurea dose should be withheld until recovery and then reduced by about 2.5 mg/kg/day, or at a dose of 2.5-5.0 mg/kg/day lower than the dose given before onset of cytopenias.

If dose escalation is warranted based on clinical and laboratory findings, typically failure to achieve mild neutrophil and reticulocyte suppression, guidance recommends to proceed as follows:

- Increase by 5-mg/kg/day increments every 8 weeks
- Give until mild myelosuppression (absolute neutrophil count of 1-3 $x10^9$ /L) is achieved. However, if ARC remains high (>200 $x10^9$/L), dose escalation may continue if clinically indicated, to achieve the optimal dosing.
- **Dose must not exceed a maximum dose of 35mg/kg/day**.
- Once a stable dose is established, laboratory safety monitoring should include CBC count with WBC differential, reticulocyte count, platelet count and blood chemistry every 4 weeks.

Doses will be calculated based on most current bodyweight (measured at each clinic visit). Doses will be rounded to the nearest 10mg. Calculations will be made using dosing calculator or by formula.
Patients will be reminded (as per standard practice) that the effectiveness of hydroxyurea depends on their adherence to daily dosing; they should be counselled not to double up doses if a dose is missed. Hydroxyurea therapy will be continued during hospitalizations or illness unless there is hematological toxicity.

FOR FULL EDIT DETAILS PLEASE SEE TRACKED CHANGES DOCUMENT (PROTOCOL VERSION 6.0- 20191121_TRACKED CHANGES)

PROTOCOL AMENDMENT NUMBER 6: (Protocol v7.0)

Reason for Amendment:

1. Risk Assessment and Responsiveness to Coronavirus (COVID-19) pandemic period, to safeguard participants and enable the trial to continue under the Government and local restrictions on travel and hospitals.
2. Increase in period between study visits by up to 12 weeks in stable participants, (60-week final visit remains unaffected by this change and maximal period on study drug remains 15 months).
3. Replacement of some on-site clinic visits with telephone calls (where applicable).
4. Provision of additional IMP supplies to participants at dispensing visits.
5. Provision of additional IMP supplies directly to participants via use of courier service.
6. Greater flexibility and pro-active investigator discretion on dosage changes in response to trends in toxicity, allowing reduction by 2.5-5mg/ml increments prior to confirmation of toxicity.
7. Flexibility in secondary outcome measures at interim visits between 20-36, which only need to be taken once.
8. Flexibility for Week 24 PK visit to occur at any on-site visit between 20-36.
9. Reduction in the overall number of PK samples required on PK days for ≥2 year olds from 5 to 3-5 (in addition to pre-dose sample).
10. The requirement for a mandatory 6-hour post-dose sample on PK days has been removed and the timing of post-dose samples will be left to investigator discretion to help reduce the time participants will spend in the hospital/clinic.
11. Reduction in the number of PK samples required on PK days for the >2 year olds, from 5 additional samples to the investigator discretional 3-5 additional samples (and a pre-dose).
12. Interim individual PK samples can be taken at any visit in which the participant is attending for safety bloods.
13. Palatability questionnaire can be carried out once at any visit from 4-60 weeks or at early WD.
14. Participant travel expenses will now include the availability of taxi services which can be arranged by the local site.
15. Recruitment during this period will be at investigator discretion.
16. On-site monitoring during this period will be adaptive based on local and Government restrictions. Remote monitoring will be used in the interim.
17. Flexibility added to requirements for interim analysis (reduction in number of patients to complete 24 weeks rather than 9 months) and potential for additional interim analysis to be added at a later date to capture additional time points.
18. Addition of BfArM to section 10.6.

Throughout Protocol:
Version and Date of the protocol was amended.

*C*** used to signify a change to the protocol in response to the Coronavirus (COVID-19) pandemic period.

FOR FULL EDIT DETAILS PLEASE SEE TRACKED CHANGES DOCUMENT (PROTOCOL VERSION 7.0_TRACKED CHANGES)

PROTOCOL AMENDMENT NUMBER 7: (Protocol v8.0) JAMAICA ONLY

1. Correction to synopsis in the Treatment Strategy section for the absolute neutrophil count from 2000 – 4000 /UL to 1-3 $x10^9$ /L. This was updated in the main body of the protocol in version 5, but had not been changed in the synopsis.
2. Correction to synopsis to include Cystatin C in the list of biomarkers, which is in the main body of the text since the trial went live but had not been included in the synopsis.
3. Change to the administrative structure to the party responsible for Pharmacokinetics, this is now being handled by BAST.
4. Change to the to the administrative structure to the party responsible for Pharmacokinetic bioanalysis/lab analysis, previously Simbec-Orion, this is now being handled by Concept Life Sciences (CLS).
5. Change to the administrative structure to the party responsible for Data management, previously NERI-Healthcore, this is now McDougall Scientific Limited who are also responsible for statistical analysis.
6. Section 1.4.1.4 Site Monitoring Visits updated to include the use of remote monitoring for remote Source Data Verification which has been supported by regulatory authorities as a risk adapted method for trials. This includes a description of several methods to achieve this which may be site/country dependent depending on local regulatory approvals and includes an example of a remote monitoring tool/system (e.g. such as the virtual operating system).
7. Addition to Section 13.1 Guidance Documents, EMA/FDA/BfARM separate regulatory guidelines for the risk-adapted management of clinical trials during COVID-19 (Mar 2020).
8. Name change throughout protocol for the Jamaican Ministry of Health (MOH), to the Jamaican 'Ministry of Health and Wellness (MOHW)'.

Throughout Protocol:
Version and Date of the protocol was amended – Protocol v8.0 for use in Jamaica only.

FOR FULL EDIT DETAILS PLEASE SEE TRACKED CHANGES DOCUMENT (PROTOCOL VERSION 8.0 JAMAICA ONLY_TRACKED CHANGES)

PROTOCOL AMENDMENT NUMBER 8: (Protocol v9.0)

Reason for Amendment:

1. Changes to administrative structure of study, change of Data Management and Statistics provider, change of Pharmacokinetic modelling provider, change of Pharmacokinetic bioanalysis provider, changes to Simbec-Orion responsibilities.
2. Removal of COVID-19 specific flexibility to the protocol denoted by (C**) throughout, this has now been changed to allow generic flexibility irrespective of COVID-19 restrictions.
3. Incorporation of Protocol v8.0 (Jamaica Protocol) specific changes (see summary of changes above for Protocol v8.0).
4. Clarification and correction of errors in the protocol synopsis in accordance with the protocol text (omission of Secondary Endpoints and correction to ANC values to match text from protocol v6.0 1-3 × 10^9/L).
5. Removal of Germany as a study site location.
6. Clarification to study procedures for Safety and Pharmacokinetics in study summary and Sections 1.3.4, Section 3.1, Figure 1, Table 1, to accommodate flexibility of in clinic visits and incorporate telephone calls.
7. Change to allow telephone visits in place of in-clinic visits at investigator discretion throughout study up to a maximum of 3months between in-clinic visits.
8. Clarification to definition of end of study requirements to attend an in-clinic Week 60 visit.
9. Removal of second planned interim analysis. Only one interim analysis is now planned due to the impact of COVID-19.
10. Addition of an Electronic Data Capture database for case report form data collection for the study.
11. Update to the rationale for the study to include the current licensing of Xromi® for > 2 years in the UK and EU.
12. Clarification to dose changes at investigator discretion in-line with in-clinic visit flexibility.
13. Update to benefits and risks to include updated information in Investigator Brochure and recent DSUR report.
14. Site Monitoring Visits 1.4.1.4 moved to Section 10.6 to incorporate the need for remote monitoring outside of COVID-19 restrictions.
15. Addition of hematocrit and clarification that it is WBC ‘differentials’ omitted in error from secondary objectives, but included elsewhere in the previously approved text.
16. Addition of mean corpuscular hemoglobin as a safety endpoint and cystatin C as a biomarker.
17. IMP deliveries to patient homes to be permitted outside of COVID-19 pandemic restrictions at investigator discretion.
18. Clarifications to IMP handling and storage.
19. Clarifications to blood sampling for pharmacokinetic analysis (Section 5) in line with global protocol flexibility.
20. Clarifications to study follow-up/final visit (Section 5.2) in line with global protocol flexibility.
21. Correction to Section 5.3 (demographics), to remove date of birth. Only age will be collected on the study database.
22. Clarification to the definition and how to record medical history.
23. Section 5.13 updated in line with previous schedule of events to include repeat of screening pregnancy if > 7 days in text for consistency.
24. In Section 5.14, it clarified that palatability and acceptability assessments can be conducted once, at any point after 8 weeks on treatment.
25. Sections 6.6, 7.4 and 7.5 were updated to clarify that participants should aim to complete study within 12-15 months or 6 months after MTD, whichever is sooner.
26. In Section 7.3, monitoring compliance was updated to clarify instructions to site on pharmacokinetic same day dosing.
27. In Section 9.2, reporting of adverse events guidance was updated to include how to report worsening in chronic conditions to aid serious adverse event/adverse reconciliation.

28. In Section 10.6, removal of written notice of urgent safety measures to BfArM following removal of Germany as a study site location.
29. In Section 11.7, patient confidentiality updated to incorporate use of EDC database and to correct the omission of date of screening date from the screening log.
30. Section 11.8 was updated to include EDC CRF data.
31. Section 12 was updated to include use of EDC database.
32. In Section 12.2, monitoring was updated to include risk adapted monitoring, specifically to incorporate the remote and central monitoring process for the study.
33. Guidance documents for the management of COVID-19 pandemic have been included.
34. Minor changes involving grammar, wordsmithing, punctuation, and other editorial changes have been made throughout the protocol. All are clearly identified in the track-changes version of the amendment.

FOR FULL EDIT DETAILS PLEASE SEE TRACKED CHANGES DOCUMENT (PROTOCOL VERSION 9.0 TRACKED CHANGES)