A comparative observational study to evaluate the safety and effectiveness of Xromi® (hydroxycarbamide oral solution 100mg/ml) for the prevention of vaso-occlusive complications of sickle cell disease in children under 2 years of age

Study Protocol

Sponsor	Nova Laboratories Ltd.
Title	A comparative observational study to evaluate the safety and effectiveness of Xromi® (hydroxycarbamide oral solution 100mg/ml) for the prevention of vaso-occlusive complications of sickle cell disease in children under 2 years of age.
Short Title and Acronym	PASS of Xromi® comparing safety and EffeCtiveness in children under 2 years with Sickle cEll disease [PRECISE PASS]
Protocol version identifier and Ref ID	NOVDD-001 v3.0; 05 Ago 2025
Date of last version	18 October 2024
EU PAS Register number	EUPAS1000000076
Active substance	Hydroxycarbamide (ATC Code L01XX05)
Medicinal product	Oral Hydroxycarbamide solution (Xromi®)
Product reference	EU/1/19/1366/001 | PLGB 13581/0003
Procedure number	EMEA/H/C/004837/0000
Joint PASS	No
Research Question & Objectives	**Research Question:** To evaluate the safety and effectiveness of hydroxycarbamide oral solution 100mg/ml (Xromi®) administered to children under 2 years of age for the prevention of complications of sickle cell disease (SCD).

	Primary Objective: • To assess the incidence of adverse events of special interest (AESIs) in children who have SCD and are treated with Xromi®, in comparison to a retrospective comparator cohort. **Secondary Objective:** • To assess the effectiveness of Xromi® for the clinical events and laboratory tests and physiological assessments of interest, in comparison to a retrospective comparator cohort. **Exploratory Objectives:** • To assess the overall safety of Xromi® by describing adverse events (AEs) by severity, seriousness, relatedness, action taken, outcome, and duration. • To describe occurrence of and reasons for discontinuation of Xromi®. • To assess the safety of Xromi® at the optimised dose. • To assess if the effectiveness of Xromi® is associated with the dose. • To assess the safety and effectiveness of Xromi® by subgroups (age, sex, β-globin genotype, country). • To assess occurrence of clinical events in comparators exposed to any formulation of hydroxycarbamide during follow-up. • To assess organ function and potential preservation in children exposed to any formulation of hydroxycarbamide.
Country(-ies) of study	Germany and the United Kingdom. Inclusion of other EU countries will be assessed.
Author(s)	This protocol was prepared by: • Dr Nawab Qizilbash, MRCP DPhil (OXON Epidemiology Ltd) • Irene Talavera Rodríguez, MSc (OXON Epidemiology Ltd) • Marina Hinojosa Campos, MSc (OXON Epidemiology Ltd) • Cono Ariti, MSc (OXON Epidemiology Ltd) • Dylan Taylor, MSc (OXON Epidemiology Ltd) • Dr Hussain Mulla, PhD (Nova Laboratories Ltd) • Dr Sarah Edwards, PhD (Nova Laboratories Ltd) • Dr Sara Trompeter, MB ChB MRCPCH FRCPath (University College London Hospital NHS Foundation Trust)

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PROTOCOL SIGNATURE PAGE PRINCIPAL INVESTIGATOR

Investigator's Declaration

- I confirm that I have read the above-mentioned study protocol and its attachments.
- I agree to conduct the study in compliance with the provisions of the protocol.
- I will conduct the study in accordance with Good Clinical Practice, the Declaration of Helsinki, Good Pharmacovigilance Practices, the applicable regulatory requirements and the moral, ethical, and scientific principles that justify medical research.
- In my formal capacity as Principal Investigator, my duties include ensuring the safety and wellbeing of the study participants enrolled under my supervision and providing Nova Laboratories Ltd. with complete and timely information, as outlined in the protocol.
- I agree to personally conduct or supervise this study and to ensure that the study team assisting in the conduct of the study is informed about their obligations in meeting these commitments.
- It is understood that all information related to the study will be kept strictly confidential and that this confidentiality requirement applies to the study participants and the study team.
- I agree to maintain adequate and accurate study records and to make those records available for audits and inspections in accordance with relevant regulatory requirements.

Signature	Date of signature

Print Full Name:	[Full Name]
Title:	[Position]
Site Name:	[Insert address of institution] [Insert phone, e-mail]

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1. LIST OF ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
AESI	Adverse Event of Special Interest
ACR	Albumin to Creatinine Ratio
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
ARC	Absolute Reticulocyte Count
ALT	Alanine Transaminase
AST	Aspartate Transaminase
CI	Confidence Interval
CrCl	Creatinine Clearance
CRO	Contract Research Organisation
CTCAE	Common Terminology Criteria for Adverse Events
DMP	Data Management Plan
EC	Ethics Committee
eCRF	Electronic Case Report Form
ED	Emergency Department
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
ePSL	Electronic Participant Screening Log
EU	European Union
EU PAS Register	European Union Electronic Register of Post-Authorisation Studies
FBC	Full Blood Count
FDA	Food and Drug Administration
GPP	Good Pharmacoepidemiology Practices
Hb	Haemoglobin
HbF	Foetal Haemoglobin
HbS	Haemoglobin S (sickle haemoglobin)
HbS/β^{+}	Sickle Beta + Thalassaemia
HbS/β^{0}	Sickle Beta-0-Thalassaemia
HbS/C	Sickle Haemoglobin-C Disease

Abbreviation	Description
HbS/S	Homozygous Sickle Cell Disease
HbSβ	Sickle Beta-Thalassaemia
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
HMA-EMA	Heads of Medicine Agencies – European Medicines Agency
ICF	Informed Consent Form
ISF	Investigator Site File
IMP	Investigational Medicinal Product
LDH	Lactate Dehydrogenase
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume
MTD	Maximum Tolerated Dose
PASS	Post-Authorisation Safety Study
PIL	Participant Identification Log
PT	Preferred Term
PV	Pharmacovigilance
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SCA	Sickle Cell Anaemia
SCD	Sickle Cell Disease
SD	Standard Deviation
SMP	Safety Management Plan
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOP	Standard Operating Procedure
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAMV	Maximum Time-Averaged Mean Velocity
TCD	Transcranial Doppler
UK	United Kingdom
USA	United States of America

2. RESPONSIBLE PARTIES

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3. ABSTRACT

Title

A comparative observational study to evaluate the safety and effectiveness of Xromi® (hydroxycarbamide oral solution 100mg/ml) for the prevention of vaso-occlusive complications of sickle cell disease in children under 2 years of age.

Rationale & Background

Sickle cell disease (SCD) is a multisystem disease, associated with episodes of acute illness and progressive organ damage, and is one of the most common severe monogenic disorders worldwide. SCD clinical manifestations include recurrent vaso-occlusive pain, splenic sequestration, aplastic crisis, acute chest syndrome, stroke, priapism, anaemia, substantial kidney injury, bacterial sepsis and meningitis, delayed growth and sexual maturation, or skin ulcers. Infants with SCD are somewhat protected from exhibiting clinical symptoms within the first 6 months of life, due to elevated levels of foetal haemoglobin (HbF), as it prevents polymerisation of the defective sickle haemoglobin (HbS) and can prevent sickling and haemolysis if present in high enough concentrations.

Hydroxycarbamide is a pharmacological therapy for SCD that increases total Hb and HbF and improves blood flow through reduced intracellular adhesion, reducing the incidence of pain episodes and acute chest syndrome episodes. Hydroxycarbamide 100mg/ml oral solution (Xromi®) was first approved for use in the European Union (EU) in July 2019 in children with SCD from the age of 2 years, and in 2024 the license was extended in both the EU and the United Kingdom (UK) to include infants from the age of 9 months. As a condition of the license extension, the authorities requested additional safety and efficacy data to further establish the risk benefit profile of hydroxycarbamide in children between the age of 9 months and 2 years. The present study is a combined Post-Authorisation Safety Study and a Post-Authorisation Efficacy Study that aims to provide data on the safety and effectiveness of hydroxycarbamide 100mg/ml oral solution administered to children under 2 years of age, over a follow-up period of 24 months.

Research Question & Objectives

The research question is to evaluate the safety and effectiveness of hydroxycarbamide oral solution 100mg/ml (Xromi®) administered to children under 2 years of age for the prevention of complications of SCD.

Primary objective:

- To assess the incidence of adverse events of special interest (AESIs) in children who have SCD and are treated with Xromi®, in comparison to a retrospective comparator cohort.

Secondary objective:

- To assess the effectiveness of Xromi® for the clinical events and laboratory tests and physiological assessments of interest, in comparison to a retrospective comparator cohort.

Exploratory objectives:

- To assess the overall safety of Xromi® by describing adverse events (AEs) by severity, seriousness, relatedness, action taken, outcome, and duration.
- To describe occurrence of and reasons for discontinuation of Xromi®.
- To assess the safety of Xromi® at the optimised dose.
- To assess if the effectiveness of Xromi® is associated with the dose.
- To assess the safety and effectiveness of Xromi® by subgroups (age, sex, β-globin genotype, country).
- To assess occurrence of clinical events in comparators exposed to any formulation of hydroxycarbamide during follow-up.
- To assess organ function and potential preservation in children exposed to any formulation of hydroxycarbamide.

Study Design

This is a non-randomised, non-interventional, comparative, observational, hybrid prospective and retrospective, post-authorisation cohort study (a matched cohort design) of children with SCD from 9 months to under 2 years of age to assess the safety and effectiveness of Xromi®. There will be 2 cohorts: i) a Xromi® exposure cohort, identified prospectively; and ii) a retrospective comparator cohort with children with SCD unexposed to any formulation of hydroxycarbamide at the index date, identified using data from the last 10 years up to the date Xromi® was first used in children under 2 years of age at the sites. Comparator participants will be matched to exposed participants following a 2:1 ratio (comparator:exposed) based on study site, age at initiation of Xromi® of the exposed participant, and β-globin genotype. Matching ratio and factors will be reviewed and adjusted (e.g., matching at country level), considering feasibility. Information will be collected from chart review. The index date will be defined as: i) prospective exposure cohort: the date of confirmed initiation of Xromi® or, if unknown, the date of the first prescription of Xromi®; or as ii) retrospective comparator cohort: the date of the recorded visit when their age was closest to the age at index date of the matched exposed participant (i.e., the selected visit for matching). All participants will be followed up for up to 24 months from their index date, regardless of changes in their treatment regimen. This includes participants in the prospective exposure cohort who discontinue Xromi® or switch to another formulation of hydroxycarbamide, as well as participants in the comparator cohort who initiate any formulation of hydroxycarbamide after their index date.

The inclusion period for the participants within the prospective exposure cohort will be from the date the site is given a site initiation greenlight until 2 years after the study start date. These participants will be identified prospectively, and the follow-up will be prospective. This period may vary depending on the time needed to achieve the recruitment target. For the retrospective comparator cohort, the inclusion period will begin up to 10 years before the date of first administration of Xromi® to children under 2 years of age at each study site. It will continue until the date of first administration of Xromi® to children under 2 years of age at each study site or 2 years before the study start date, whichever occurs first. These participants will be identified retrospectively, and the follow-up will be retrospective.

Study Population

The study will seek the participation of European specialist sites. The study population will consist of children with SCD who meet all the inclusion criteria and none of the exclusion criteria.

Prospective exposure cohort

- Inclusion criteria:
  - Aged from 9 months to under 2 years at the index date.
  - Diagnosis of SCD.
  - Known β-globin genotype at the index date.
  - Prescribed Xromi® for the prevention of complications of SCD.
  - Parent(s) (or a legal representative(s)) provides written informed consent to participate in the study, unless there is a waiver, non-opposition, or blanket written informed consent by the parent for research studies.
- Exclusion criteria:
  - Previous use of hydroxycarbamide of any formulation before the index date.
  - Receiving regular blood transfusions (occurring every 8 weeks or more frequently) at the index date.
  - Known hypersensitivity to any of the excipients of Xromi® at the index date.
  - Contraindications to the drug at the index date:
    - Severe hepatic impairment (Child-Pugh classification C).
    - Severe renal impairment (creatinine clearance: CrCl <30 ml/min).
    - Presence of at least one of the following: Absolute neutrophil count (ANC) < 1.0 x 10^9/L, absolute reticulocyte count (ARC) <80 x 10^9/L, platelets <80 x 10^9/L.
  - Participating in another clinical study of an investigational medicinal product (IMP) at the index date.
  - Anti-retroviral medicinal products for human immunodeficiency virus (HIV) at the index date.
  - Active malignancy at the index date.

Retrospective comparator cohort

- Inclusion criteria:
  - Aged from 9 months to under 2 years at the index date.
  - Diagnosis of SCD.
  - Known β-globin genotype.
  - Matched to an exposed participant.

- Parent(s) (or a legal representative(s)) provides written informed consent to participate in the study, unless there is a waiver, non-opposition, or blanket written informed consent by the parent for research studies.

- Exclusion criteria:
  - Use of hydroxycarbamide of any formulation before or at the index date.
  - Receiving regular blood transfusions (occurring every 8 weeks or more frequently) at the index date.
  - Presence at the index date of any of the following:
    - Severe hepatic impairment (Child-Pugh classification C).
    - Severe renal impairment (CrCl <30 ml/min).
    - Presence of at least one of the following: ANC < 1.0 x 10^9/L, ARC < 80 x 10^9/L, platelets < 80 x 10^9/L.
  - Participating in another clinical study of an IMP at the index date.
  - Anti-retroviral medicinal products for HIV at the index date.
  - Active malignancy at the index date.

Variables

Information on demographics, anthropometrics, and other medical history will be collected at baseline and during follow-up. Information to describe SCD will be collected at baseline (e.g. age at first SCD symptoms, β-globin genotype, history of SCD manifestations and hospitalisations). Data about SCD management, including previous and concomitant use of treatments, will be collected at baseline and during follow-up.

To assess safety of Xromi®, occurrence of AEs, including AESIs (e.g. myelosuppression), will be collected during follow-up. To assess effectiveness of Xromi®, occurrence of the clinical events of interest (e.g. painful vaso-occlusive events, dactylitis, splenomegaly, acute chest syndrome, hospitalisations, blood transfusions, cerebrovascular accident [stroke]) will be collected during follow-up, and results from laboratory tests and physiological assessments conducted will be collected at baseline and during follow-up.

Data Sources

The data will be collected by the investigational team in participating sites from clinical records. Data obtained as part of participants' routine clinical practice will be collected during clinic visits for prospective data and from chart review for retrospective data.

Study Size

The study aims to recruit at least 180 participants in total: 60 participants administered Xromi® and 120 comparator participants, during a 4-year study period. Initially, up to 10 sites in the UK and up to 5 sites in Germany are planned to be invited to participate in the study. The inclusion of other potential European countries and sites will be assessed if needed.

Sample sizes were estimated assuming that 60 participants will be recruited to the Xromi® cohort, and each exposed participant will be matched to 2 comparators. All calculations were conducted using 80% power and a two-sided type I error rate of 5%. For safety, the minimum detectable hazard ratio (HR) was estimated by assuming the log rank test as the basis of the comparison. As it is possible that not all participants will be followed up for the full 24-month period, the calculations were repeated for average follow-up durations of 18 months and 12 months. For effectiveness, a similar approach was used.

Data Analysis

This study will be mainly exploratory and there are no previous hypotheses to test. It will use a comparative matched cohort design. Descriptive analyses will describe the study participants using summary statistics, tabulations, and descriptive figures.

The primary safety analysis will examine the occurrence of safety events of interest. For each AESI the number of events, person-years of exposure and number of participants experiencing an AESI will be reported. The incidence rate of the AESI in each study cohort will be calculated and expressed per 100 person years, with 95% confidence intervals (CIs) calculated using the exact Poisson formula. The percentage of participants experiencing each AESI will be reported. Kaplan-Meier curves, stratified by cohort, with risk tables, will be provided. Time-to-AESI will be summarised using Kaplan-Meier estimates of the median time-to-event and 95% CI. A Cox proportional hazards model, including cohort as its only covariate, will be used to derive crude HRs and 95% CIs. A second confounder-adjusted multivariable Cox model will be used to report adjusted HR, 95% CIs and p-value.

For clinical events and biochemical/physiological factors, a similar approach will be applied, including descriptive analyses and, for the examination of clinical events, Kaplan-Meier/Cox model evaluations. Longitudinal changes in biochemical/physiological factors will be examined using linear mixed effects models, with time treated as a continuous variable. The mean difference in the response variable between the exposed and comparator participants will be calculated from the model at fixed time points after index date. The corresponding 95% CI and p-value will also be calculated from the model results. A fully adjusted model will then further include the confounding variables described in the statistical analysis plan (SAP). Mean differences 95% CIs and p-values will be reported at each time point.

AEs will be tabulated separately with the number and proportion of participants, and number of events, with 95% CIs calculated using the Clopper-Pearson method for each of the exposed and comparator groups. Time-to-discontinuation of Xromi® will be described using Kaplan-Meier analysis, with discontinuation reasons summarised by counts and percentages. The analysis of AESIs will be repeated comparing participants on and not on optimised dose, using appropriate survival curve estimates and Cox models with a time-dependent variable for dose attainment. HRs comparing the exposed participants with the comparator participants and corresponding 95% CIs will also be derived. The same approach will be used to analyse effectiveness at the optimised dose. Additionally, the primary safety and effectiveness analyses will be repeated for each subgroup of interest.

Poisson regression models will be used to compare incidence rates of clinical events in the comparator group comparing periods of exposure to any formulation of hydroxycarbamide with periods with no exposure by calculating incidence rate ratios and 95% CIs. The time to splenic sequestration, stroke

and acute chest syndrome will be used to measure organ damage in the pooled cohort of participants who received any formulation of hydroxycarbamide. Kaplan-Meier methods will be used to describe survival curves and median time to organ damage if sufficient data allow. Differences between survival curves will be assessed using the log-rank test. HRs and 95% CIs will be calculated using a Cox proportional hazards model. An unadjusted and adjusted Cox model will be used to calculate HRs and 95% CIs.

4. AMENDMENTS AND UPDATES

Amendment Number	Planned date	Section	Reason
1	21 October 2024	Sections 5, 7, 8, 10. Other sections have been updated to reflect these changes, if needed.	Changes implemented after receiving European Medicines Agency (EMA) PRAC Rapporteur Assessment Report.
	Amendment/update		
	Section 5: Milestones have been updated. Section 7: The only secondary objective is: "*To assess the effectiveness of Xromi® for the clinical events and laboratory tests and physiological assessments of interest, in comparison to a retrospective comparator cohort*". All other secondary objectives have been moved to exploratory. Section 8: Deletion of the retrospective exposure cohort. Definition of the baseline visit added. Inclusion criteria updated to children aged 9 months to under 2 years. Addition of 'Screening status' to be collected at baseline and 'Fluid retention, including oedema' to be collected at baseline and follow-up. Definition of myelosuppression updated. The criteria for assessing the enrolment of the comparator cohort have been updated to align with the eligibility criteria. Analysis section updated to reflect the above changes. Limitations expanded to include the impact of changes in standard clinical care. Section 10: Relatedness will be assessed using the WHO-UMC system. Minor wording adjustments have been made throughout the document to enhance clarity.		
Amendment Number	**Planned date**	**Section**	**Reason**
2	31 Jul 2025	Protocol signature page QPPV. Sections 2 and 5.	Staffing changes to the EUQPPV and UKQPPV.
	Amendment/update		
	Protocol signature page QPPV and Section 2: EUQPPV and UKQPPV updated. Section 5: Milestones planned dates updated. Typographical and/or grammatical errors corrected.		

5. MILESTONES

Milestone	Planned date
Registration in the EU PAS register	March 2024
Start of study (first site Greenlight)	29 Apr 2025
Start of data collection (first patient enrolled)	June 2025
End of data collection	June 2029
Interim progress reports	May 2026 and May 2028
Final report of study results	June 2030

6. RATIONALE & BACKGROUND

6.1. Background

Sickle Cell Disease (SCD) is a multisystem disease, associated with episodes of acute illness and progressive organ damage, and is one of the most common severe monogenic disorders worldwide. Globally, the incidence is estimated to be 300,000-500,000 newborns/year, and most cases occur in historically malaria-endemic regions of Africa, Middle East, the Caribbean and South Asia [1,2]. In Europe, it is estimated that SCD affects fewer than 5 in 10,000 people [3].

The term SCD is used to denote all the different genotypes responsible for the characteristic clinical syndrome. SCD is caused by a mutation in the β-globin gene that produces a defective sickle haemoglobin (HbS) [4,5]. The most common β-globin genotype is HbS/S, homozygosity for the β^S allele. Other β-globin genotypes include sickle haemoglobin-C disease (HbS/C, co-inheritance of β^S and β^C alleles) and sickle beta-thalassaemia (HbSβ) that can be sickle beta-0-thalassaemia (HbS/β^0) if a HbS gene and a β-zero-globin thalassaemia gene are inherited resulting in a complete loss of β-globin chains in one gene; or sickle beta+ thalassaemia (HbS/β^+) if a HbS gene and a β-globin-plus thalassaemia gene are inherited resulting in a partial loss of β-globin chains [4,6,7].

The clinical manifestations of SCD exhibit a broad spectrum, encompassing both acute and chronic manifestations. Chronic clinical manifestations include anaemia, chronic pain, substantial kidney injury, delayed growth and sexual maturation, skin ulcers, retinopathy, avascular necrosis or pulmonary hypertension [7]. Acute manifestations include recurrent vaso-occlusive pain, splenic sequestration, aplastic crisis, acute chest syndrome, stroke, gallstones, bacterial sepsis and meningitis, and priapism. In children under 6 years of age, about 30% experience splenic sequestration, increasing their risk for severe life-threatening anaemia in addition to infection by encapsulated organisms. Vaso-occlusive pain is the hallmark of SCD and generally presents first in the small bones of the hands and feet (i.e. dactylitis). Approximately half of children with SCD develop dactylitis by age 2, as young infants are generally protected because of their elevated foetal haemoglobin (HbF) concentrations [5]. The frequency of vaso-occlusive pain crisis is extremely variable, although each individual typically has a consistent pattern for crisis frequency. Some have as many as 6 or more episodes annually, whereas others may have episodes infrequently, or not at all. Pain crises may last several hours to several days. Patients usually present to the emergency department (ED) after self-treatment fails, which includes bed rest, oral analgesia, and hydration. Acute chest syndrome is also a frequent cause of morbidity and hospitalisation.

Disease severity is mainly determined by HbS polymerisation. HbF prevents polymerisation of HbS and can prevent intracellular sickling and resulting haemolysis if present in high enough concentrations. In adults, increased HbF levels are inversely associated with mortality and pain crises [5]. Infants are somewhat protected from exhibiting clinical symptoms in the first 6 months of life by elevated levels of HbF after which the condition becomes evident clinically. However, it is still difficult to foresee which infants will experience the most severe consequences of the disease. Considering the diverse types of SCD and their prognostic factors, e.g. presence of comorbidities, frequency of painful vaso-occlusive

events, and severity of anaemia, is important when managing the disease in paediatric patients, and dosing of therapies.

Effective management of SCD in children involves regular monitoring, infection prophylaxis, and follow-up to ensure appropriate disease control and early introduction of hydroxycarbamide. Regular comprehensive assessments in specialist settings, including routine laboratory tests and imaging studies, are important for monitoring organ function, identifying complications, and guiding treatment decisions. Long-term follow-up studies have demonstrated the importance of continuity of care in reducing morbidity and mortality in paediatric SCD patients [8]. Early treatment may have a protective effect to help delay or prevent clinical worsening and improve prognosis and quality of life.

Treatment for SCD in Children

Treatment aims to alleviate symptoms, prevent complications, and improve overall quality of life. This often involves a multidisciplinary approach, including medications to manage pain, prevent infections, and reduce the risk of stroke. Blood transfusions may be necessary, particularly for children with severe anaemia or acute complications. Among the recommendations to prevent complications of SCD, are the use of daily oral prophylactic penicillin up to age 5 to prevent infections, and annual transcranial Doppler (TCD) examinations to predict primary stroke risk between ages of 2 and 16 years. The use of long-term transfusion therapy is also recommended to prevent stroke in children with abnormal TCD velocity (≥200 cm/s) and to delay the time of splenectomy in children with a severe form of SCD presenting with splenic sequestration. In children with HbS/S, transfusion therapy may be considered pre operatively to prevent complications from the disorder in concordance with national guidelines [9]. Rapid initiation of effective analgesia is common for the treatment of severe pain associated with vaso-occlusive crisis, and for pain associated with avascular necrosis, analgesics and physical therapy are recommended. The pneumococcal vaccine is also recommended to be initiated soon after birth in line with national guidance [10-14].

Hydroxycarbamide (also known as hydroxyurea) is a pharmacological therapy for SCD that improves clinical outcomes and has been increasingly used in the recent years. Hydroxycarbamide increases total Hb and HbF in individuals with SCD and improves blood flow through reduced intracellular adhesion, reducing the incidence of pain episodes and acute chest syndrome episodes. Patients receiving hydroxycarbamide require regular dose review and assessment of HbF and other laboratory parameters with periodic blood testing and monitoring, with special attention to the development of excessive neutropenia and/or thrombocytopenia, although in practice such dose-limiting toxicities are uncommon.

In 1995, Charache et al. [15] published the results of a multicentre phase III trial of hydroxycarbamide showing a 40% reduction in the incidence of acute pain episodes, acute chest syndrome and hospitalisations in adults with sickle cell anaemia (SCA). The United States Food and Drug Administration (FDA) approved the use of hydroxycarbamide in symptomatic SCD patients in 1998. Since then, several studies assessing hydroxycarbamide for SCA have been published [16]. In the paediatric population, hydroxycarbamide has been assessed in the HUG KIDS studies [17-19], HUSOFT trial [20], and the BABY HUG trial [21-23], showing a decreased occurrence of pain episodes, acute chest syndrome, hospitalisations and transfusion. In the TWiTCH trial [24], it was shown that hydroxycarbamide use in children with SCD was non-inferior for maintaining TCD velocities compared to continued transfusions in children with abnormal TCD velocities. The efficacy of hydroxycarbamide has been

confirmed in long-term studies [20, 25-27], and observational studies have also shown a reduction in clinical events and in participant admissions in a cohort in Italy [28], and improvement in haematological parameters in a cohort in the United Kingdom (UK) [29].

Hydroxycarbamide 100mg/ml Oral Solution (Xromi®)

Hydroxycarbamide 100mg/ml oral solution (Xromi®) was first approved for use in the European Union (EU) in July 2019 in children with SCD from the age of 2 years, and in 2024 the license was extended in both the EU and UK to include infants from the age of 9 months [30]. The summary of product characteristics (SmPC) for hydroxycarbamide 100mg/ml oral solution [31] states that treatment should be supervised by a physician experienced in the management of SCD and is indicated for the prevention of vaso-occlusive complications in patients over 9 months of age. The dose is based on the patient's body weight (kg) with usual starting dose being 15 mg/kg/day, maintenance dose 20-25 mg/kg/day and maximum dose 35 mg/kg/day. If dose escalation is warranted based on clinical and laboratory findings, dose should be increased by 5 mg/kg/day increments every 8 weeks, until mild myelosuppression is achieved, up to a maximum of 35 mg/kg/day. Full blood cell counts with white cell differential and reticulocyte should be monitored once a month for the first 2 months following treatment initiation and at least every 4 weeks when adjusting dosage. If neutropenia or thrombocytopenia occurs, hydroxycarbamide dosing should be temporarily withheld, and full blood cell count with white cell differential should be monitored weekly.

Once a maximum tolerated dose (MTD) is established, laboratory safety monitoring should include full blood cell count with white cell differential, reticulocyte count, and platelet count every 2-3 months. Red blood cells, mean corpuscular volume (MCV), and HbF levels should be monitored for evidence of consistent or progressive laboratory response. However, a lack of increase in MCV, HbF, or both, is not an indication to discontinue therapy if the patient responds clinically (e.g. decreased incidence of pain or hospitalisation). A clinical response to treatment with hydroxycarbamide may take 3-6 months and therefore, a 6-month trial on the MTD is required prior to considering discontinuation due to treatment failure (whether due to lack of adherence or failure to respond to therapy).

6.2. Rationale

The safety and effectiveness of hydroxycarbamide in paediatric patients ranging from birth to 24 months of age have not yet been fully established. While limited data suggest that a 20 mg/kg/day dosage is safe and reduces painful episodes in children under 2 years of age [23], the long-term safety of this treatment remains to be determined.

The present study is a combined Post-Authorisation Safety Study (PASS) and a Post-Authorisation Efficacy Study that aims to provide data on the safety and effectiveness of hydroxycarbamide 100mg/ml oral solution administered to children under 2 years of age, over a follow-up period of 24 months.

7. RESEARCH QUESTIONS & OBJECTIVES

7.1. Research Question

The research question is:

- To evaluate the safety and effectiveness of hydroxycarbamide oral solution 100mg/ml (Xromi®) administered to children under 2 years of age for the prevention of complications of SCD.

7.2. Research Objectives

The **primary objective** of the study is:

- To assess the incidence of adverse events of special interest (AESIs) in children who have SCD and are treated with Xromi®, in comparison to a retrospective comparator cohort.

The **secondary objective** of the study is:

- To assess the effectiveness of Xromi® for the clinical events and laboratory tests and physiological assessments of interest, in comparison to a retrospective comparator cohort.

The **exploratory objectives** of the study are:

- To assess the overall safety of Xromi® by describing adverse events (AEs) by severity, seriousness, relatedness, action taken, outcome, and duration.
- To describe occurrence of and reasons for discontinuation of Xromi®.
- To assess the safety of Xromi® at the optimised dose.
- To assess if the effectiveness of Xromi® is associated with the dose.
- To assess the safety and effectiveness of Xromi® by subgroups (age, sex, β-globin genotype, country).
- To assess occurrence of clinical events in comparators exposed to any formulation of hydroxycarbamide during follow-up.
- To assess organ function and potential preservation in children exposed to any formulation of hydroxycarbamide.

8. RESEARCH METHODS

8.1. Study Design

8.1.1. General Aspects

This is a non-randomised, non-interventional, comparative, observational, hybrid prospective and retrospective, post-authorisation cohort study (a matched cohort design) of children with SCD from 9 months to under 2 years of age to assess the safety and effectiveness of Xromi® based on primary and secondary data collection *(Figure 1)*. This study will be mainly exploratory and there are no previous hypotheses to test.

This comparative observational study will include 2 cohorts:

- Prospective exposure cohort: Children with SCD aged 9 months to under 2 years of age who are newly prescribed Xromi®, will be identified prospectively. These participants will be followed up for 24 months from their index date, regardless of whether they continue treatment with Xromi®, discontinue all hydroxycarbamide treatment, or switch to another formulation of hydroxycarbamide. The decision to prescribe Xromi® will be made solely by the physician independently of the study, as part of standard care.
- Retrospective comparator cohort: Children with SCD and naïve to any hydroxycarbamide formulation at the index date. These participants will be identified retrospectively using the data from the last 10 years up to the date Xromi® was first used in children from 9 months to under 2 years of age at each individual site. The 24-month follow-up will be retrospective from the date they are matched to the exposed participant, irrespective of whether they start on any formulation of hydroxycarbamide during the follow-up.

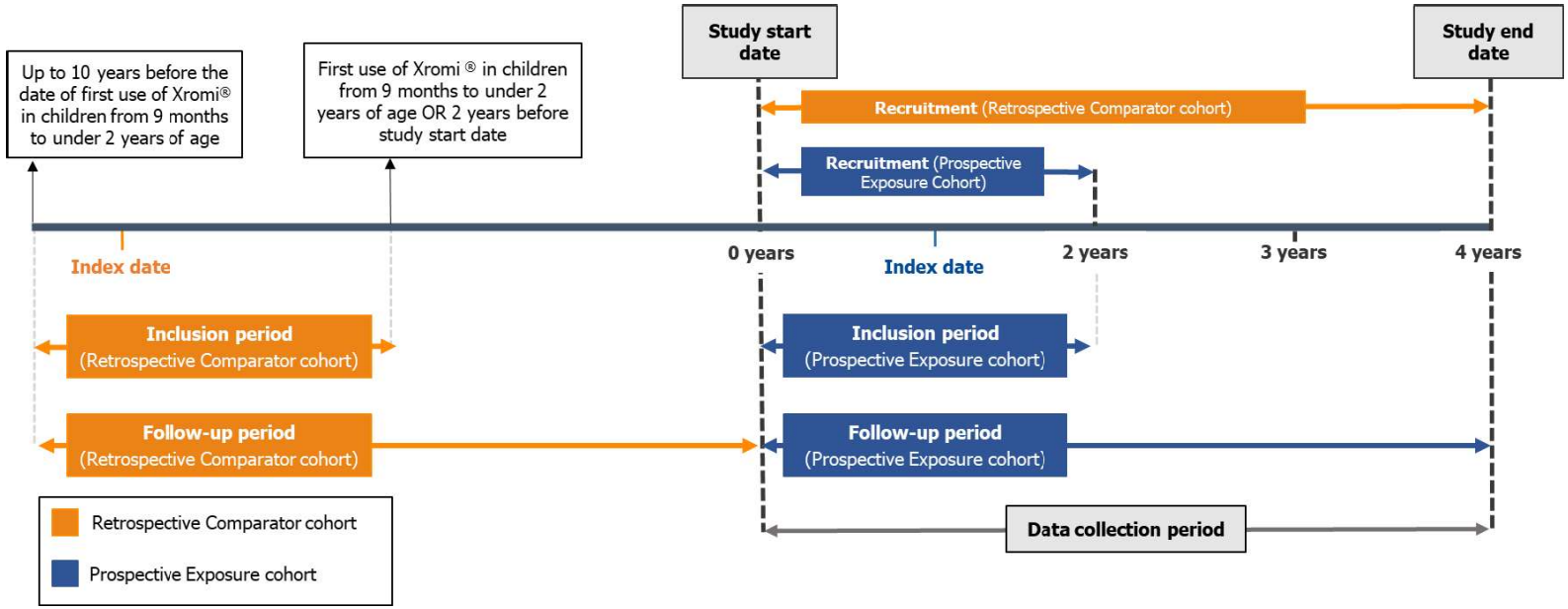


Figure 1 – Study Design

8.1.1.1. Matching

Children with SCD prescribed Xromi® will be matched with hydroxycarbamide naïve children (retrospective comparator cohort), following a 2:1 ratio (comparator:exposed) by the following variables:

- study site,
- β-globin genotype,
- age at initiation of Xromi® of the exposed participant.

Matching ratio and factors will be reviewed and adjusted (e.g., matching at country level), considering feasibility. For further details, see Section *8.9.3*.

8.1.2. Study Periods

8.1.2.1. General Study Conduct Period

The study start date will be defined as the date the first site is given a site initiation greenlight to begin study activities.

The study end date will be defined as the end of the 24-month follow-up period of the last participant included in the prospective exposure cohort to ensure a 24-month follow-up for all participants. The study end date will be 4 years after the study start date, although this may vary depending on the time needed to achieve the recruitment target.

The overall study period is from up to 10 years before Xromi® was first used in children from 9 months to under 2 years of age at the sites until the study end date.

8.1.2.2. Recruitment Period (Cohorts)

The recruitment period refers to the specific timeframe during which participants are screened, invited to participate in the study and enrolled. The recruitment periods for the cohorts will be defined as follows:

- Prospective exposure cohort: The recruitment period will begin at the study start date and will continue for 2 years. In this period, participants will be enrolled, and their data collected prospectively. If the recruitment target is not met, the recruitment period will be extended and additionally, more sites may be invited to participate in the study.
- Retrospective comparator cohort: Screening and recruitment of the comparators will begin at the study start date and will continue until the study end date. In this period, potential comparators will be pre-screened, matched, and enrolled in the study. All comparators' data will be collected retrospectively.

For further details about the recruitment process see *Section 8.2.4.*

8.1.2.3. Inclusion Period

The inclusion period will be site-specific and refers to the period during which eligible participants are allowed to enter the study based on the predefined eligibility criteria.

- Prospective exposure cohort: Participants will be selected from the date the site is given a site initiation greenlight until 2 years after the study start date. If the recruitment target is not met, this period will be extended in line with the recruitment period.
- Retrospective comparator cohort: The inclusion period for the comparator cohort will begin up to 10 years before the date of first use of Xromi® in children from 9 months to under 2 years of age at each study site. The inclusion period will continue until the date of first use of Xromi® in children from 9 months to under 2 years of age at each study site or 2 years before the study start date, whichever occurs first.

The date of first use of Xromi® in children from 9 months to under 2 years of age at each study site will be determined by e.g. examining pharmacy logs or prescription records at the site.

8.1.2.4. Baseline Visit

The baseline visit will be the visit at which all information needed to describe the study participants at baseline will be collected. The baseline visit will be participant-specific and is defined as follows:

- Prospective exposure cohort: The date of the visit of the first recorded prescription of Xromi®.
- Retrospective comparator cohort: The selected visit for matching (i.e., the date of the visit at which the patient's age was closest to the age of the matched exposed participant at the index date).

8.1.2.5. Index Date

The index date will be participant-specific:

- Prospective exposure cohort: Defined as the confirmed date of first use of Xromi®. If the date of first use is unknown, the visit with the first recorded prescription of Xromi® will be the index date.
- Retrospective comparator cohort: The selected visit for matching (i.e., the date of the visit at which the patient's age was closest to the age of the matched exposed participant at the index date).

8.1.2.6. Follow-Up Period

The follow-up period will be participant-specific and will be defined as follows:

- Prospective exposure cohort: Exposed participants will be followed from the index date to the earliest date of the following: death, loss to follow-up, completion of 24-month follow-up, or end of study. Exposed participants will be followed up for up to 24 months irrespective of whether treatment with Xromi® is permanently discontinued.

- Retrospective comparator cohort: Matched participants who are hydroxycarbamide naïve at the index date will be followed from the index date to the earliest date of the following: death, loss to follow-up, completion of 24-month follow-up, or end of study. Comparators will be followed up for up to 24 months irrespective of whether they initiated any formulation of hydroxycarbamide after the index date.

8.2. Setting

8.2.1. Site Selection

The study will seek the participation of the EU and UK specialist sites, which have long-term follow-up of SCD children and a known use of Xromi® in children under 2 years of age. SCD is largely managed by specialist sites or nurse led clinics and good record-keeping will be a key factor in ensuring good data quality.

Initially, up to 10 sites in the UK and up to 5 sites in Germany are planned to be invited to participate in the study for the identification of the exposure and the retrospective comparator cohorts. The inclusion of additional sites will be assessed if insufficient exposed participants are identified. In the UK participant identification centres may also be used to identify potential participants even in Local Hospital Teams. Initially, each site is expected to recruit approximately 5 exposed participants, allowing for variations to recruit a total of at least 60 exposed participants. The inclusion of other potential European countries will be assessed if needed.

8.2.2. Study Exposure

Xromi® is indicated for the prevention of vaso-occlusive complications of SCD in patients over 9 months of age. Xromi® is a clear, colourless to pale yellow, oral solution. The active substance is hydroxycarbamide and the other ingredients are xanthan gum, sucralose (E955), strawberry flavour, methyl parahydroxybenzoate (E218), sodium hydroxide, and purified water. See the SmPC for further details [31].

8.2.3. Study Population

The study population will consist of children with SCD who meet all the inclusion criteria and none of the exclusion criteria. Identification of participants will be conducted as follows:

- Prospective exposure cohort: Potential participants will be identified as they attend the participating sites where eligibility criteria will be assessed.
- Retrospective comparator cohort: Potential participants will be selected from clinical chart review.

8.2.3.1. Eligibility Criteria

a) Prospective exposure cohort :

Inclusion criteria

- Aged from 9 months to under 2 years at the index date.
- Diagnosis of SCD.
- Known β-globin genotype at the index date.
- Prescribed Xromi® for the prevention of complications of SCD.
- Parent(s) (or a legal representative(s)) provides written informed consent to participate in the study, unless there is a waiver, non-opposition, or blanket written informed consent by the parent(s) for research studies.

Exclusion criteria

- Previous use of hydroxycarbamide of any formulation before the index date.
- Receiving regular blood transfusions (occurring every 8 weeks or more frequently) at the index date.
- Known hypersensitivity to any of the excipients of Xromi® at the index date.
- Contraindications to the drug at the index date:
  - Severe hepatic impairment (Child-Pugh classification C).
  - Severe renal impairment (creatinine clearance: CrCl <30 ml/min).
  - Presence of at least one of the following: ANC < 1.0 x 10^9/L, absolute reticulocyte count (ARC) <80 x 10^9/L, platelets <80 x 10^9/L.
- Participating in another clinical study of an investigational medicinal product (IMP) at the index date.
- Anti-retroviral medicinal products for human immunodeficiency virus (HIV) at the index date.
- Active malignancy at the index date.

Participants in the prospective exposure cohort who are prescribed Xromi® but do not initiate treatment will be excluded from the dataset.

b) Retrospective comparator cohort:

Inclusion criteria

- Aged from 9 months to under 2 years at the index date.
- Diagnosis of SCD.
- Known β-globin genotype.

- Matched to an exposed participant.
- Parent(s) (or a legal representative(s)) provides written informed consent to participate in the study, unless there is a waiver, non-opposition, or blanket written informed consent by the parent(s) for research studies.

Exclusion criteria

- Use of hydroxycarbamide of any formulation before or at the index date.
- Receiving regular blood transfusions (occurring every 8 weeks or more frequently) at the index date.
- Presence at the index date of any of the following based on the clinician decision:
  - Severe hepatic impairment (Child-Pugh classification C).
  - Severe renal impairment (CrCl <30 ml/min).
  - Presence of at least one of the following: ANC <1.0 x 10^9/L, ARC <80 x 10^9/L, platelets <80 x 10^9/L.
- Participating in another clinical study of an IMP at the index date.
- Anti-retroviral medicinal products for HIV at the index date.
- Active malignancy at the index date.

8.2.4. Recruitment Process (Participants)

The process to screen potential participants, assess their eligibility and enrol them in the study is detailed below (see *Figure 2*).

8.2.4.1. Screening and Enrolment Assessment

The screening will be conducted by the investigational and/or clinical team. The screening process will take into consideration the recruitment and inclusion periods as defined in *Section 8.1.2.* Once potential participants (i.e. SCD patients from 9 months to under 2 years of age) have been identified through the screening process, the clinical team will assess if the patient is eligible to be included in the study, following the eligibility criteria as described in *Section 8.2.3.1,* except for the informed consent, which will be provided at a later stage.

Prospective Exposure Cohort

For the prospective exposure cohort, potential participants will be identified once the site has received a site initiation greenlight. Their eligibility to participate in the study will be assessed consecutively as they are initiated on/are first prescribed Xromi®. If eligible, the parent(s)/legal guardian(s) of eligible children will be invited to participate, provided a patient information sheet (PIS), and given time to consider and ask questions before informed consent is obtained.

To guide the screening and eligibility assessment process of the prospective exposure cohort, the sites will complete an electronic participant screening log (ePSL) in which the following limited information

will be collected: eligibility criteria (yes/no), and informed consent obtained (yes/no). Screened children will be assigned a unique participant screening log number (i.e., screening ID).

Retrospective Comparator Cohort

Sites will conduct a pre-screening process to identify and consent **potential** comparators, followed by a matching process conducted by OXON and a final enrolment assessment conducted by the sites to confirm eligibility of the comparators at the selected visit for matching. This process is detailed below.

a) Pre-screening

The pre-screening will be conducted to ensure there are sufficient data available in the medical records and the pre-screening requirements are met (see below). Sites will identify children with a known SCD β-globin genotype that were treated at the site when their ages were between 9 months and under 2 years of age during the retrospective comparator cohort inclusion period (see *Section 8.1.2.3*). Children will be entered onto an ePSL and assigned a unique participant screening log number (i.e., screening ID).

Sites will identify a minimum number of eligible children meeting the pre-screening requirements, depending on the estimated number of exposed participants they can provide. Potential comparators will be identified by moving backwards from the date before the date of first use of Xromi® in children from 9 months to under 2 years of age (or from 2 years before the study start date, whichever occurred first) and in a consecutive manner. Parent(s) or legal guardian(s) of identified potential comparators will then be contacted, informed about the study, and provided the PIS. If willing to participate, consent will be taken by the clinical team.

The pre-screening requirements are as follows:

- Access to the full medical records with data about clinical visits, AEs, hospitalisations, or transfusions (including red cell exchange).
- Access to full blood count (FBC) test results and HbF.

The potential comparators for which an informed consent has been obtained will have their matching criteria entered onto the ePSL. No further data entry will be completed for these participants, and they will be kept on hold until all exposed participants have been enrolled in the study to determine if they are a suitable match to any of the exposed participants. Parent(s) or legal guardian(s) will be informed that not all potential comparators invited to participate who provided consent will be finally matched and enrolled in the study.

b) Matching and enrolment assessment

After enrolling all Xromi®-treated participants (prospective exposure cohort), the pre-screened potential comparators who have provided consent will be re-evaluated to assess whether they can be matched with the exposed participants and if they meet the study's inclusion/exclusion criteria. OXON will be responsible for conducting and overseeing the matching process, based on the matching algorithms designed by the OXON statisticians.

During the matching and enrolment assessment, the following criteria will be assessed:

- Comparator is matched with an exposed participant:
  - within the same site (or at a country level, if unfeasible),
  - with the same β-globin genotype,
  - the comparator has a recorded visit when his/her age was closest to the age at index date of the matched exposed participant,
  - the comparator has a FBC and HbF test result available in the medical record at or close to that recorded visit.
- Comparator meets the protocol's inclusion/exclusion criteria at the chosen index date.

Initially, each exposed participant will be matched to 2 comparators within sites. Depending on the number and characteristics of the comparator participants available, this may need to be flexible in some circumstances, adapting matching ratio and/or matching factors as needed (see *Section 8.9.3*). If necessary, sites could potentially conduct another pre-screening to identify additional children meeting the pre-screening requirements. Once identified, sites will follow the same process presented above.

To guide the assessment of the pre-screening requirements, the potential visits available for matching and eligibility criteria, the clinical team at sites will be provided with an ePSL. Screened children will be assigned a unique participant screening number (i.e., screening ID).

8.2.4.2. Informed Consent Provision

For the prospective exposure cohort, eligible participants will be invited to participate in the study by the clinical team, parent(s) or legal representative(s) will be provided with the PIS and if willing, consent will be taken by the clinical team during the recruitment period.

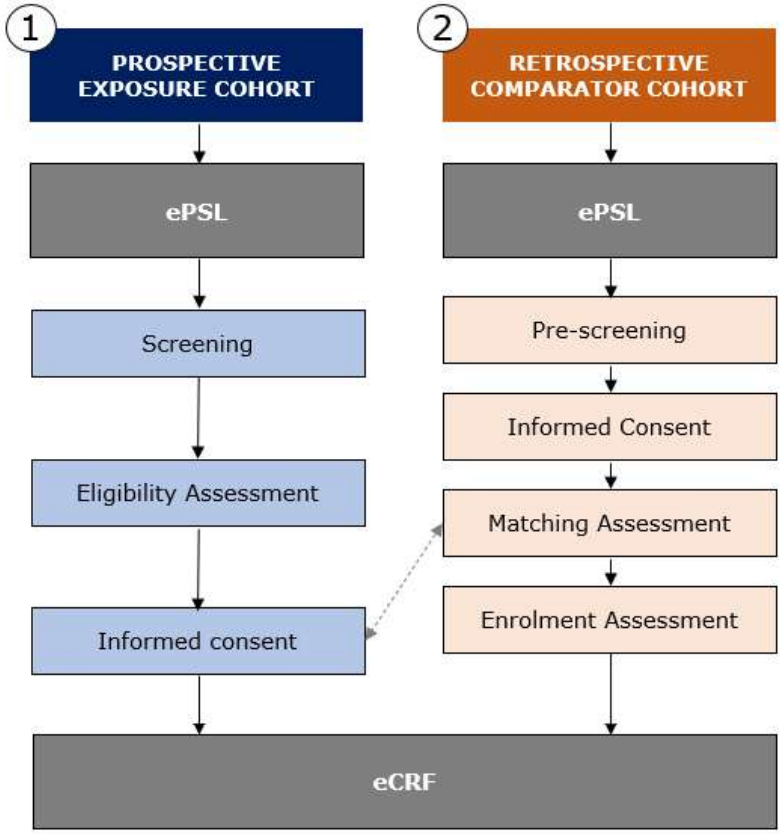
For the retrospective comparator cohort, parent(s) or legal representative(s) of children meeting the pre-screening requirements will be invited to participate in the study by the clinical team. Parent(s) or legal representative(s) will be provided a PIS and if willing to take part in the study, consent will be taken by the clinical team. An age-appropriate PIS will be provided to potential comparators as needed when invited to participate, and assent will be obtained by the clinical team where appropriate. Not all potential comparators invited to participate in the study will be finally matched and enrolled. Parent(s) or legal representative(s) will be informed accordingly before they provide informed consent, and will be notified by the site if their child has been matched and they are enrolled in the study, at which point they are also provided with their participant ID.

8.2.4.3. Enrolment

For the prospective exposure cohort, once their eligibility has been confirmed and informed consent obtained, they will be entered onto the main electronic case report form (eCRF) for the study. For the retrospective comparator cohort, once informed consent has been obtained, their matching eligibility criteria will be entered onto the ePSL. Once matching has been confirmed by the OXON statisticians, and the clinical team have confirmed their eligibility at the selected visit for matching, the clinical site team will enter their complete dataset onto the main eCRF for the study.

The eCRF is hosted on a secure, access-restricted study specific electronic data capture (EDC) system. The EDC will assign participants a unique anonymised participant ID. The participant ID will be cross-checked with the participant screening log number from the screening log (ePSL).

A participant identification log (PIL) will be provided to the sites and will contain the linkage information to identifiable participant data. The PIL will only be available to the Sponsor or their delegates for monitoring purposes for which explicit consent will be obtained and will be retained only in the Investigator Site File (ISF).



Abbreviations: eCRF, electronic case report form; ePSL, electronic participant screen log.

Figure 2 – Participant Enrolment Flow Diagram

8.3. Study Visits and Procedures

The study will adhere to established clinical practices (standard care), and no non-routine procedures or investigations will be required. Participants will not be requested to attend additional medical visits beyond routine clinical practice. All assessments will be conducted at the discretion of the investigator as part of routine medical care.

8.4. Study Variables

The following variables will be collected by the investigators to address the study objectives. *Table 1* below provides an overview of the data planned to be recorded at the baseline visit and during follow-up.

8.4.1. Demographics, Anthropometrics, and Other Medical History

The following variables will be collected at the baseline visit. Length/height and weight, as well as vaccines received will also be collected (when available) during follow-up.

- Age
- Sex assigned at birth
- Self-declared race/Ethnic origins
- Length/Height and Weight
- Vaccinations received up to the baseline visit (e.g. pneumococcal vaccine [including type of vaccine], haemophilus influenzae type B vaccine, meningococcal ACWY vaccine, meningococcal B vaccine, BCG vaccine)
- Other medical history and findings up to the baseline visit:
  - Previous infections (proven/treated), including previous sepsis and bacteraemia
  - Asthma
  - TCD velocities (maximum time-averaged mean velocity (TAMV))
  - Developmental delay
  - Seizures
  - Growth retardation
  - Myelosuppression event, defined as presence of one or more of the following:
    - neutropenia defined as ANC <1.0 x 10^9/L, and
    - reticulocytopenia defined as ARC <80 x 10^9/L, unless Hb >90 g/L, and
    - thrombocytopenia defined as platelets <80 x 10^9/L and
    - anaemia defined as Hb <45 g/L.
  - Renal impairment
  - Skin and subcutaneous tissue disorders (e.g. alopecia/ other hair loss, skin ulcer, rash)
  - Sleep disorder
  - Surgery
  - Fluid retention, including oedema
  - Other (e.g. iron deficiency, allergy, pulmonary hypertension).

8.4.2. SCD-Specific Data

The following information will be collected at the baseline visit to describe SCD :

- Screening status (yes/no, date of confirmed diagnosis)
- β-globin genotype (HbS/S; HbS/C; HbS/β^0; HbS/β^+; Other)
- Age at first appearance of SCD complications, including identification of the first symptom/s observed:
  - Bacteraemia
  - Splenic sequestration
  - Painful vaso-occlusive event
    - Dactylitis
  - Abnormal/conditional TCD
  - Other SCD symptoms
- Number of hospitalisations (i.e., admitted to a ward) for SCD up to the baseline visit, including duration of hospitalisation
- Number of non-hospitalised (i.e., not admitted for overnight stay) ED visits/treatment centres/day unit/paediatric ward for SCD up to the baseline visit
- History of complications of SCD up to the baseline visit:
  - Painful vaso-occlusive events, including number of events up to baseline and presence of any of the following clinical presentations:
    - Dactylitis
    - Analgesic use (opiate/ non-opiate)
    - Acute presentation to hospital (e.g. ED, day unit, paediatric ward)
    - Hospital admission
    - General practitioner review
    - Pulse oximetry (low oxygen saturation)
    - Vitals (low blood pressure / low heart rate / fever)
  - Acute chest syndrome, including number of events
  - Anaemia (defined as a drop in Hb 20g/L from baseline outside the normal drift down of Hb in infancy)
  - Splenomegaly (spleen palpable in the abdomen)
  - Splenic sequestration
  - Cerebrovascular accident (including silent stroke)
  - Cholelithiasis including biliary colic, cholestasis, cholangitis, presence of gallstones on ultrasound
  - Priapism
  - Other

SCD Management

The following information about SCD management, including previous and concomitant use of treatments, will be collected at baseline and during follow-up:

- Previous and concomitant SCD treatments:
  - Beta-lactam prophylactic antibacterials
  - Folic acid
  - Blood transfusions
  - Hydroxycarbamide
  - Analgesics (opiate and non-opiate)
  - Other SCD treatments

The following information will be collected for the prospective exposure cohort only, at baseline, to characterise participants' use of Xromi®:

- Type of prescriber (e.g. consultant/SCD specialist, junior doctor, nurse, GP)
- Initial dose

The following information will be collected for the prospective exposure cohort only, during follow-up, to characterise participants' use of Xromi®:

- Changes in dosage during follow-up (doses and dates)
- Optimised dose (dose (mg/kg/day), date and time at optimised dose)*
- MTD (dose (mg/kg/day), date and time at MTD)**
- Date of discontinuation
- Reasons for discontinuation
  - Switch to tablet / capsule formulation
  - Adverse event (investigator or other health care professional decision)
  - Lack of drug effect (including underdose, treatment non-adherence, and/or other)
  - Improved condition or remission
  - Parental decision
  - Lost to follow-up
  - Other

* The optimised dose will be defined as the highest stable dose (mg/kg/day) of Xromi® that achieves a clinically acceptable response. A stable dose (mg/kg/day) is one that is maintained for at least 6 consecutive months.

** The MTD will be defined as the highest stable and tolerated dose (mg/kg/day) of Xromi® that achieves a target range of mild marrow suppression most commonly determined by the ANC and ARC levels (otherwise known as the maintenance dose). This will be assessed by the Investigator and is usually

achieved 6 months after initiating the drug and is typically 25-30 mg/kg/day but with a maximum dose of 35 mg/kg/day.

8.4.3. Safety Related Data

Occurrence of all AEs will be collected during follow-up.

8.4.3.1. Adverse Events of Special Interest (AESIs)

In this study, the following AEs will be considered AESIs:

- Myelosuppression, presence of one or more of the following:
  - neutropenia defined as ANC <1.0 x 10^9/L, and
  - reticulocytopenia defined as ARC <80 x 10^9/L, unless Hb >90 g/L, and
  - thrombocytopenia defined as platelets <80 x 10^9/L and
  - anaemia defined as Hb <45 g/L.
- Abnormal weight gain
- Abnormal loss of weight
- Hepatic enzymes increased, including:
  - Alanine transaminase (ALT) increased
  - Aspartate transaminase (AST) increased
- Skin and subcutaneous tissue disorders, defined as presence of any of the following:
  - Alopecia or other hair loss
  - Skin hyperpigmentation, including oral and nail hyperpigmentation
  - Rash
  - Skin ulcer, including leg ulcer
- Growth retardation
- Infection (proven/treated): bacterial, fungal or viral

The participants' height, weight, and the laboratory parameters (including white blood cells (WBC) count) necessary to describe these AESIs will be collected during follow-up, whenever available in the participant's clinical record. Growth retardation will be defined as a drop of 2 or more centiles on growth charts over time [32, 33]. Further details will be provided in the statistical analysis plan (SAP).

8.4.3.2. Other Adverse Events

Additionally, occurrence of other AEs will be collected during follow-up, including but not limited to:

- Anaemia (not including protocol-defined myelosuppression), iron deficiency, platelet count decreased (but not protocol defined thrombocytopenia), leukopenia, constipation, cutaneous lupus erythematosus, diarrhoea, dizziness, dry skin, fever, fluid retention

(including oedema), gastrointestinal disturbances, gastrointestinal ulcer, haemorrhage, headache, hepatotoxicity, leukaemia, macrocytosis, nausea, severe hypomagnesaemia, skin cancer, stomatitis, systemic lupus erythematosus, vomiting, irritability/ crying, feeding disorder, oxygen saturation decreased, other unforeseen AE.

8.4.4. Effectiveness Related Data

8.4.4.1. Clinical Events

Occurrence of the following clinical events will be collected during follow-up by the investigator. Descriptions of these clinical events are provided as guidance in *ANNEX 3 – DEFINITIONS OF CLINICAL EVENTS*, and are mainly based on the BABY HUG study [23]. The final determination will depend on the recorded occurrence of the event in the participant's clinical record and the investigator's clinical judgment.

- Painful vaso-occlusive event, (including dactylitis)
- Acute chest syndrome
- Splenomegaly
- Priapism
- Hepatobiliary disorder
- Splenic sequestration crisis
- Surgery (planned, emergency, due to pre-existing conditions (e.g. SCD))
- Blood transfusion
- Cerebrovascular accident (including silent stroke)
- Abnormal/conditional TCD
- Other events:
  - Hospitalisations for SCD (including reason for hospitalisation and duration)
  - Non-hospitalised ED visits/treatment centres/paediatric ward/day unit attendances for SCD
  - Other

8.4.4.2. Laboratory Tests and Physiological Assessments

Results and dates from the following laboratory tests and physiological assessments will be collected, whenever available. At baseline, the most recent results, prior to Xromi® initiation in the prospective exposure cohort, will be collected. Results from laboratory tests and physiological assessments conducted during follow-up will also be collected.

TCD may not be available at all sites for this age group (under 2 years of age). Previous TCD velocities, prior to Xromi® initiation in the prospective exposure cohort, will be collected at the baseline visit. TCD velocities will also be collected at any point during follow-up.

- Haematological parameters:
  - Absolute neutrophil count (ANC)
  - Absolute reticulocyte count (ARC)
  - Haemoglobin (Hb)
  - Haemoglobinopathy screen results including: HbF, HbA, and all other Hb fractions
  - Mean corpuscular volume (MCV)
  - Mean corpuscular Hb (MCH)
  - Platelet count
  - White blood cell count (WBC)
- Iron profile
  - Ferritin
  - Iron binding saturation or transferrin saturation
- Liver parameters will be collected to describe:
  - Liver function tests:
    - Alanine Transaminase (ALT)
    - Alkaline phosphatase (ALP)
    - Aspartate Transaminase (AST)
    - Total bilirubin
    - Lactate dehydrogenase (LDH)
    - Gamma-glutamyl transferase (GGT)
- Liver and spleen size on examination (number of cm at maximum from costal margin)
- Renal parameters will be collected to describe:
  - Renal function tests:
    - Serum creatinine
    - Estimated glomerular filtration rate (eGFR)
    - Albumin and creatinine for albumin-to-creatinine ratio (ACR) (urine parameters)
- Cerebrovascular function will be assessed by:
  - TCD velocities (maximum time-averaged mean velocity (TAMV))

Table 1 – Planned data extraction schedule

Variable	Before/at baseline	During follow-up
Eligibility criteria	X	N/A
Informed consent (if applicable)	X	N/A
Index date	X	N/A
Withdraw of consent (if applicable)	N/A	X
Demographics, anthropometrics, and other medical history		
Age	X	N/A
Sex	X	N/A
Self-declared race/ Ethnic origins	X	N/A
Length/height and weight	X	X
Vaccinations	X	X
Other medical history	X	N/A
SCD specific information		
Age at first appearance of SCD symptoms	X	N/A
β-globin genotype	X	N/A
Number of hospitalisations for SCD	X	N/A
Number of non-hospitalised ED visits/treatment centres/paediatric ward/day unit attendance for SCD	X	N/A
History of SCD complications	X	N/A
SCD Management		
SCD treatment and management	X	X
Xromi® (prospective exposure cohort only)	N/A	N/A
Type of prescriber	X	N/A
Initial dose	X	N/A
Dose changes during follow-up	N/A	X
Optimised dose	N/A	X
MTD	N/A	X
Discontinuation of Xromi®	N/A	X
Safety related data		
AESIs	N/A	X
Other AEs	N/A	X
Effectiveness related data		
Clinical events	N/A	X
Laboratory tests and physiological assessments	X	X

Abbreviations: AEs: adverse events; AESIs: adverse events of special interest; ED: Emergency department; MTD: maximum tolerated dose; NA: not applicable; SCD: sickle cell disease.

8.5. Study Endpoints

8.5.1. Primary Endpoint

The primary endpoint is occurrence of AESIs (see *Section 8.4.3.1*) over the follow-up period. The incidence of AESIs will be compared between the participants receiving Xromi® and retrospective comparator participants.

8.5.2. Secondary Endpoints

To assess the effectiveness of Xromi®, the endpoints are the occurrence of clinical events (see *Section 8.4.4.1*), and defined biochemical and physiological factors (see *Section 8.4.4.2*), to be compared between the exposed and comparator participants.

For biochemical and physiological factors, the following endpoints will be defined:

- Haematological parameters:
  - Absolute levels of ANC, ARC, Hb, HbF, HbA and other Hb fractions, MCV, MCH, WBC count and platelets.
  - Occurrence of:
    - Hb <70 g/L
    - Hb 20% decrease from baseline
    - HbF < 10% and HbF < 20%
- Iron profile:
  - Absolute levels of ferritin, and iron binding saturation or transferrin saturation (as available)
- Liver parameters:
  - Absolute levels of ALT, ALP, AST, total bilirubin, LDH and GGT
- Change in liver or spleen size (below the costal margin) in comparison to baseline
- Renal parameters:
  - Absolute eGFR, creatinine level, ACR (urine parameters)
  - Change in eGFR
    - ≥25% reduction in eGFR from baseline
    - eGFR below age-appropriate normal range
- Cerebrovascular function:
  - Absolute values of TCD Velocities (maximum time-averaged mean velocity (TAMV))
  - Occurrence of abnormal TCD Velocities (≥200 cm/s non-imaging), conditional TCD (170-199 cm/s non-imaging)

8.5.3. Exploratory Endpoints

8.5.3.1. Overall Safety

Occurrence of AEs in the prospective exposure and the retrospective comparator cohorts will be described by severity, seriousness, relatedness, action taken, outcome, and duration of each event. For haematological and biochemical toxicities, AEs will be assessed by clinical significance and normal ranges. Further details about management and reporting of AEs are provided in *Section 10.*

8.5.3.2. Discontinuation Of Xromi®

Occurrence of discontinuation of Xromi® in the prospective exposure cohort including reasons for discontinuation.

8.5.3.3. Safety at the Optimised Dose

Occurrence of AESIs and AEs during the period the participant is at the optimised dose.

8.5.3.4. Effectiveness at the Optimised Dose

Occurrence of clinical events and event-based biochemical and physiological factors during the period the participant is at the optimised dose.

8.5.3.5. Safety And Effectiveness by Subgroups

The event-based safety and effectiveness endpoints will be calculated as described in *Section 8.5.1* and *Section 8.5.2* by the following subgroups of interest:

- Age group
- Sex designated at birth
- β-globin genotype (HbS/S; HbS/C; HbS/β^0; HbS/β^+; Other)
- Country

8.5.3.6. Clinical Events in Comparators Exposed to any Formulation of Hydroxycarbamide During Follow-Up

Occurrence of clinical events (see *Section 8.5.2*) in the retrospective comparator group, during the periods of exposure to any formulation of hydroxycarbamide.

8.5.3.7. Organ Function and Potential Preservation

Occurrence of splenic sequestration, cerebrovascular accident (stroke) and acute chest syndrome in children exposed to any formulation of hydroxycarbamide.

8.6. Data Sources

All data will be collected by the investigational team in participating sites from clinical records obtained as part of participants' routine clinical practice.

Data collected at baseline and during follow-up listed in *Section 8.4* will be transcribed from the source clinical records and captured via eCRF by the Investigator or delegated site staff. Biochemical and haematology data from laboratory reports will be transcribed from paper or electronic records onto the eCRF by the investigator or delegated site staff. The eCRFs will be retained within a fully validated, EDC system (see *Section 8.8* and *Section 8.11*).

For safety follow-up data, data collection will include information on AEs, adverse drug reactions (ARs), serious adverse events (SAEs), serious adverse drug reactions (SARs), and AESIs. Methods for collection will include participant reported information during clinic visits, and data obtained from medical records (e.g. hospital admissions, ED/day unit/paediatric ward attendances, transfusions, routine procedures), which may trigger additional follow-up of participants to obtain further information, where possible, to validate or establish the diagnosis associated with the event. Further details about the management and reporting of AEs will be provided in the Safety Management Plan (SMP). The SMP will include the reconciliation processes of the EDC data and safety database, and medical review processes by the medical monitors (see *Section 10*).

8.7. Study Size

The study aims to recruit at least 180 participants in total: 60 participants prospectively administered Xromi® and 120 retrospective comparator participants during a 4-year study period. Sample sizes were estimated assuming that 60 participants will be recruited to the prospective exposure cohort, and each exposure participant matched to 2 comparator participants. All calculations were conducted using 80% power and a two-sided type I error rate of 5%.

Incidence estimates are derived from the BABY-HUG trial [23], a US-based trial. The incidence rate reported in the tables below will act as an upper bound (and thus the reported minimum detectable HR will be a best-case scenario) for what we might expect with this different threshold. The definitions used in the BABY-HUG trial do not exactly match those used in our study. Details of the definitions used in our study can be found in Sections *8.4* and *8.5*.

8.7.1. Safety

Using the results from the BABY-HUG trial [23] it will be possible to detect the following increase in risk of toxicity or AEs as estimated by the hazard ratio (HR) comparing the incidence of the safety event in the exposed cohort versus the comparator group. The minimum detectable HR was estimated by assuming the log rank test as the basis of the comparison. As it is possible not all participants will be followed up for the full 24-month period the calculations were repeated for average follow-up durations of 1.5 years (18 months) and 1 year (12 months).

The estimated sample size allows for the detection of minimum detectable HR below 2 for myelosuppression (as a composite of severe anaemia, thrombocytopenia, and ANC < 1250 / µL [1.25

10^9/L]) and neutropenia alone (ANC <1250 /µL [1.25 10^9/L]), even for an average follow-up of 1 year. Full details of the calculations are shown in *Table 2* below.

Table 2 – Minimum detectable hazard ratios for safety events of interest at different average follow-up durations

Outcome	Estimated incidence (per 100 PY)	Minimum detectable HR		
		Average 2 years of FU	Average 1.5 years of FU	Average 1 year of FU
Creatinine (≥2×baseline and ≥10 mg/L [88.4 µmol/L])	0.0	N/A	N/A	N/A
ALT (>150 U/L)	0.5	6.48	7.19	8.20
Bilirubin (>100 mg/L [1710 µmol/L])	0.5	6.48	7.19	8.20
Severe anaemia (haemoglobin <70 g/L and ARC <80×10^3/µL [80x10^9/L])	1.1	4.93	5.42	6.11
Thrombocytopaenia (platelet count <80×10^3/µL [80x10^9/L])	4.3	2.65	2.82	3.07
ANC <1250 /µL (1.25 10^9/L)	19.5	1.83	1.89	1.98
Myelosuppression (composite of severe anaemia, thrombocytopenia, and ANC < 1250 / µL [1.25 10^9/L])	24.9	1.76	1.81	1.88

Abbreviations: ALT: alanine aminotransferase; ANC: absolute neutrophil count; ARC: absolute reticulocyte count; FU: follow-up; HR: hazard ratio; N/A: not applicable; PY: person-years.

8.7.2. Effectiveness

For effectiveness, a similar approach was used as for the safety outcomes by estimating the minimum detectable reduction in HR using the log rank test. Calculations were repeated for assumed average follow-up durations of 2 years (24 months), 1.5 years (18 months), and 1 year (12 months). Estimated incidence rates for each outcome were calculated from the BABY HUG trial except for all-cause infections which was taken from Thorburg et al. [21]. The results of the calculations are shown in *Table 3* below.

Table 3 – Minimum detectable hazard ratios for effectiveness outcomes at different average follow-up durations

Outcome	Estimated incidence (per 100 PY)	Minimum detectable HR		
		Average 2 years of FU	Average 1.5 years of FU	Average 1 year of FU
Stroke (cerebrovascular accident)	0.5	<0.01	<0.01	<0.01
Priapism	1.1	<0.01	<0.01	<0.01
Sepsis or bacteraemia	3.2	0.05	0.02	<0.01

Outcome	Estimated incidence (per 100 PY)	Minimum detectable HR		
		Average 2 years of FU	Average 1.5 years of FU	Average 1 year of FU
Splenic sequestration	6.5	0.21	0.17	0.11
Acute chest syndrome	14.6	0.40	0.36	0.31
Transfusion	34.1	0.54	0.52	0.48
Splenomegaly	47.6	0.58	0.56	0.53
Dactylitis	66.5	0.61	0.59	0.57
Skin and subcutaneous disorders	89.2	0.62	0.61	0.60
Hospitalisations (for any cause)	175.1	0.64	0.64	0.63
Pain (all reports)	202.7	0.64	0.64	0.63
Infection (all cause)	217.3	0.64	0.64	0.64

Abbreviations: FU: follow-up; HR: hazard ratio; PY: person-years.

8.8. Data Management

A data management plan (DMP) will be developed to guide all aspects of data handling. It will describe all specifications for data collection, cleaning, and data validation processes. The DMP will include all data collection forms (CRF) and variables annotations, data dictionaries, data base specifications including any necessary country-specific modifications, transfer of files, and interim analyses specifications if applicable. For more information on the EDC and its validation and quality processes please refer to *Section 8.11*.

Data entered into the eCRF will be subject to programmed flows, built-in edit checks and manual and automatic queries to control missing data, out of range values and discrepancies to ensure consistency and high-quality data. The eCRF will be subject to remote monitoring and query resolution procedures that will also be documented in the monitoring plan and will be reconciled regularly against the safety database.

The investigator or qualified designee is responsible for recording and verifying the accuracy of participant data.

Medical history, clinical events, and AEs will be mapped to MedDRA thesaurus terms, and exposures to medications will be coded using ATC code dictionaries.

As discussed in *Section 9*, the identities of participants taking part in the study will be pseudonymised using unique identification codes, and only age not date of birth will be captured in the EDC.

8.9. Data Analysis

This study will be mainly exploratory and there are no previous hypotheses to test. The study will be analysed as a comparative matched cohort design. A descriptive analysis will be performed to describe the study participants using summary statistics, tabulations, and descriptive figures.

A detailed SAP will be developed and finalised prior to the first interim analysis and will include methods of analysis and presentation and table shells. All analyses will be performed using SAS 9.4 (or higher) statistical software (SAS, Cary, North Carolina, USA). Full details on handling of all missing data, which are common in observational studies, will be described separately in the SAP.

The sub-sections below are an outline of the types of analyses that are currently being considered.

8.9.1. General Considerations

Categorical data will be summarised by counts and percentages. Continuous data will be summarised using number, mean, standard deviation (SD), median, quartiles, minimum and maximum and, in the case of non-normally distributed data, range and interquartile range. All statistical tests will be 2-sided and conducted at the 0.05 alpha level. P-values will be presented to 3 decimal places.

For descriptive analyses, missing data will be omitted from the percentages. However, the frequencies will be presented as a separate category for categorical data, and the amount of missing data for continuous variables will be summarised.

Time-to-event outcomes will be described using a Kaplan-Meier approach. Median time-to-events will be reported with 95% confidence intervals (CIs), estimated using Greenwood's formula, alongside the 25th and 75th percentiles of the time-to-event. Log-rank tests will be performed to formally compare groups. Adjustments for covariates would use a Cox proportional hazards regression approach, and univariable and covariate-adjusted HRs and 95% CIs will be provided. The confounding variables to be included in these multivariable models will be specified in the SAP.

Binary outcomes will be described by number and frequency. For comparison between groups, logistic regression will be used to calculate crude and covariate-adjusted odd ratios and 95% CIs; if these variables are measured repeatedly during follow-up, mixed effects logistic regression models will be used to account for the within-participant correlation. For continuous outcomes, descriptions will be provided by means, SDs, medians, upper and lower quartiles, minimum and maximum. Non-normal outcomes will further be reported by the overall and interquartile ranges. Crude comparisons between cohorts will be conducted using univariable linear regression and the mean difference in measurements, together with 95% CIs, will be reported. A covariate-adjusted multivariable linear regression model will also be performed, and the adjusted mean difference in measurements and associated 95% CIs will be reported. If the linear regression model diagnostics exhibit a substantial deviation for the required model assumptions, then an equivalent non-parametric analysis will be explored. For continuous outcomes that are measured repeatedly over time, repeated measures regression models will be developed; typically, this will extend the model to include a within-participant, between-observation correlation structure.

8.9.2. Study Conduct

A description of the study populations will be provided, and a study flow chart produced showing the number of participants at each stage of the study process.

If available, reasons for non-participation in the study will be tabulated and presented in the flow chart in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [34]. A CONSORT type diagram will be prepared showing the flow of participants at each stage of the study together with their reasons for exclusion.

The study sample will be summarised by the number of participants, duration of follow-up, losses to follow-up, participant withdrawals, and deaths.

8.9.3. Matching

Participants will be matched on the factors described in *Section 8.1.1.1*. Matching is planned to be in the ratio 1 exposed participants to 2 comparator participants. The matching algorithm will be designed by the OXON statisticians. Depending on the number of comparator participants available to be matched this matching ratio may need to be flexible in some circumstances. Additional matching ratios of 1:1 and 1:3 (exposed:comparator) will be considered in those circumstances. It is currently planned that matching will be performed without replacement, but this may be reconsidered depending on the number of potential comparator participants.

Comparators will aim to be matched within sites. In cases where this is not possible, comparators from other sites/countries which fulfil the matching criteria may be used.

Full details of the matching process and final matching algorithms will be detailed in the SAP.

8.9.4. Description of the Sample

Participants' sociodemographic and clinical characteristics at baseline will be described overall, by country and by site for each of the study cohorts, i.e. exposed and comparator participants.

8.9.5. Analysis of the Primary Endpoint

The primary safety analysis will examine the occurrence of safety events of interest presented in *Section 8.4.3.1*.

For each AESI, the number of events, person years of exposure and number of participants experiencing an AESI will be reported. The incidence rate of the AESI in each study cohort (exposed and comparator) will be calculated and expressed per 100 person years. 95% CIs will be calculated using the exact Poisson formula. The percentage of participants who experience a particular AESI will also be reported.

For each of the AESIs, Kaplan-Meier curves, stratified by cohort, will be produced. These figures will be accompanied by risk tables showing the number of participants still at risk at the index date, and at 6, 12, 18 and 24 months of follow-up. Time-to-AESI will be summarised using Kaplan-Meier estimates of the median time-to-event and its 95% CI. A Cox proportional hazards model will be constructed, including cohort as its only covariate, from which crude HRs and 95% CIs will be derived. A second confounder-adjusted multivariable Cox model will be used to report adjusted HR, 95% CI and p-value.

8.9.6. Analysis of the Secondary Endpoints

8.9.6.1. Clinical Events

The frequency and time-to-first occurrence of the clinical events listed in *Section 8.4.4.1* will be analysed using the same approach outlined in *Section 8.9.5*.

8.9.6.2. Biochemical and Physiological Factors

A descriptive analysis of the laboratory tests and physiological assessments described in *Section 8.4.4.2* will be provided using summary statistics. Visualisations of trends will also be provided as a description.

Where obtained, the frequency and time to first occurrence of abnormal and conditional TCD Velocities, Hb <70 g/L, 20% decrease in Hb, HbF <10% and <20%, change in liver and spleen size in comparison to index date, and changes in eGFR of ≥25% reduction in eGFR from baseline or eGFR below age-appropriate normal range will be reported using the same approach described in *Section 8.9.5*.

Longitudinal changes in ANC, ARC, platelets, WBC count, Hb, HbF, HbA and other Hb fractions, MCV, MCH, iron, ferritin or iron binding saturation, ALT, ALP, AST bilirubin level, LDH, GGT, creatinine, eGFR, ACR, liver, and spleen size, and TCD Velocities will be examined using linear mixed effects models. Time will be considered as a continuous variable and will be modelled as a smooth curve after inspecting the data for the best functional form. Unadjusted models will be constructed with each of the continuous biochemical and physiological factors as the response variable, and with cohort and time and the interaction of cohort and time as covariates. The models will include random effects for the intercept and functional shape variables. The mean difference in the response variable between the exposed and comparator participants will be calculated from the model at fixed time points (e.g. 3, 6, 12 and 24 months after index date). The corresponding 95% CI and p-value will also be calculated from the model results. A fully adjusted model will then further include the confounding variables described in the SAP. Mean differences, 95% CIs and p-values will be reported at each time point. Full details of the modelling approach will be described in the SAP.

8.9.7. Exploratory Analyses

8.9.7.1. Overall Safety Assessment

Verbatim descriptions of AEs will be mapped to MedDRA® thesaurus terms and be presented in a data listing. AEs will be summarised using frequency, percent, and two-sided 95% Clopper-Pearson CIs for each MedDRA® System Organ Class (SOC) and Preferred Term (PT) within each SOC. Within each cohort, the count and proportion of each AEs at every grade of severity, seriousness, relatedness, action taken, outcome and duration will be reported. Safety data will also be tabulated separately by MedDRA® categories SOC and PT. Each participant will only be counted once within each SOC or PT.

AEs, SAEs, AESIs, hospitalisations and ED/paediatric ward/day unit/treatment centre visits for SCD, and deaths reported will be tabulated separately with the number and proportion of participants, and number of events, with 95% CIs calculated using the Clopper-Pearson method for each of the exposed and comparator groups. Hospitalisations and ED visits will be described by frequency and proportions

of participants. Length of stay of hospitalisations will be summarised using means, SD, median, IQR and minimum and maximum.

A listing of all deaths will be provided including date, cause of death, relatedness, MedDRA® SOC and PT, and a brief narrative description. Pregnancy will not be tested/captured in this study due to the age of the participants.

8.9.7.2. Discontinuation of Xromi®

Among participants exposed to Xromi®, time-to-discontinuation will be described using a Kaplan-Meier analysis, with the median time-to-discontinuation, its 95% CI, and the upper and lower quartiles reported. The number of participants discontinuing Xromi® before the end of follow-up and the reasons cited for discontinuation will be summarised by counts and percentages.

8.9.7.3. Safety at the Optimised Dose

The analysis of the AESIs (as described in *Section 8.9.5*) will be repeated comparing participants on and those not on the optimised dose. As the optimised dose is a time-dependent variable, survival curve estimates, stratified by attainment of the optimised dose, will be produced using the methods of Schultz et al. [35]. HRs comparing the exposed participants with the comparator participants and corresponding 95% CIs will be derived from Cox models with a binary, time-dependent variable denoting the period on which there is attainment of optimised dose (1 = attained, 0 = not attained).

An exploratory analysis will be performed using all dosage data in a regression model where the dose response relationship is flexibly modelled over time. Full details will be provided in the SAP.

8.9.7.4. To Assess if the Effectiveness of Xromi® is Associated with the Dose

The clinical effectiveness endpoints (all event-based endpoints described in *Section 8.5.2*) will be analysed using the approach described in *Section 8.9.7.3*.

8.9.7.5. Safety and Effectiveness by Subgroups

The primary safety and effectiveness analyses (all event-based endpoints described in *Section 8.5.1* and *Section 8.5.2*) will be repeated for each of the subgroups outlined in *Section 8.5.3.5*. Definitions of subgroup membership will be provided in the SAP.

8.9.7.6. To Assess Occurrence of Clinical Events in Comparators Exposed to Any Formulation of Hydroxycarbamide During Follow-Up

The occurrence of clinical events will be assessed in the retrospective comparator group comparing periods of exposure to any formulation of hydroxycarbamide to periods with no exposure, with exposure to hydroxycarbamide treated as a time updated binary covariate (0 = unexposed, 1 = exposed). Poisson regression models will be used to compare incidence rates in the exposed versus unexposed periods by calculating incidence rate ratios and 95% CIs. Full details of the analyses will be provided in the SAP.

8.9.7.7. To Assess Organ Function and Potential Preservation in Children Exposed to Any Formulation of Hydroxycarbamide

The time to splenic sequestration, cerebrovascular accident (stroke) and acute chest syndrome will be used to measure organ damage in the pooled cohort of participants who received any formulation of hydroxycarbamide. The analysis will be stratified by age at initiation of hydroxycarbamide. The analysis will use Kaplan-Meier methods to describe survival curves and median time to organ damage if sufficient data allow. Differences between survival curves will be assessed using the log-rank test. HRs and 95% CIs will be calculated using a Cox proportional hazards model. An unadjusted and adjusted Cox model will be used to calculate HRs and 95% CIs. Full details of the analyses will be provided in the SAP.

8.9.8. Interim Analyses

An analysis on the cumulative enrolled participants is planned to be performed on a yearly basis along with a short statistical interim report every 2 years. Analyses included in each of these reports will be defined in the SAP. Reports will be submitted as standalone reports.

8.10. Limitations of the Research Methods

In the context of being a non-interventional, non-randomised, descriptive study, its primary limitation lies in the absence of randomisation. Nevertheless, the comparative analysis is superior to a one-arm safety study. This comparative design offers an added benefit by allowing the assessment of effectiveness.

The study relies on a sample of children with SCD identified in specialised sites in 2 or more European countries, and the recruitment rate is limited by the number of births of SCD in the study sites and the locations where Xromi® is used in children with SCD under 2 years of age in the participating countries. While the inclusion of at least 2 countries and the identification of participants through specialised sites limit potential information/detection bias and may increase generalisability, the non-randomised nature of the study and the limited number of countries and participants included could introduce selection bias, meaning the final study population may not be representative of the target population. This may impact the generalisability of the results, potentially impacting the applicability of findings to other regions. The small sample size may also mean very rare AEs will not be detectable. However, the sample size calculated allows for the detection of a minimum detectable HR below 2 for neutropenia and myelosuppression, even if the average follow-up is one year instead of the 2-year planned follow-up.

For the prospective exposure cohort, sites will be asked to enrol consecutive participants who meet the inclusion criteria, reducing potential selection bias. Ideally, both exposure and comparator groups would be concurrent. However, in the last few years many infants with SCD have been initiated onto Xromi® (off-label) irrespective of severity after the publication of the BABY HUG trial results in 2011 and the fact that children with HbS/S and HbS/β^0 aged 9 months and above should be offered hydroxycarbamide regardless of the clinical severity of their illness based on the UK guidelines (2018) [36]. Hence it would be difficult to prospectively and concurrently recruit both exposed and comparator participants, have sufficient follow-up in the retrospective comparator cohort and include comparable participants in both cohorts. The proposed design of non-concurrent comparators overcomes this challenge, but assumes that clinical management of these participants has not otherwise changed dramatically in the last 5-10

years of the study period. However, this assumption may not hold true, as clinical practices can evolve rapidly, particularly in fields like paediatric care.

For example, a reduction in the incidence of invasive pneumococcal disease has been observed with the uptake of pneumococcal conjugate vaccines covering a broader range of serotypes, and since the publication of the 'Prophylaxis with oral penicillin in children with sickle cell anaemia' (PROPS) and PROPS-II studies in 1986 and 1995 [37, 38], as well as the review of guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen by the British Society for Standards in Haematology in 2011 [39]. Additionally, there have been a change in the management of the disease since the implementation of newborn screening. In England, newborn screening for SCD has been implemented since 2006 [40], whereas in Germany, nationwide newborn screening for SCD was implemented in September 2021 [41]. Prior to this, SCD diagnosis was often clinical, with some hospitals and clinics conducting targeted screenings. Moreover, four pilot studies were conducted in Germany since 2011 to evaluate the impact and feasibility of introducing newborn screening for SCD.

It is important to note that the beneficial effects of these changes in SCD care were well-established in the UK by 2010, the earliest point of our retrospective data collection. By limiting our retrospective data collection to a maximum of 10 years, we ensure that these key interventions were standard practice in the UK throughout our study period.

While Germany only adopted nationwide newborn screening for SCD in September 2021, after the start of our study period, this does not indicate a complete lack of early diagnosis efforts. Prior to 2021, many regions and hospitals in Germany conducted targeted screenings for SCD in high-risk populations, providing a pathway for early diagnosis in many cases.

In this study, we will collect screening status and data on vaccinations received, including pneumococcal vaccines. To mitigate potential biases arising from these changes, comparators will be selected in a backwards consecutive manner, since the date of first Xromi® use in children from 9 months to under 2 years of age at the sites. This approach aims to include the most recent comparators possible, thereby enhancing comparability between the exposed group and the comparator group. As the abovementioned changes in standard clinical care were instigated many years ago, this approach would lessen the difference in the management between exposed and comparators. Sensitivity analyses will be conducted to evaluate the extent to which the evolving standard of care might undermine these analyses. These will be described in depth in the SAP.

Finally, the study relies on the data available in the medical records, which may vary in quality and completeness. Also, given the observational nature of the study, consideration was given to the possibility of inaccurately measured or unmeasured confounding. The data will be reviewed to identify potential confounding factors. As described above adjusted analyses will be performed to account for the identified confounding variables. This approach, while robust, may not fully account for all forms of confounding, especially those that are unknown or difficult to measure. Sensitivity analyses to understand the impact of unmeasured confounding will be performed and these analyses will be detailed in the SAP.

8.11. Quality Control

The electronic data collection tool that will be used in this study is OXONeDC. This EDC has been developed and validated by OXON. OXONeDC is a web-based application that supports global observational studies with real-time access to study data and reports.

In collaboration with Nova Laboratories, OXON will be responsible for the development and testing of the eCRF system. For this purpose, the amount and type of data to be collected will be assessed according to the study protocol and its objectives. The EDC will implement several layers of quality checks on the data entered into the database.

- Input validations: These validations are performed as data is entered into the EDC. These are built-in edit checks that display alerts/messages in real time, e.g.:
  - warnings if the type of value entered is incorrect,
  - alerts for missing mandatory fields,
  - alerts for data inconsistencies,
  - out of range values, or
  - guided data entry flow.
- Programmed validation rules for automated queries: These validation rules are programmed to perform more complex checks.

Training will be provided to study staff in the sites on the protocol, procedure, safety reporting and EDC. Systematic validation checks will be performed via the EDC with automatic range and logical consistency checks, and resolution of queries by clinical research associates with remote and on-site monitoring of sites. Concurrent manual data review will be performed using a risk-based approach.

Data quality control will be performed at site level by qualified designated personnel. During the site initiation visit, training will be provided to the investigator, sub-investigator(s), and all site staff involved in the study. Remote and on-site monitoring will be performed to examine compliance with the protocol, consent process, data collection procedures and accuracy and completeness of submitted data. Proper maintenance of records and documents for the duration of the study will also be verified. Source data verification by review of original patient records will be performed. The monitor will also validate expedited reporting of AE/SAE per local regulations.

Site close-out will be completed after the last participant's final study visit/follow-up is completed, all data have been entered and all outstanding monitoring issues have been resolved or addressed. All monitoring procedures and frequency of monitoring visits will be described in the monitoring guidelines. Representatives of the Sponsor quality assurance unit/monitoring team and competent regulatory authorities must be permitted to audit/inspect all study-related documents and other materials at the site, including the investigator site file, the completed eCRFs and the participants' original medical records. Audits/Inspections may be conducted at any time during or after the study to ensure the validity and integrity of the study data.

8.12. Other Aspects

8.12.1. Study Management

The study is sponsored by Nova Laboratories Ltd. a UK based pharmaceutical company. The Sponsor has contracted a Contract Research Organisation (CRO), OXON Epidemiology Ltd., for the design, conduct management, data collection and analysis of the study.

8.12.2. Good Pharmacoepidemiology Practice

The Sponsor and Investigator will ensure that the 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 ICH-GCP and Good Pharmacoepidemiology practices (GPP) for the conduct of post authorisation safety studies.

The Sponsor and its delegated CRO have implemented quality management systems to manage quality throughout all stages of the study process using a risk-based approach. A Project Plan will be developed and will include a risk assessment and risk register for the identification, evaluation, control, communication, review and reporting of the associated study risks. The risk assessment will be reviewed as appropriate during the study.

8.12.3. Protocol Deviations

Deviations from the procedures and processes outlined in this protocol will be recorded and reported to the Sponsor/delegate, and the corresponding regulatory authorities and Ethics Committees (EC) when applicable. It is the Investigator's responsibility to make all reasonable efforts to avoid protocol deviations in order to avoid possible exclusion of the participant from the study and/or analyses. Significant deviations or repeated non-compliance with the protocol could result in discontinuation from the study of the site involved.

8.12.4. Study or Site Discontinuation

The Sponsor may temporarily or permanently discontinue the study at a single site or all sites for safety, ethical, compliance or other reasons. If this is necessary, the Sponsor will endeavour to provide advance notification to the site(s) involved. If the site or study is suspended or discontinued, the Sponsor will be responsible for informing the EC and Competent Authority where required by local regulations. In such cases all study data must be returned to the Sponsor and documentation archived in accordance with applicable legislation and this protocol requirements. See *Section 9.4* for further information.

8.12.5. Registration of Study on Public Websites

This study will be registered on the Heads of Medicine Agencies – European Medicines Agency (HMA-EMA) Catalogues of Real-world data (RWD) sources and studies (previous EU electronic Register of Post-Authorisation Studies [EU PAS Register] and European Network of Centres for

Pharmacoepidemiology and Pharmacovigilance [ENCePP] Resource Database), and the study summary results will be posted to this public website no later than 12 months after the end of the study.

9. PROTECTION OF HUMAN SUBJECTS

9.1. Ethics Committee

This type of study requires review and approval by EC and applicable local authorities to conduct the study in the country/hospital. To collect and analyse study data, informed consent will be obtained from the parent(s)/guardian(s) and where applicable assent for retrospective children based on their age during study conduct. Thus, the study will be conducted under the auspices of an independent EC (and any local EC if applicable) in each of the participating countries, as defined in local regulations, and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

Nova Laboratories (or delegate) will ensure that an appropriately constituted EC that complies with the requirements of the applicable country-specific regulations will be responsible for the approval of the study. Prior to initiation of the study, Nova Laboratories and OXON will submit the study protocol and the relevant age-appropriate PIS and the informed consent forms (ICFs) (both for retrospective and prospective data collection) to be used for the study to the relevant EC for its review and approval.

Before initiating the study, the Sponsor will have written and dated full approval from the responsible EC for the protocol. The Sponsor will also be responsible to promptly report to the EC all changes in the study, and to provide a final report to the EC following study completion.

Apart from seeking favourable opinion from an independent EC (and any other local EC that may apply in each of the participating countries), the Sponsor (or delegate) must ensure that any other required approvals are obtained before study initiation at the site, such as independent review committees, regulatory authorities, and/or other local governance bodies.

9.2. Informed Consent

In accordance with local regulations, parent(s)/guardian(s) should provide consent before enrolment into the study. Investigators must ensure that parent(s)/guardian(s) are clearly and fully informed about the purpose of the study, potential risks, the participant's rights, and responsibilities when participating in this study. If local regulations do not require an informed consent document to be signed by the parent(s)/guardian(s) or there is a waiver in place, the site staff should document key elements of the informed consent process in the participant's chart. For those retrospective participants, age-appropriate assent for access to their data will be obtained where possible (if required by the local EC).

Prospective participants will be screened, and their eligibility assessed as they attend the participating sites. Eligible prospective participants will then be invited to participate in the study, provided the PIS and if willing, consent will be taken. For the retrospective comparator cohort, participants will be screened, and their eligibility assessed from clinical chart review. Those pre-screened children that are selected as potential matched comparators, will be invited to participate in the study, an age-appropriate PIS will be provided, and if willing, consent (or assent if applicable) will be obtained either in clinic or via postal invitation or remotely via telephone.

If important new information becomes available which may be relevant to the participants, any written material that they may receive must be revised to reflect this information, the PIS will be updated accordingly, participants notified in a timely manner and a new consent (or assent if applicable) obtained.

All patient information leaflets, and consent or assent forms required will be translated for use in Germany and provided in local languages. Where additional languages are required, this can be requested by the sites, or hospital translation services could be provided.

9.2.1. Withdrawal from the Study

The participant must be withdrawn from the study if:

- The participant and/or parents or legal representative explicitly withdraws consent.
- The investigator considers that, for study compliance reasons, it is in the best interests of the participant that he or she be withdrawn from the study.

All participants/parents or legal representative must be informed that their participation in the study is voluntary, and that they are free to withdraw from the study at any time without justifying their decision. The participants/parents or legal representative must be informed that their personal study-related data will be used by the sponsor in accordance with local data protection law. The actions to be taken as a result of the withdrawal of consent will be described in the informed consent.

The date of withdrawal of consent and the rationale (if provided) will be collected in the eCRF. Information collected from participants prior to withdrawal will remain in the study data set. No new data will be collected from the participant. Participants who discontinue treatment with Xromi® during the study are still eligible to continue in the study and will follow the same reporting procedure until the end of follow-up.

9.3. Data Protection and Confidentiality

The confidentiality of records that could identify participants within the database must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

Participants will be assigned a screening ID in the ePSL prior to enrolment, and a study ID following confirmation of eligibility and consent to participate (see *Section 8.2.4.3*). The study ID will be a unique code assigned by the EDC to each participant, such as a series of numbers and/or letters which will identify site and country. The data that is recorded with the participant's assigned study ID code is called “key-coded data.” Key-coded study data will be managed by the sponsor and/or its delegates in a study-specific electronic database (the “study database”). Only the investigator and the site research staff have access to the link between participant’s assigned code and the participant’s identity. However, in case of an audit or inspection, subject to local laws and regulations, government officials, Regulatory Bodies, Institutional EC representatives and sponsor representatives may access this information at the study site. If the study requires on-site monitoring, subject to local laws and regulations, sponsor representatives will also access the primary data source at the study site (see *Section 8.11*). Data that

could directly identify the participant will not be collected in the study database and only retained in the ISF.

9.4. Database Retention and Archiving of Study Documents

The investigator and Sponsor must retain all study records and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for 25 years as specified by the sponsor, whichever is longer. The investigator must contact Nova Laboratories prior to destroying any records associated with the study. Location of database and supporting documentation will be outlined in the final study report.

10. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ ADVERSE REACTIONS AND OTHER SAFETY INFORMATION

AE collection and reporting will be managed in line with current legislation. All AEs occurring during the study, regardless of the assumption of causal relationship, must be collected and documented on the eCRF. AEs should be documented from the time the parent(s) or legal representative(s) provides informed consent until the participant's participation in the study has been completed.

As the study has a design based on both primary data collection (prospective) and secondary use of data (retrospective), the submission of Adverse Drug Reaction Report (Individual Case Safety Reports) to the Competent Authority is required exclusively for the data obtained through primary data collection.

For the retrospective data, the information will be documented on the eCRF as described in the protocol and the reports of AEs will be summarised as part of any interim safety analysis and in the final study report.

In *Section 10.1* are provided the definitions of AEs, ARs, seriousness, severity and relatedness currently considered, as described in the Guideline on Good Pharmacovigilance Practices, Annex 1 [42]. These definitions will be further described in the SMP (*Section 10.4*).

10.1. Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product that does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Adverse Reaction (AR)

An AR is a response to a medicinal product which is noxious and unintended.

Response in this context means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility.

ARs may arise from:

- The use of a medicinal product within the terms of the marketing authorisation.
- The use of a medicinal product outside the terms of the marketing authorisation, including overdose, off-label use, misuse, abuse, and medication errors.

- Occupational exposure (this refers to the exposure to a prescribed treatment as a result of one's professional or non-professional occupation).

Serious Adverse Event (SAE)/ Serious Adverse Reaction (SAR)

An AE or AR is serious if one or more of the following criteria are met:

- Death.
- Life threatening: an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- In-patient hospitalisation or prolongation of existing hospitalisation: if a hospitalisation is planned, prior to the participant receiving the first dose of medicinal product it is not classified as serious. However, if a hospitalisation is unplanned and is a result of an adverse experience, this is considered a SAE. Hospitalisations due to procedures for pre-existing conditions (e.g. SCD) that were planned before the participant's enrolment are not considered SAEs unless they last > 7 days, there is worsening of the pre-existing condition, or any complication occurs after the participant signs the ICF.
- Results in persistent or significant disability or incapacity.
- Congenital anomaly or birth defect.
- Medical and scientific judgement should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalisation but might jeopardise the participant or might require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation or development of dependency or abuse.
- Any suspected transmission via a medicinal product of an infectious agent is also considered a SAR.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A suspected unexpected serious adverse drug reaction (SUSAR) is a SAR which nature, severity or outcome is not consistent with the SmPC. This includes class-related reactions mentioned in the SmPC but not specifically described as occurring with this product.

Severity

The severity of the AEs will be graded using the general guidelines from Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 [43]. Due to the characteristics of the study, CTCAE AE-specific grading will not be applied. Therefore, the investigator will assess the severity based on the following:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only, intervention not indicated.

- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

A semi-colon indicates 'or' within the description of the grade. Not all grades are appropriate for all AEs.

Relatedness

A clinical assessment of causality must be provided for all AEs (serious and non-serious). Causality will be assessed using the WHO-UMC system for standardise case causality assessment [44]. The causality terms and assessment criteria are as follows:

- Certain:
  - Event or laboratory test abnormality, with plausible time relationship to drug intake
  - Cannot be explained by disease or other drugs
  - Response to withdrawal plausible (pharmacologically, pathologically)
  - Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognised pharmacological phenomenon)
  - Rechallenge satisfactory, if necessary
- Probable / likely:
  - Event or laboratory test abnormality, with reasonable time relationship to drug intake
  - Unlikely to be attributed to disease or other drugs
  - Response to withdrawal clinically reasonable
  - Rechallenge not required
- Possible:
  - Event or laboratory test abnormality, with reasonable time relationship to drug intake
  - Could also be explained by disease or other drugs
  - Information on drug withdrawal may be lacking or unclear
- Unlikely:
  - Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible)
  - Disease or other drugs provide plausible explanations
- Conditional / unclassified:
  - Event or laboratory test abnormality

- More data for proper assessment needed, or
- Additional data under examination

- Unassessable / unclassifiable:
  - Report suggesting an adverse reaction
  - Cannot be judged because information is insufficient or contradictory
  - Data cannot be supplemented or verified

10.2. Collection of Adverse Events

All observed or volunteered AEs regardless of suspected causal relationship to Xromi® will be recorded in an eCRF on the study EDC and reported as described below and in more detail in the study SMP.

The clinical investigator will record all directly observed AEs and all AEs spontaneously reported by the participants. Each participant will be questioned during their routine clinical visits about any AEs experienced. AE information will be followed up to determine the final outcome of the event and classification for seriousness and relatedness. Any SAR occurring would require immediate notification to the Sponsor delegate (OXON), for reporting to the relevant Competent Authority.

AEs, ARs and other safety information will be individually collected by OXON Pharmacovigilance (PV). PV will exercise due diligence in following up the case with the investigators to collect any missing data elements of any reported case and ensure the completeness and accuracy of the information received.

10.3. Reporting of Adverse Events

Safety information for reportable ARs will be submitted to the Competent Authorities within the required timelines and according to the current legislation.

Where an Investigator does not become aware of the occurrence of a SAR immediately, the Investigator is to report the event to PV within 24 hours of becoming aware of it.

AE data arising from retrospective participants will not be subject to reporting, in line with current regulatory guidance and legislation. These cases will be recorded and captured in the EDC.

Due to the frequency of the expected AESI myelosuppression (as defined in *Section 8.4.3)* in this study population (under 2 years of age), only those events that meet the protocol definition and are categorised as related SARs will be subject to reporting to the Competent Authorities by PV. These cases will be collected and listed in the study reports.

10.4. Safety Management Plan

PV will develop a study-specific SMP, in compliance with Nova Laboratories requirements, and will include the following elements to ensure a comprehensive approach to safety information collection and reporting:

- Definitions (AEs, SAEs, non-serious AEs, ARs, SARs, medication error, severity, relatedness, off-label use, etc).
- Safety-specific roles and responsibilities.
- PV training to the study team.
- Safety information (AEs, ARs, AESIs, etc.) collection and reporting processes.
- AEs, ARs, other study-specific questionnaires.
- Medical monitoring.
- Study-specific PV monitoring process.
- Reporting process and timelines.
- Relevant Safety Issues: management and communication to Nova Laboratories.
- Reconciliation process.

11. PLANS FOR DISSEMINATION AND COMMUNICATION OF STUDY RESULTS

11.1. Ownership and Use of Data and Study Results

The ownership of data arising from the study resides with Nova Laboratories Ltd. (Nova). OXON will provide a full copy of the anonymised data to Nova. No use of the data will be possible without the authorisation of Nova.

11.2. Publication

The protocol, study status updates and report(s) will be included in regulatory communications according to the risk minimisation plan, periodic benefit-risk evaluation reports and other regulatory milestones and requirements.

The study summary results will be posted on the HMA-EMA RWD Catalogues after study termination. Nova Laboratories retains the right to publish at their discretion material relating to the conduct and conclusion of the study. A summary results document may also be provided to the participants.

A clinical study report will be developed and will serve as a basis for the development of publications and presentations in scientific journals and press releases and for submission to Regulatory Authorities to satisfy licensing requirements (e.g. PRAC and FDA, MHRA).

Abstracts, summaries, presentations and manuscripts will be prepared in line with dissemination guidelines of the International Committee of Medical Journal Editors [45] and Guidelines for GPP [46] to help ensure the quality and integrity of pharmacoepidemiological research and to provide adequate documentation of research methods and results.

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ANNEX 1 – LIST OF STAND-ALONE DOCUMENTS

None.

ANNEX 2 – HMA-EMA CATALOGUE OF REAL-WORLD STUDIES CHECKLIST FOR STUDY PROTOCOLS

Study title: A comparative observational study to evaluate the safety and effectiveness of Xromi® (hydroxycarbamide oral solution 100mg/ml) for the prevention of vaso-occlusive complications of sickle cell disease in children under 2 years of age.
EU PAS Register® number: EUPAS1000000076 **Study reference number (if applicable):**

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[1]	☒	☐	☐	5
1.1.2 End of data collection[2]	☒	☐	☐	5
1.1.3 Progress report(s)	☒	☐	☐	5
1.1.4 Interim report(s)	☒	☐	☐	5
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	5
1.1.6 Final report of study results.	☒	☐	☐	5
Comments:				

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:				
2.1.1 Why the study is conducted? (e.g., to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7
2.1.2 The objective(s) of the study?	☒	☐	☐	7
2.1.3 The target population? (i.e., population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	7
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☐	☒	
2.1.5 If applicable, that there is no a priori hypothesis?	☒	☐	☐	8.1

[1] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.

[2] Date from which the analytical dataset is completely available.

Comments:

Section 3: Study design	Yes	No	N/A	Section Number
3.1 Is the study design described? (e.g., cohort, case-control, cross-sectional, other design)	☒	☐	☐	8.1
3.2 Does the protocol specify whether the study is based on primary, secondary, or combined data collection?	☒	☐	☐	8.1
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	8.5
3.4 Does the protocol specify measure(s) of association? (e.g., risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☒	☐	☐	8.9
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g., adverse events that will not be collected in case of primary data collection)	☒	☐	☐	10
Comments:				

Section 4: Source and study populations	Yes	No	N/A	Section Number
4.1 Is the source population described?	☒	☐	☐	8.2
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period	☒	☐	☐	8.1
4.2.2 Age and sex	☒	☐	☐	8.2
4.2.3 Country of origin	☒	☐	☐	8.2
4.2.4 Disease/indication	☒	☐	☐	8.1
4.2.5 Duration of follow-up	☒	☐	☐	8.1
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g., event or inclusion/exclusion criteria)	☒	☐	☐	8.2
Comments:				

Section 5: Exposure definition and measurement	Yes	No	N/A	Section Number
5.1 Does the protocol describe how the study exposure is defined and measured? (e.g., operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☒	☐	☐	8.4

5.2 Does the protocol address the validity of the exposure measurement? (e.g., precision, accuracy, use of validation sub-study)	☐	☐	☒	
5.3 Is exposure categorised according to time windows?	☐	☐	☒	
5.4 Is intensity of exposure addressed? (e.g., dose, duration)	☒	☐	☐	8.4
5.5 Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	
5.6 Is (are) (an) appropriate comparator(s) identified?	☒	☐	☐	8.1
Comments:				

Section 6: Outcome definition and measurement	Yes	No	N/A	Section Number
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	8.5
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	8.5
6.3 Does the protocol address the validity of outcome measurement? (e.g., precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☐	☐	☒	
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g., HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☐	☐	☒	
Comments:				

Section 7: Bias	Yes	No	N/A	Section Number
7.1 Does the protocol address ways to measure confounding? (e.g., confounding by indication)	☒	☐	☐	8.9
7.2 Does the protocol address selection bias? (e.g., healthy user/adherer bias)	☒	☐	☐	8.9
7.3 Does the protocol address information bias? (e.g., misclassification of exposure and outcomes, time-related bias)	☐	☐	☒	
Comments:				

Section 8: Effect measure modification	Yes	No	N/A	Section Number
8.1 Does the protocol address effect modifiers? (e.g., collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☒	☐	☐	8.9
Comments:				

Section 9: Data sources	Yes	No	N/A	Section Number
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g., pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	8.6
9.1.2 Outcomes? (e.g., clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	8.6
9.1.3 Covariates and other characteristics?	☒	☐	☐	8.6
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g., date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☒	☐	☐	8.6
9.2.2 Outcomes? (e.g., date of occurrence, multiple events, severity measures related to event)	☒	☐	☐	8.6
9.2.3 Covariates and other characteristics? (e.g., age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	8.6
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g., WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☒	☐	☐	8.8
9.3.2 Outcomes? (e.g., International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	8.8
9.3.3 Covariates and other characteristics?	☒	☐	☐	8.8
9.4 Is a linkage method between data sources described? (e.g., based on a unique identifier or other)	☒	☐	☐	9.3
Comments:				

Section 10: Analysis plan	Yes	No	N/A	Section Number
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	8.9
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	8.7
10.3 Are descriptive analyses included?	☒	☐	☐	8.9
10.4 Are stratified analyses included?	☒	☐	☐	8.9
10.5 Does the plan describe methods for analytic control of confounding?	☒	☐	☐	8.9
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☐	☒	
10.7 Does the plan describe methods for handling missing data?	☒	☐	☐	8.9
10.8 Are relevant sensitivity analyses described?	☒	☐	☐	8.10
Comments:				

Section 11: Data management and quality control	Yes	No	N/A	Section Number
11.1 Does the protocol provide information on data storage? (e.g., software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	8.8, 9
11.2 Are methods of quality assurance described?	☐	☒	☐	
11.3 Is there a system in place for independent review of study results?	☐	☒	☐	
Comments:				

Section 12: Limitations	Yes	No	N/A	Section Number
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☒	☐	☐	8.10
12.1.2 Information bias?	☒	☐	☐	8.10
12.1.3 Residual/unmeasured confounding? (e.g., anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☒	☐	☐	8.10
12.2 Does the protocol discuss study feasibility? (e.g., study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☒	☐	☐	8
Comments:				

Section 13: Ethical/data protection issues	Yes	No	N/A	Section Number
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☒	☐	☐	9.1
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	
13.3 Have data protection requirements been described?	☒	☐	☐	9.3
Comments:				

Section 14: Amendments and deviations	Yes	No	N/A	Section Number
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	4
Comments:				

Section 15: Plans for communication of study results	Yes	No	N/A	Section Number
15.1 Are plans described for communicating study results (e.g., to regulatory authorities)?	☒	☐	☐	11
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	11
Comments:				

Name of the main author of the protocol:	Hussain Mulla
Date:	06-Aug-2025
Signature:	[redacted]

ANNEX 3 – DEFINITIONS OF CLINICAL EVENTS

The following definitions for the clinical events of interest are intended as guidance. However, the final determination will depend on the recorded occurrence of the event in the participant's clinical record and the investigator's clinical judgment.

- Acute chest syndrome: A clinical syndrome that includes at least 3 of the following symptoms: chest pain, temperature elevation over 38.5°C/101.5°F, tachypnoea, wheezing or cough.
- Blood transfusion: The provision of red blood cells.
- Cerebrovascular accident: Acute neurologic syndrome secondary to occlusion of an artery or haemorrhage with resultant ischemic and neurologic symptoms and signs. Can be:
  - Stroke, haemorrhagic: Injury to brain tissue resulting from disturbance of blood supply to the brain due to haemorrhage.
  - Stroke, infarctive: Injury to brain tissue consistent with occlusion of vessel(s) by thrombus or embolus which results in neurologic abnormalities on physical examination that persist beyond 24 hours.
  - Transient ischemic attack: Temporary interference with blood supply to the brain. The symptoms include neurologic signs that clear within 24 hours (48 hours if basilar system is involved). After the attack, no evidence of residual neurologic damage remains on physical examination.
  - Silent: so visible on imaging without overt neurological symptoms
- Painful vaso-occlusive event: Pain in the extremities, back, abdomen, chest, or head for which no other explanation can be found, and which is not classified as one of the other special events. The pain shall have lasted for at least 2 hours and for which any analgesic agent is taken. In a young child, pain or tenderness on palpation will be considered appropriate evidence of event.
- Dactylitis: Pain and tenderness with or without swelling in hands and/or feet. Also known as 'Hand foot syndrome.'
- Priapism: A painful erection of the penis lasting for more than 2 hours (sustained) or repeated episodes of shorter duration (stuttering).
- Hepatobiliary disorder: It can be any of the following:
  - Cholangitis: Inflammation of the bile ducts.
  - Cholecystitis: Inflammatory condition of the gallbladder causing right upper quadrant pain that may or may not be associated with gallstones.
  - Cholelithiasis: Formation or presence of calculi or bile stones in the gallbladder or common bile duct, with minimal or no symptoms.
  - Hepatitis: An inflammation of the liver.

  - Hepatic sequestration/intrahepatic sequestration: Jaundice and pain in the liver not due to gallstones. May include intrahepatic sickling crisis.
  - Other: Any other hepatobiliary event not listed above.

- Splenic sequestration crisis: The event is characterised by an increase in spleen size and firmness, reduction of Hb level by at least 20% and may include drop in platelet or white counts.
- Splenomegaly: An increase in spleen size and firmness.
- Surgery: Any operative procedure.
- Other events: Including, hospitalisations for SCD and non-hospitalised ED /treatment centres/paediatric ward/day unit visits, or any other clinical event not listed above.