26 April 2019
EMA/CHMP/170631/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Xromi

International non-proprietary name: hydroxycarbamide

Procedure No. EMEA/H/C/004837/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Administrative information

Name of the medicinal product:	XROMI
Applicant:	Nova Laboratories Ireland Limited 3rd Floor, Ulysses House Foley Street Dublin 1 D01 W2T2 IRELAND
Active substance:	HYDROXYCARBAMIDE
International Nonproprietary Name/Common Name:	hydroxycarbamide
Pharmaco-therapeutic group (ATC Code):	Other antineoplastic agents (L01XX05)
Therapeutic indication(s):	Prevention of vaso-occlusive complications of Sickle Cell disease in patients over 2 years of age.
Pharmaceutical form(s):	Oral solution
Strength(s):	100 mg/ml
Route(s) of administration:	Oral use
Packaging:	bottle (HDPE)
Package size(s):	1 bottle

Table of contents

List of abbreviations

ACS	Acute Chest Syndrome
AE	Adverse event
AF	Application Form
ALT	Alanine Transaminase
AML	Acute Myeloid Leukaemia
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
AR	Assessment Report
ARC	Absolute Reticulocyte Count
AS	Active Substance
AUC	Area Under (Plasma Concentration-Time) Curve
AUC_{0-inf}	Plasma Concentration Time Curve Extrapolated to Infinity
AUC_{0-t}	Plasma Concentration Time Curve Calculated to the Last Measured Concentration
$AUC_{\%extrap}$	Percentage of the Area Under the Curve extrapolated to infinity
BABY HUG	A phase III double-blinded, multi-centre, randomised, placebo-controlled infant hydroxycarbamide study
BCS	Biopharmaceutical Classification System
BP	British Pharmacopoeia
b.w.	Body weight
CAPA	Corrective and Preventive Actions
CAS	Chemical Abstracts Service
CEP	Certificate of Suitability to the monographs of the European Pharmacopoeia
cfu	Colony-forming unit
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CL/F	Clearance
C_{max}	Concentration Maximum
CMDh	Coordination Group for Mutual Recognition and Decentralised Procedures – Human
COMP	Committee for Orphan Medicinal Products
CRS	Chemical Reference Standard
CTD	Common Technical Document

CV	Co-efficient of Variation
DAD	Diode-Array Detector
DMT	Disease modifying therapy
DNA	Deoxyribonucleic acid
EC	European Commission
ECG	Electrocardiogram
EDQM	European Directorate for the Quality of Medicines
EDTA	Ethylenediaminetetraacetic acid
EEA	European Economic Area
EMA	European Medicines Agency
EPO	Erythropoietin
ESRD	End Stage Renal Disease
EU	European Union
EUDRA-GMDP	EU database on manufacturing, import and wholesale-distribution authorisations, and good manufacturing-practice (GMP) and good-distribution-practice (GDP) certificates
FDA	Food and Drug Administration
FP	Finished Product
FT-IR	Fourrier Transform Infrared Spectroscopy
g	Gram
GCP	Good Clinical Practice
GD	Gestation Day
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GVP MV	Good Vigilance Practice Module V
Hb	Haemoglobin
HbF	Foetal Haemoglobin
HbS	Sickle Haemoglobin
HC	Hydroxycarbamide
HDPE	High-density polyethylene
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HSCT	Haematopoietic Stem Cell Transplantation
HU	Hydroxyurea
HUSOFT	Hydroxyurea Safety and Organ Toxicity study

HUSTLE	Hydroxyurea Study of Long-Term Effects
IARC	International Agency for Research on Cancer
ICH	International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IMP	Investigational Medicinal Product
INN	International Non-proprietary Name
IV	Intravenous
k_{el}	Elimination rate constant
KF	Karl Fischer titration
kg	Kilogram
L	Litres
LaSHS	Laikon Study of Hydroxycarbamide in Sickle Cell Syndromes
LC-MS	Liquid Chromatography–Mass Spectrometry
LIC	Liver Iron Concentration
LLOD	Lower Limit Of Detection
LLOQ	Lower Limit Of Quantification
LS	Least Squares
m^2	Square meter
MA	Marketing Authorisation
MAA	Marketing Authorisation Application
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
MIAH	Manufacturer and Importer Authorisation Holder
mL	Millilitre
MO	Major Objection
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
MSH	Multicentre Study of Hydroxycarbamide in Patients with Sickle Cell Anaemia
MTD	Maximum Tolerated Dose
n	Number
NA	Not Available
NHLBI	National Heart, Lung, and Blood Institute

NIH	National Institute of Health
NMRI	Naval Medical Research Institute
NMT	Not More Than
NO	Nitric Oxide
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program
OC	Other Concern
OOS	Out Of Specification
PAC	Post-Approval Commitment
PBT	Persistence, Bioaccumulation, and Toxicity
PD	Pharmacodynamic
PDCO	Paediatric Committee
PEC	Predicted Environmental Concentration
PET	Preservative Efficacy Testing
Ph. Eur	European Pharmacopoeia
PhV	Pharmacovigilance
PIL	Patient Information Leaflet
PK	Pharmacokinetic
PNEC	Predicted No Effect Concentration
PRAC	Pharmacovigilance Risk Assessment Committee
PSUR	Periodic Safety Update Report
PSUSA	Periodic Safety Update report Single Assessment
Q	Quarter
QP	Qualified Person
RBC	Red Blood Cell
RCT	Randomized Controlled Trial
RH	Relative Humidity
RLD	Reference Listed Drug
RMP	Risk Management Plan
RR	Ribonucleotide Reductase
SCA	Sickle Cell Anaemia
SCATE	Sparing Conversion to Abnormal TCD Elevation study

SCD	Sickle Cell Disease
SCF	Stem Cell Factor
SD	Standard Deviation
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
SWiTCH	Stroke With Transfusions Changing to Hydroxycarbamide
TAMC	Total Aerobic Microbial Count
TAMMV	Time Averaged Maximum Mean Velocity
TCD	Transcranial Doppler
$t_{1/2}$	Half-Life
TEAE	Treatment Emergent Adverse Event
t_{max}	Amount of time that a drug is present at the maximum concentration
TWiTCH	Transfusions Changing to Hydroxyurea
TYMC	Total Combined Yeasts/Moulds Count
UK	United Kingdom
USA	United States of America
USAN	United States Adopted Name
USP	United States Pharmacopoeia
UV	Ultraviolet
V_d	Volume of distribution
VDJ	Variable, diverse, and joining
V_{max}	Maximum rate of metabolism
VOC	Vaso-Occlusive Crisis
WBC	White Blood Count
WHO	World Health Organization
µg	Microgram
µM	Micromolar

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Nova Laboratories Ireland Limited submitted on 9 February 2018 an application for Marketing authorisation to the European Medicines Agency (EMA) for Hydroxycarbamide Nova Laboratories, through the centralised procedure under Article 3 (2) (b) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 12 October 2017. The eligibility to the centralised procedure under Article 3(2)(b) of Regulation (EC) No 726/2004 was based on demonstration of interest of patients at Community level.

The application concerns a hybrid medicinal product as defined in Article 10(3) of Directive 2001/83/EC and refers to a reference product, as defined in Article 10 (2)(a) of Directive 2001/83/EC, for which a marketing authorisation is or has been granted in a Member State on the basis of a complete dossier in accordance with Article 8(3) of Directive 2001/83/EC.

The applicant applied for the following indication: Prevention of complications of Sickle Cell disease including recurrent painful vaso-occlusive crises, dactylitis, acute chest syndrome, anaemia and stroke, in adults, adolescents and children older than 2 years regardless of the severity of disease.

The legal basis for this application refers to:

Hybrid application (Article 10(3) of Directive No 2001/83/EC).

The application submitted is composed of administrative information, complete quality data, a bioequivalence study with the reference medicinal product Hydrea and appropriate non-clinical and clinical data.

The chosen reference product is:

Medicinal product which is or has been authorised in accordance with Union provisions in force and to which bioequivalence has been demonstrated by appropriate bioavailability studies:

- Product name, strength, pharmaceutical form: Hydrea, 500mg, capsule hard
- Marketing authorisation holder: E.R.Squibb & sons Ltd
- Date of authorisation: (25-05-1986)
- Marketing authorisation granted by:
- Member State (EEA): UK
- National procedure
- Marketing authorisation number(s): PL 11184/0139
- Bioavailability study number(s): RD 729/26118

Medicinal product authorised in the Union/Member State where the application is made or European reference medicinal product:

- Product name, strength, pharmaceutical form: Hydrea, 500mg, capsule hard
- Marketing authorisation holder: E.R.Squibb & sons Ltd

- Marketing authorisation granted by:
- Member State (EEA): Ireland
- National procedure
- Marketing authorisation number(s): PA 0002/27/001
- Bioavailability study number(s): RD 729/26118

Information on paediatric requirements

Not applicable.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Scientific advice

The applicant received Scientific Advice on 01 April 2016 (EMEA/H/SA/3241/1/2016/SME/III) and 23 March 2017 (EMEA/H/SA/3241/1/FU/1/2017/SME/III) for the development programme supporting the indication granted by the CHMP. The Scientific Advice pertained to the following quality, non-clinical, and clinical aspects:

- Suitability of the proposed oral liquid formulation for children. Acceptability of the proposed limit of an identified impurity of NMT 0.5% in the drug product specification during shelf-life.
- Requirement for non-clinical pharmacology or toxicology studies with respect to the excipients proposed for the intended marketed formulation and the drug substance.
- Sufficiency of published efficacy and safety data to support an indication in infants aged 9 months – 2 years, at a dose of 20 mg/kg/day without escalation, regardless of the severity of the disease. Need for additional efficacy and safety data in adults, adolescents and children aged >2 years. Sufficiency of a relative bioavailability study in healthy adult volunteers comparing Nova's hydroxycarbamide 100mg/mL oral solution to the innovator Hydrea 500mg capsule to establish the bridge between the new formulation and Hydrea-based literature. Appropriateness of the proposed clinical study protocol for generating PK data in infants and children, and for providing the reassurance for weight based dosing across the paediatric age range.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Constantinos Markopoulos Co-Rapporteur: Koenraad Norga

The application was received by the EMA on	9 February 2018
The procedure started on	1 March 2018
The Rapporteur's first Assessment Report was circulated to all CHMP members on	22 May 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	22 May 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	4 June 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	28 June 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	13 Sept 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	22 October 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	31 October 2018
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	15 November 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	28 January 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	15 February 2019
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	28 February 2019
The applicant submitted the responses to the CHMP List of Outstanding Issues on	04 March 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	19 April 2019
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	27 March 2019
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Hydroxycarbamide Nova Laboratories on	26 April 2019

2. Scientific discussion

2.1. Introduction

This application was submitted under a 10(3) hybrid legal basis, using Hydrea (containing hydroxycarbamide) as a reference product. Hydrea is authorised for treatment of chronic myeloid leukaemia and cancer of the cervix in conjunction with radiotherapy, but not for the indication applied for by Nova Laboratories for Xromi. Another hydroxycarbamide containing medicinal product, Siklos, was granted a MA in EU in 2007 and is indicated for the following indication: "Prevention of recurrent painful vaso-occlusive crises including acute chest syndrome in adults, adolescents and children older than 2 years suffering from symptomatic Sickle Cell Syndrome."

The applicant has conducted a systematic review of the published literature relating to the efficacy and safety of hydroxycarbamide in the treatment of Sickle Cell Disease (SCD). Based on an evaluation of the totality of data reported in the literature, the applicant justified that the proposed oral solution formulation is expected to perform in the same way as the oral liquid formulation and capsules used in the reported studies. No non-clinical pharmacological or toxicological studies have been performed considering the long clinical history of hydroxycarbamide.

Xromi 100 mg/ml is an oral solution, one ml of which contains 100 mg hydroxycarbamide. It belongs to the pharmacotherapeutic group of other antineoplastic agents and its ATC code is L01XX05. Hydroxycarbamide (international non-proprietary name, INN), also known as Hydroxyurea, has the chemical formula CH4N2O2 and a molecular weight of 76.05 and is classified as a cytotoxic, antimetabolite, antineoplastic agent. The main mechanism of action of hydroxycarbamide is the reversible inhibition of ribonucleotide reductase, which causes temporary arrest of haematopoiesis and induces the so called "stress erythropoiesis". The marrow responds to this stressed state by enhanced erythropoiesis and increased HbF production. HbF inhibits intracellular HbS polymerization and prevents the sickling process within erythrocytes. Another action of hydroxycarbamide is improving blood flow through reduced intercellular adhesion. Decreased expression of RBCs, white blood cells, and endothelial integrins and other adhesion molecules probably improves microvascular blood flow and reduces pro-inflammatory cell–cell interactions. Hydroxycarbamide may also stimulate nitric oxide (NO) production as an NO donor or through stimulation of intermediates. The primary pharmacodynamic effect of hydroxycarbamide is the increase of HbF, preventing thus the formation of HbS and ameliorating the clinical effect of SCD. Optimal results are achieved when the dose is escalated to MTD.

Xromi 100 mg/mL oral solution's posology is based on the patient's body weight. The usual starting dose of hydroxycarbamide is 15 mg/kg/day, the usual maintenance dose is between 20-25 mg/kg, and the maximum dose is 35 mg/kg/day. Full blood cell count with white cell differential and reticulocyte count should be monitored every 2 weeks for the first 2 months following treatment initiation. If neutropenia or thrombocytopenia occurs, hydroxycarbamide dosing should be temporarily withheld and full blood cell count with white cell differential should be monitored weekly. When blood counts have recovered, hydroxycarbamide should be reinstated at a dose 5 mg/kg/day lower than the dose given before onset of cytopenias. If dose escalation is warranted based on clinical and laboratory findings, dosing 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. Once a stable dose is established, laboratory safety monitoring should include full blood cell count with white cell differential, reticulocyte count, and platelet count every 2–3 months. 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).

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as oral solution containing 100 mg/ml of hydroxycarbamide as active substance.

Other ingredients are: xanthan gum (E415), sucralose (E955), strawberry flavour, methyl parahydroxybenzoate (E218), sodium hydroxide (E524), and purified water.

The product is available in Amber type III glass bottle with tamper evident child-resistant closure (HDPE with expanded polyethylene liner) as described in section 6.5 of the SmPC.

Each pack contains an HDPE bottle adaptor and 2 polyethylene dosing syringes (a red syringe graduated to 3 ml and a white syringe graduated to 12 ml).

2.2.2. Active substance

General information

The chemical name of hydroxycarbamide is N-hydroxyurea corresponding to the molecular formula $CH_4N_2O_2$ It has a relative molecular mass of 76.05 g/mol and the following structure:

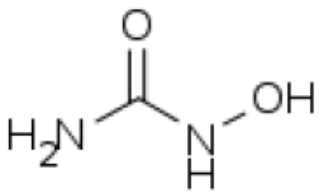


Figure 1: Active substance structure

The relevant information of the chemical structure of active substance has been assessed by the EDQM before issuing the Certificate of Suitability (CEP).

The active substance is a hygroscopic white or almost white, crystalline powder, soluble in water, and practically insoluble in alcohol.

Hydroxycarbamide has a non - chiral molecular structure.

The European Pharmacopoeia monograph for hydroxycarbamide (monograph 1616) states that the substance shows polymorphism. There was no evidence of this property having any significant effect on hydroxycarbamide dissolution rate and solubility during development studies and in pilot-scale manufacture of the finished product.

As there is a monograph of the active substance in the European Pharmacopoeia, the manufacturer of the active substance has been granted a Certificate of Suitability of the European Pharmacopoeia (CEP) for which has been provided within the current Marketing Authorisation Application.

Manufacture, characterisation and process controls

The relevant information has been assessed by the EDQM before issuing the Certificate of Suitability.

The active substance is packed into sealed a double “food-grade” polyethylene inner bag which complies with the EC directive 2002/72/EC and EC 10/2011 as amended put in fibre drums with metal cover.

Specification

The active substance specification includes tests for identification (FT-IR, TLC), assay (HPLC), urea (TLC), impurities (HPLC), chlorides (Ph. Eur.), water (KF), and sulphated ash (Ph. Eur.).

The control tests were carried out to comply with the specifications and test methods of the Ph. Eur. monograph. In addition, any impurity by HPLC to not more than 0.05% is controlled, in accordance with Ph. Eur. 2034.

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

Stability

The CEP submitted does not include any information on the active substance retest period, additional information was submitted on stability.

Stability data from 7 commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 60 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided.

The following parameters were tested*:* appearance, loss on drying, assay and degradation products.

All tested parameters under long term and accelerated conditions were within the specifications.

Results on stress conditions were also provide on one batch. Forced degradation was performed by the exposure to the following stress conditions: acid- and base-mediated degradation (1N HCl, 0.1N HCl, 1N NaOH, and 0.1N NaOH for 72 hours), oxidative (peroxide, 0.5% and 2% H2O2 for 72 hours) degradation, temperature (~80 °C for 72 hours) degradation, moisture degradation (40 °C/ 75% RH for 72 hours), and photodegradation.

The active substance shows minimal degradation, indicating a reasonable level of stability in these conditions when exposed to acidic or oxidative conditions, as well as heat.

Photostability testing following the ICH guideline Q1B was performed on one batch. All tested parameters were within the specifications.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 36 months without storage conditions in the proposed container.

2.2.3. Finished medicinal product

Description of the product and Pharmaceutical development

The finished product is an oral solution. Its composition is given in Table 1 below.

Table 1: Composition of finished product

Component	Quality standard
Hydroxycarbamide	Ph.Eur., USP
Xanthan Gum	Ph. Eur., NF
Sucralose	Ph. Eur., NF
Strawberry liquid flavour	Manufacturer's specification
Methyl para-hydroxybenzoate	Ph. Eur., USP
1.0 N Sodium Hydroxide	Ph. Eur., USP
Water	Ph. Eur.

Hydroxycarbamide is a cytotoxic, antimetabolite, and antineoplastic agent used for several decades to treat a variety of medical disorders and was approved for the treatment of adults with sickle cell anaemia (SCA) (Hydrea) which is manufactured as capsules. The aim of the pharmaceutical development of the finished product was to improve the acceptability of the formulation in children under 6 years old who have difficulties swallowing tablets or capsules. Hydroxycarbamide oral solution administered using an oral syringe will allow the dose of hydroxycarbamide to be individualised to the patient's maximum tolerated dose, both accurately and safely. Moreover, improved ease of administration for children will enhance medication acceptability and adherence.

From previous experience, hydroxycarbamide was known to be compatible with most of the excipients used in this formulation. Hydroxycarbamide stability has since been confirmed by formal stability testing. Hydroxycarbamide is described as "freely soluble in water" in the European Pharmacopoeia. As such it is an ideal candidate for a solution formulation. The hydroxycarbamide solution is formulated at a pH which provides maximum stability. The powder is odourless or almost odourless. The active substance is cytotoxic, and as such formulation design has taken into account minimisation of exposure during use. This is particularly important for a finished product designed for use in the paediatric population, where a guardian is likely to be frequently involved in dosing.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards, with the exception of strawberry liquid flavour which complies with 'In-house' specifications. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

The provided literature data indicated that chemical stability would be a challenge since hydroxycarbamide is highly soluble and unstable in aqueous environment. Initial formulation stability studies confirmed this. The degradation pathways are not clearly defined in literature, although pH induced hydrolysis was considered the most plausible and likely degradation pathway. Thus, pH was likely to be a critical parameter of the formulation. Degradation was significantly reduced by storing samples at 5°C and hence subsequent stability testing was conducted at 5°C for long-term and 25°C/60%RH accelerated conditions.

The level of sucralose was selected based on levels typically used in oral pharmaceuticals and the level of strawberry liquid flavor was determined based on the range recommended by the manufacturer. Palatability has been assessed by a questionnaire as part of the bioequivalence study.

The manufacturing process involves successive dissolution of the formulation components by stirring to form a clear solution.

A study to assess the bioequivalence of an oral hydroxycarbamide solution 500 mg/5 mL versus two formulations of oral hydroxycarbamide capsule 500 mg (Hydrea) was performed showing bioequivalence between the formulations.

The primary packaging is Amber type III glass bottle with tamper evident child-resistant closure (HDPE with expanded polyethylene liner). The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. Each pack contains an HDPE bottle adaptor and 2 polyethylene dosing syringes (a red syringe graduated to 3 ml and a white syringe graduated to 12 ml). The medical devices are CE marked. Although the active is present in solution in the formulation, given that the contact period between the oral solution and the syringe is brief, no significant interaction is anticipated.

Manufacture of the product and process controls

The manufacturing process consists of 4 main steps: weighing of ingredients, preparation of solution, filling and closure. The process is considered to be a standard manufacturing process.

Major steps of the manufacturing process have been validated by a number of studies. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

Product specification

The finished product release and shelf –life specifications include appropriate tests for this kind of dosage form: appearance (visual), pH (Ph. Eur.), viscosity (Ph. Eur.), uniformity of mass of delivered doses (Ph. Eur.), hydroxycarbamide content (HPLC), identification hydroxycarbamide (HPLC, UV), related substances (HPLC), methyl hydroxybenzoate content (HPLC), identification methyl hydroxybenzoate (HPLC, diode array detection), fill volume, TAMC (Ph. Eur), TYMC (Ph. Eur)and specified microorganism, *Escherichia coli* (Ph.Eur.).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for 3 production scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing

Stability of the product

Stability data from 3 pilot scale batches of finished product stored for up to 24 months and 1 pilot batch stored for up to 21 months under long term conditions (2-8 ºC) and for up to 3 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. The batches of the medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, pH, assay of hydroxycarbamide, related substances, assay of methyl hydroxybenzoate, viscosity, total viable count, microbial limit test (absence of *E. coli*), and preservative efficacy.

Initial formulation studies indicated that data from long term (2-8°C) showed that the product meets the proposed shelf life specification. For the pilot scale batch stability studies, a significant change occurred at the accelerated condition (25°C/60%RH) with regards to the level of an unknown related substance which exceeds the acceptance limit in the first 3 months' testing. Therefore, stability testing at the accelerated condition was not conducted after 3 months.

Two additional studies were performed: a hold time study evaluated the stability of the formulation during manufacture at room temperature and a thermal cycling study to evaluate temperature excursions which may occur during commercial product distribution. The conclusion from both of these studies is that short term excursions above 5°C will not have a detrimental effect on the stability of the finished product.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The data indicate that hydroxycarbamide is not susceptible to photodegradation. Results indicate that storage in amber glass bottles is sufficient protection for the finished product against photo-degradation.

An in-use stability study was conducted on one batch at 9 months after manufacture and on another batch at 3 months. Five bottles were used from each batch and the study was conducted over 12 weeks. Bottles were stored at 2-8°C for the duration of the study. On each day of sampling the bottle was removed from storage, the sample removed, and then the bottle returned to 2-8°C storage. This allowed the withdrawal of product on up to 60 occasions from a single bottle of product. As the study was conducted over an extended period of time the product was subjected to a maximum challenge. There was no detectable bioburden at the end of the study for both batches. A further study has been conducted on one batch at 20 months after manufacture. The study design is similar to that mentioned, however, chemical testing was only performed every four weeks and includes viscosity and microbiological testing is performed at 8 weeks and 12 weeks and includes preservative efficacy. To support this study design eighteen bottles were used. There was no loss of potency of hydroxycarbamide during the studies. All analytical and microbiological parameters remained within specification over the duration of the studies. Microbiological contamination remained at very low levels.

Based on available stability data, the proposed shelf-life of 24 months and store in a refrigerator (2°C - 8°C) and the in-use shelf-life "After first opening to be used within 12 weeks", as stated in the SmPC (section 6.3), are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendation for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

No formal non-clinical development programme was undertaken for hydroxycarbamide, the non-clinical data is based on relevant information identified from the literature. The use of hydroxycarbamide over many decades has provided much information on this compound that has led to its use, first in oncology and then in haematological indications, such as sickle cell disease. Due to its historic use in the clinic, this has also resulted in limited pre-clinical studies being published in the literature. However, there have been several comprehensive reviews including IARC and NTP, which have contributed significantly to the general information available in evaluating hydroxycarbamide.

2.3.2. Pharmacology

The principle and most well understood mechanism of action of hydroxycarbamide, which was first demonstrated *in vitro*, is the reversible inhibition of ribonucleotide reductase, a critical enzyme that converts ribonucleosides into deoxyribonucleosides, which are required for the synthesis and repair of DNA. Other benefits include increased levels of nitric oxide and cyclic nucleotides that may facilitate vascular dilatation and also induce HbF, and even direct salutary effects on the vascular endothelium, reduced expression of surface molecules that adhere to the endothelium, decreased neutrophil adhesion and effects on sickle red blood cell rheology. The applicant was requested and submitted data from more recent studies on the mechanism of action of hydroxycarbamide, such as those presented at Silva-Pinto 2014 and Cokic 2007. Data argued that although various mechanisms of action of hydroxycarbamide have been identified in the literature, it is generally agreed that its precise role and/or contribution of each mechanism is currently unclear.

Bibliographical references concerning *in vivo* pharmacology study results, strongly suggested that hydroxycarbamide (an S-phase–specific cytotoxic) increases HbF by a mechanism that does not involve inhibition of DNA methylation. Furthermore, the combination of SCF+EPO+hydroxycarbamide resulted in an additional two-fold increase in HbF, whereas F-cells and F-reticulocytes increased only 25% compared to the SCF + EPO regimen. Further studies have examined the possible modes of action by which hydroxycarbamide may be acting resulted that induction of foetal haemoglobin was required for beneficial clinical effects.

Safety pharmacology programme

The cardiovascular safety of hydroxycarbamide was examined in a safety pharmacology study in dogs resulting that all cardiovascular effects reported with hydroxycarbamide were reversible within 24 hours. Other toxicity studies (not only cardiovascular) were presented and analysed chronic hydroxycarbamide administration in animals. The available data in animals have identified a range of toxicities in rats, dogs, monkeys. However, the results from non-clinical studies have not been reflected in data from clinical

studies. A chronic administration for up to 15 years of hydroxycarbamide in clinical studies has demonstrated an absence of the toxicities previously observed in non-clinical data.

Pharmacodynamic Drug Interactions

There are limited studies in the non-clinical literature regarding pharmacodynamic drug interactions with hydroxycarbamide. However it is known that hydroxycarbamide interacts with antiretroviral drugs when used concomitantly leading to increased toxicity. Although hydroxycarbamide is not indicated for the treatment of HIV infection, patients with HIV infection that are administered with hydroxycarbamide and didanosine, with or without stavudine, have experience fatal and nonfatal pancreatitis. Additionally, hepatotoxicity and hepatic failure resulting in death have been reported during postmarketing surveillance in patients with HIV infection treated with hydroxycarbamide and other antiretroviral drugs. Peripheral neuropathy, has been reported in patients with HIV infection receiving hydroxycarbamide in combination with antiretroviral drugs.

There is also evidence that there is an analytical interference of hydroxycarbamide with the enzymes (urease, uricase, and lactate dehydrogenase) used in the determination of urea, uric acid, and lactic acid, rendering falsely elevated results of these in patients treated with hydroxycarbamide.

2.3.3. Pharmacokinetics

Absorption, distribution, metabolism and excretion data are based upon studies in the literature and no formal development programme was undertaken for hydroxycarbamide by the applicant.

Methods of analysis are not always described in the publications on hydroxycarbamide pharmacokinetics. In cases where analytical methods are mentioned these include high performance liquid chromatography (HPLC), liquid scintillation counting, and gas chromatography-mass spectrometry (GC-MS).

Absorption and elimination was found to follow first-order kinetics after intraperitoneal administration of hydroxycarbamide in a study comparing wild type to transgenic sickle cell mice. Studies *in vitro* have identified that hydroxycarbamide is a substrate for organic anion transporting polypeptide (OATP1B) transporters.

Investigation using OATP1B homozygous knockout mice also suggested that OATP1B transporters have a role in the absorption and the distribution of hydroxycarbamide and revealed accumulation of hydroxycarbamide in the kidney and liver of wild type mice. Hydroxycarbamide ability to cross the blood - brain barrier and the dependence of this movement on organic anion transportation was shown in anaesthetised guinea-pigs with the use of non-clinical distribution data.

In a recent metabolism study with wild type and transgenic sickle cell mice, HPLC analysis of the hydroxycarbamide extracted from the urine showed that there was no change in the parent compound. The non-clinical metabolism data derived from previous distribution and excretion studies, concluded that basically 100% of the excreted C14 would be accounted for as unchanged parent, urea and carbonate.

In vitro investigation of the effect of CYP450 (human cytochromes) and P-glycoprotein on hydroxycarbamide metabolism concluded that hydroxycarbamide is unlikely to cause clinically relevant drug interactions with the substrates of these enzymes/transporters.

Excretion, following intraperitoneal (mice and rats) and oral administration (mice) of C14-hydroxycarbamide, was found to be predominantly and rapidly via the urine.

2.3.4. Toxicology

Single dose toxicity

Table 2: single dose toxicity

Study	Species/Sex/Number/Group	Dose/Route	LD_{50}	Major Findings
Single Dose Toxicity				
Hydrea FDA Prescribing Information, 2010	Mouse	p.o.	7,330 mg/kg bw	Aplasia
Hydrea FDA Prescribing Information, 2010	Rat	p.o.	5,780 mg/kg/bw	Aplasia
Malec et al 1989	Male Rabbit, n=15	i.v. 150 mg/kg	NS	Significant transient decrease in blood erythrocyte count

NS=not stated

In single-dose studies, mice and rats were exposed to a single oral dose of hydroxycarbamide followed by a 14-day observation period. The acute median lethal dose was within 5,000 to 15,000 mg/kg b.w. depending on animal species and route of administration.

Deaths were observed after several days. The major clinical sign observed was aplasia (no further specifics available). The oral LD50 of hydroxycarbamide was 7,330 mg/kg b.w. in mice and 5,780 mg/kg b.w. in rats (Hydrea Bristol-Myers Squibb Patient Information 2010).

Additionally, in male rabbits receiving a single intravenous injection of 150 mg/kg b.w. hydroxycarbamide solution, there was an early but transient significant decrease in blood erythrocyte count (approximately 30% lower at day 3 after hydroxycarbamide) with a gradual increase in their osmotic resistance, a suppression of granulocyte phagocytic capacity (approximately 50% at day 3), and a rapid increase in the proportion of lymphocytes without detectable lysosomes (2-fold at 24 hours). Furthermore, serum acid phosphatase activity was elevated at day 7 after hydroxycarbamide administration, which may have reflected a manifestation of cell injury. Histological analysis of the liver of BALB/c mice analogously treated with hydroxycarbamide, revealed a marked hepatotoxicity, manifested as dilation of the intercell cord spaces and bile capillaries, the appearance of numerous balloon cells, cells with pyknotic nuclei, undetectable nucleoli and necrosis; particularly in the centres of hepatic lobules. To explain the observed toxicities, it was hypothesized that hydroxycarbamide initiated free radical mediated reactions (Malec et al, 1989).

Repeat dose toxicity

Table 3: Repeat dose toxicity

Study	Species/Sex/Number/Group	Dose/Route	Duration	NOEL/NOAEL (mg/kg/day)	Major Findings
Repeat Dose Toxicity					
Morton et al 2015	Sprague Dawley Rat, n=5/sex/group	p.o. 50, 500, 1,500 mg/kg day	10 days	NOAEL = 50 mg/kg/day	Haematology
Morton et al 2015	Beagle Dog, n=1/sex/group	p.o. 50, 250, 1,000 mg/kg/day	14 days	NOAEL = 50 mg/kg/day	Clinical Pathology
Morton et al 2015	Beagle Dog, n=3/sex	p.o. 50 mg/kg/day	Up to 29 days	NS	Haematology, ↑ bone marrow cellularity, ↓ maturing granulocytes, ↑ creatinine activity, iron pigment in bone marrow and hepatic sinusoidal cells

NS=not stated

In both the rat and dog models 50 mg/kg/day dose was considered well tolerated and as the no adverse effect level due. In the one month study in dogs results showed that a dose of 50 mg/kg/day administered once-daily for one month was tolerated with no adverse clinical signs or effects on body weight, food consumption, or ophthalmic assessments (Morton et al., 2015). However, long-term hydroxycarbamide treatment in dogs, (up to four months), with daily doses of up to 30 mg/kg, the following effects have been noted: myelosuppression, gastrointestinal complications and, rarely, onychomadesis. Within a month of cessation of treatment, all nails had healed completely (Marconato et al., 2007).

Genotoxicity

An IARC monograph was published in 2000, which concluded that hydroxycarbamide neither bonds chemically or otherwise to DNA. However, it is known to inhibit ribonucleotide reductase, which converts ribonucleoside diphosphates to deoxyribonucleotide diphosphate precursers for de-novo DNA synthesis (IARC 2000). A study in 2011 concluded that hydroxycarbamide showed mutagenic and genotoxic potential (Santos *et al* 2011).

Carcinogenicity

Table 4: Carcinogenicity studies:

Study	Dose/Route	Species/Sex/No. of animals	Negative Control	Treated	Positive Control
Carcinogenicity					
IARC, 2000	1mg at 2 days old, 3mg at 8 days old, 5mg at 15 days old, 10mg from 30 days old – 1 year, i.p.	XVII/G mice, n=50 mice/sex/group	Pulmonary tumours 30/50 (60%)	Pulmonary tumours 16/35 (46%)	Urethane: pulmonary tumours 28/30 (93%)
IARC, 2000	5µg 7,12-dimethylbenz[a]anthracene dermally; 4 weeks later 1% croton oil then 500 mg/kg hydroxycarbamide i.p. once at 24 hrs or twice at 24 and 48 hrs after croton oil for 14 weeks	Swiss mice	NS	Two treatments of hydroxycarbamide resulted in reduced incidence of skin papilloma compared with no hydroxycarbamide treatment	NS
IARC, 2000	Pre-treatment (30 min prior) with 5mg hydroxycarbamide i.p. then dermal 2mg N-methyl-N-nitrosurea	Hairless Oslo mice	NS	Increased incidence of skin tumours from 50-80%	NS
IARC, 2000	I.p. injections in 23 fractionated consecutive doses of 0.1 mg/kg each shortly before and during maximal urothelial cell proliferation (33-55 hr after partial cystectomy) produced by N-methyl-N-nitrosurea given as a single intravesicular pulse dose of 5 mg/kg during various cell cycle phases.	Female Wistar rats, n=36-43	14/43 urothelial bladder tumours in G0 phase	Inhibited N-methyl-N-nitrosurea induced urothelial bladder tumour development. Degree of inhibition depended on cell cycle phase that N-methyl-N-nitrosurea was given where 7/37, 4/43, 10/46, 10/38, 9/36, 12/40 in late G1, early and late S, G2+M and early and late postmitotic phases respectively.	NS
Study	**Dose/Route**	**Species/Sex/No. of animals**	**Negative Control**	**Treated**	**Positive Control**
Hydrea FDA Prescribing Information 2016	125 – 250 mg/kg i.p. thrice weekly for 6 months	Female rats (strain not stated)	NS	Increased incidence of mammary tumours in rats surviving to 18 months	NS

NS=not stated

The IARC concluded that there was inadequate evidence in both humans and experimental animals for the carcinogenicity of hydroxycarbamide, and as such gave an overall evaluation that hydroxycarbamide was not classifiable as to its carcinogenicity to humans (Group 3, IARC, 2000).

Given the nature of the product, even with limited data, the possibility of carcinogenicity remains as hydroxycarbamide is presumed to be a trans-species carcinogen. In patients receiving long-term hydroxycarbamide for myeloproliferative disorders, secondary leukaemia has been reported. It is unknown whether leukaemogenic effects are secondary to the drug or associated with the underlying SCD. Skin cancer has also been reported in patients receiving long-term hydroxycarbamide treatment.

Reproduction toxicity

Hydroxycarbamide has been shown to cause testicular atrophy, reducing sperm production in male rats, and hydroxycarbamide at 500 mg/kg b.w. causes up to 50% loss of testicular weight in male mice by day 29.

Hydroxycarbamide crosses the placental barrier and damages embryos in rats. Repeated oral administration of hydroxycarbamide during the organogenesis period at dose levels ranging from 50 to 450 mg/kg b.w. leads to a dosedependent embryo-lethal and teratogenic effect with an evident dose response relationship. Live foetuses at term generally show severe ocular and craniofacial anomalies; hydrocephalus, cardiovascular abnormalities, vertebral and costal defects. Rat embryos are teratogenically more sensitive to hydroxycarbamide than monkey embryos. Several other studies have been conducted in NMRI mice, rats, golden hamsters, New Zealand White rabbits, European and Persian breeds of cats and Rhesus monkeys at varying stages of gestation. The IARC concluded that hydroxycarbamide was a teratogen and caused postnatal behavioural deficits after prenatal exposure in all species tested (IARC, 2000).

In 2008 the NTP Center for the Evaluation of Risks to Human Reproduction published the results of a review evaluating the potential for hydroxycarbamide to cause adverse effects on reproduction and development in humans.

Evidence in the literature after publication of the NTP review has been in agreement with the NTP review regarding the reproductive effects of hydroxycarbamide.

Jones *et al.,* 2009 reported hydroxycarbamide administration to male transgenic sickle cell mice was shown to exacerbate the existing hypogonadism to gonadal failure.

Treatment with hydroxycarbamide to C57Bl/6J mice compromised folliculogenesis and the ability of generated embryos to develop (Sampson *et al.,* 2010). Others reported that hydroxycarbamide was also able to induce neural tube defects in a murine model via perturbation of deoxynucleoside triphosphate metabolism and associated abnormal cell balance between proliferation and apoptosis (Guan *et al.,* 2015). New Zealand White rabbits were shown to be sensitive to hydroxycarbamide on gestation days 8 to 13 regarding axial skeletal development and on gestation days 11 to 16 and 11 to 12 regarding disruption of appendicular and cranio-facial skeletal development respectively (Campion *et al* 2012).

Following extensive review of the literature, the NTP concluded that there is serious concern that exposure of therapeutic doses of hydroxycarbamide to men (who have reached puberty) may affect sperm production.

The NTP concluded that there is concern that exposure of pregnant women to hydroxycarbamide could result in birth defects, abnormalities of foetal growth or abnormal postnatal development in offspring. However the NTP expressed minimal concern that exposure of therapeutic doses of hydroxycarbamide to children aged 5 to 15 years of age would adversely affect growth (NTP, 2008).

The NTP found that hydroxycarbamide can cross the placenta and is found in breast milk and as such use of hydroxycarbamide in pregnancy/lactation could lead to exposure to the unborn child and infant (NTP, 2008).

Local tolerance

No local tolerance studies/data were submitted.

Other toxicity studies

The applicant submitted the results of a 10-day toxicity study in rats (conducted upon FDA request), as committed. The objective of the study was to provide adequate safety information in order to permit qualification of the identified impurity at a limit of 0.5%. A Scientific Advice (EMA/CHMP/SAWP/172730/2017) had already been addressed to the CHMP, concluding that the proposed limit of the identified impurity of NMT 0.5% in the drug product specification during shelf-life, is considered justified. The toxicity of the Sponsor's formulation of hydroxyurea containing an identified impurity, was compared with the same formulation in the absence of the impurity, and also the listed drug form of hydroxyurea, Hydrea, following daily gavage administration for 10 days. In the absence of a clear difference in toxicity between the applicant's formulations of hydroxyurea, with or without impurity, it was concluded that the impurity was considered not to have exacerbated the toxicity of hydroxyurea or to have a novel toxicity profile of its own. Therefore, the qualification limit of 0.5% of the identified impurity, suggested by the toxicology study, is in agreement to the already agreed qualification limit based on literature data and safety data from clinical use in children.

A toxicology study in rats was submitted by the applicant during the assessment phase, intended to qualify the levels of this impurity at the drug product specification. The qualification limit of 0.5% concluded by the study is in agreement to the previously agreed qualification limit of this impurity based on literature data and safety data from clinical use in children.

2.3.5. Ecotoxicity/environmental risk assessment

The applicant has suggested that Hydroxycarbamide Nova Laboratories 100 mg/ml oral solution's PECSURFACEWATER value is below the action limit of 0.01 µg/L and is not a PBT substance as log Kow does not exceed 4.5.

The applicant was asked to provide further clarification on the calculation of DOSEai, used to estimate the Predicted Environmental Concentration (PEC) in surface water, taking into account the maximum daily dose of active ingredient described in the proposed SPC (35 mg/kg/day, also used for adults). The Applicant was also informed that since PEC value is greater than 0.01 µg/ml, Phase II assessment is expected to be conducted.

A clarification, regarding the calculation of DOSEai and Fpen, was provided by the applicant, based on data obtained from the use of hydroxycarbamide in children.

The calculation of the DOSEai (Maximum daily dose consumed per inhabitant) by the applicant, was not accepted. For DOSEai calculation the highest recommended dose should be used, which should be based on a bodyweight of an adult patient and not of a child patient (as proposed by the applicant), taking into account that Hydroxycarbamide Nova Laboratories is proposed for indication to both children and adults. Furthermore, the proposed SmPC describes the maximum daily dose at 35mg/kg/day. Even if the product is primarily designed with children in mind, the analysis of the potential risk that the use of the medicine poses to the environment has to be calculated based on the target group described by the indication of the product.

Fpen represents the fraction of a population receiving the drug substance during a given time. The Fpen value was calculated by the applicant based on the impact of the use of hydroxycarbamide in children 2-9 years of age. Although, taking into account that Hydroxycarbamide Nova Laboratories is proposed for indication to both children and adults, it is the Committee's view that Fpen value should be derived directly by the prevalence of SCD in the EU, which is 1.5 in 10000.

Given the above calculations, the PECSURFACEWATER value is estimated to be above the limit of 0.01 µg/l.

Table 5: Summary of main study results

Substance (INN/Invented Name): hydroxycarbamide			
CAS-number (if available): 127-07-1			
PBT screening		***Result***	***Conclusion***
Bioaccumulation potential- log K_{ow}	experimental and calculated (literature)	Log Kow = -1.27	No potential PBT
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}		B/not B
	BCF		B/not B
Persistence	DT50 or ready biodegradability		P/not P
Toxicity	NOEC or CMR		T/not T
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.21 µg/l	µg/L	> 0.01 threshold
Other concerns (e.g. chemical class)			(Y/N)

In the context of the obligation of the MAH to take due account of technical and scientific progress, the CHMP recommends the following points to be addressed:

- The applicant should conduct an OECD 309 test on biodegradability and submit the results in 18 months. In addition, the applicant should also conduct a full Phase II study including an OECD 308 test with results to be provided in 3 years following approval of Xromi 100 mg/ml oral solution.

2.3.6. Discussion on non-clinical aspects

As Hydroxycarbamide is a well-known active substance, no further studies on pharmacology, pharmacokinetics and toxicology are required. Since hydroxycarbamide is already in use in humans for a long time, the metabolism in humans is well understood. Therefore, this approach is considered

acceptable, according to the EMA Guideline on the Non-Clinical Documentation for Mixed Marketing Authorisation Applications (CHMP/SWP/799/95, 2005), particularly given the extensive clinical experience with hydroxycarbamide.

Pharmacology

The pharmacology studies sourced from published literature demonstrate proof of concept that the primary effect of hydroxycarbamide is to increase HbF levels. HbF inhibits intracellular HbS polymerisation and prevents the sickling process within erythrocytes.

Pharmacokinetics

Absorption, distribution, metabolism and excretion data are based upon studies in the literature and no formal development programme was undertaken for hydroxycarbamide by the Applicant. Since hydroxycarbamide is already in use in humans for a long time, the metabolism in humans is well understood. Therefore, this approach is considered acceptable, according to the EMA Guideline on the Non-Clinical Documentation for Mixed Marketing Authorisation Applications (CHMP/SWP/799/95, 2005), particularly given the extensive clinical experience with hydroxycarbamide.

Toxicology

Single dose, repeated dose toxicity, genotoxicity, carcinogenicity and reproductive/developmental toxicity studies (Male Fertility, Female fertility, Embryo-foetal Development, Peri & postnatal) were presented in a tabular format, giving information for the species/sex/number/group, dose/route, duration/dosing period, major finding, NOAEL(mg/kg). Information concerning breastfeeding and adverse effects in the offspring was also adequately provided.

Published studies in single dose toxicity studies considered hydroxycarbamide of low acute toxicity after oral administration in mice and rats. A single IV injection of hydroxycarbamide in male rabbits and BALB/c mice, revealed an early but transient significant decrease in blood erythrocyte count in rabbits and a marked hepatotoxicity in mice, suggesting that hydroxycarbamide could have initiated free radical mediated reactions.

Repeated dose toxicity includes a 10-Day (Exploratory) Study in Rats, a 2-Week (Dose Range Finding) Study and a 1-Month Toxicity Study in Dogs, as well as the IARC monography and reports from veterinary practice with long-term treatment of dogs for up to 4 months. Study in rats ended with all animals in the highest dose group dead or euthanised. Clinical signs included reduction in body weight and food consumption. Necropsy revealed dark lungs in some female animals and gelatinous content or water content in small and large intestines of males and females; spleens and thymuses were also noted to be small. The cause of deaths and severe clinical signs in the 1,500 mg/kg/day dose group were not determined. The median dose group of 500 mg/kg/day exhibited decreased body weight gain, decreased circulating leukocytes, erythrocytes, and platelets, decreased cellularity of thymus, lymph nodes and bone marrow, and epithelial degeneration and/or dysplasia of the stomach and small intestine. Overall, the 50 mg/kg/day dose was considered as the no adverse effect level (rat NOAEL) due to haematologic findings that were of limited severity and not adverse, and the absence of clinical observations or microscopic findings.

In the Dose Range Finding all animals in the medium and high dose groups were euthanised in moribund condition within hours of the first dose. Clinical signs in the medium (250 mg/kg/day) and high dose (1,000 mg/kg/day) group animals showed emesis, decreased activity, prostration, inability to rise, blue gums, salivation and ocular mucus accumulation. In the medium dose group of 250 mg/kg/day, blood oxygen saturation was evaluated due to clinical signs of prostration and blue gums suggesting low blood oxygenation and elevated erythrocyte mass parameters were consistent with haemoconcentration and shock. However, serum clinical chemistry and necropsies for dogs in the high dose group were not

conducted. The 50 mg/kg/day dose level was considered as the no adverse effect level (dog NOAEL) as the magnitude of clinical pathology changes were modest and not adverse.

Results from 1-Month Toxicity Study in dogs showed that a dose of 50 mg/kg/day hydroxycarbamide administered in purified water once daily for 1 month was tolerated with no adverse clinical signs or effects on body weight, food consumption, or ophthalmic assessments. Haematology parameters showed decreased circulating leukocytes, erythrocytes and platelets, and microscopic evaluations showed increased bone marrow cellularity with decreased maturing granulocytes, increased creatinine activity and increased iron pigment in bone marrow and hepatic sinusoidal cells. Treatment with hydroxycarbamide did not show any changes in urinalysis, organ weight or macroscopic evaluations.

According to the International Agency for Research on Cancer (IARC, 2000) monograph, the main toxicity of hydroxycarbamide is neutropenia.

Reports from veterinary practice revealed that long-term treatment of dogs with hydroxycarbamide for up to 4 months with doses generally in the order of 30 mg/kg can lead to myelosuppression, gastrointestinal complications and, rarely, onychomadesis, which led to discontinuation of hydroxycarbamide therapy. Within a month of cessation of treatment, all nails had healed completely. Melanonychia, myelosuppression, and gastrointestinal have been included in section 4.8 of the SmPC.

Carcinogenicity

Conventional long-term studies to evaluate the carcinogenic potential of hydroxycarbamide have not been conducted. However, hydroxycarbamide is presumed a trans-species carcinogen. Therefore, a carcinogenic risk to humans under the treatment with hydroxycarbamide cannot be excluded. This is reflected in the SmPC of the reference product Hydrea (hydroxycarbamide) and is adequately reflecting in the proposed in Sections 5.3 and Section 4.4 of the SmPC of Xromi 100 mg/mL oral solution.

Section 5.3 (Preclinical safety data) of the proposed SmPC states the following, which is supported by the scientific literature: “Hydroxycarbamide is unequivocally genotoxic.”

Hydroxycarbamide adversely affects reproductive performance in rats and causes testicular atrophy, reducing sperm production in male rats while high doses induce high incidence of resorptions in rats on Day 7-11 of gestation. In male mice, hydroxycarbamide at 500mg/kg b.w. causes up to 50% loss of testicular weight by day 29. Hydroxycarbamide crosses the placental barrier and damages embryos in rats. Repeated oral administration of hydroxycarbamide during the organogenesis period at dose levels ranging from 50-450 mg/kg b.w. leads to a dose-dependent embryo-lethal and teratogenic effect with an evident dose response relationship. Live foetuses at term generally show severe ocular and craniofacial anomalies; hydrocephalus, cardiovascular abnormalities, vertebral and costal defects. Rat embryos are teratogenically more sensitive to hydroxycarbamide than monkey embryos.

Reproduction toxicity

The National Toxicology Program (NTP) Centre for the Evaluation of Risks to Human Reproduction (2008) published the results of a review evaluating the potential for hydroxycarbamide to cause adverse effects on reproduction and development in humans. Following extensive review of the literature, the NTP concluded that there is “serious concern” that exposure of therapeutic doses of hydroxycarbamide to men (who have reached puberty) may affect sperm production, that there is “concern” that exposure of pregnant women to hydroxycarbamide could result in birth defects, abnormalities of foetal growth or abnormal postnatal development in offspring, and a “minimal concern” that exposure of therapeutic doses of hydroxycarbamide to children aged 5-15 years of age would adversely affect growth.

The NTP found that hydroxycarbamide can cross the placenta and is found in breast milk and, as such, use of hydroxycarbamide in pregnancy/lactation could lead to exposure in the unborn child and infant.

In humans, hydroxycarbamide has been shown to affect spermatogenesis with cryptozoospermia and azoospermia, but the impact on fertility is not clear. These disorders are also associated with the underlying disease, as confirmed clinically.

The reproductive effects noted in this pre-clinical study are reflected in human use and are included in existing product profiles and are included in sections 4.8 and 5.3 of the SmPC.

With the evidence of teratogenic effects of hydroxycarbamide in the pre-clinical studies, Hydroxycarbamide Nova Laboratories, 100 mg/mL oral solution is contraindicated in the pregnancy and breastfeeding and it documented in the Hydroxycarbamide Nova Laboratories, 100 mg/mL oral solution SmPC (paragraph 4.6).

Paediatrics

Treatment of children is intended with hydroxycarbamide. It is not known if pharmacokinetics of hydroxycarbamide in children will show the same pattern as in adults. Since no juvenile toxicity studies with concomitant toxicokinetics were performed to compare possible toxicities and exposure levels of hydroxycarbamide, the safe use of hydroxycarbamide in children cannot be confirmed from the non-clinical point of view.

The applicant addresses sufficiently the appropriateness of the proposed formulation for the paediatric population, taking into account the toxicological properties of the formulation excipients.

Toxicokinetics

No toxicokinetic analysis was provided based on the bibliographical toxicology package and the well-established use of hydroxycarbamide.

Environmental Risk assessment

The PECSURFACEWATER value is estimated to be above the limit of 0.01 µg/l.

In the context of the obligation of the MAH to take due account of technical and scientific progress, the CHMP recommends the following points to be addressed:

- The applicant should conduct an OECD 309 test on biodegradability and submit the results in 18 months. In addition, the applicant should also conduct a full Phase II study including an OECD 308 test with results to be provided in 3 years following approval of Xromi 100 mg/ml oral solution.

2.3.7. Conclusion on the non-clinical aspects

The non-clinical data supports the authorisation of Xromi 100 mg/ml oral solution in the proposed indication

In the context of the obligation of the MAH to take due account of technical and scientific progress, the CHMP recommends the following points to be addressed:

The applicant should conduct an OECD 309 test on biodegradability and submit the results in 18 months. In addition, the applicant should also conduct a full Phase II study including an OECD 308 test with results to be provided in 3 years following approval of Xromi 100 mg/ml oral solution.

2.4. Clinical aspects

2.4.1. Introduction

This is an application for Xromi oral solution containing hydroxycarbamide. To support the marketing authorisation application the applicant conducted a bioequivalence study with cross-over design under fasting conditions.

Formal scientific advice by the CHMP was given for this medicinal product. For the clinical assessment Guideline on the Investigation of Bioequivalence CPMP/EWP/QWP/1401/98 Rev.1) in its current version is of particular relevance.

Hydroxycarbamide was introduced into clinical practice soon after animal studies showed it was active against tumours in mammals. Hydroxycarbamide has been used for over 50 years in the oncology arena, due to its properties as a cytotoxic, antimetabolite, and antineoplastic agent, to treat a variety of medical disorders, such as myeloproliferative neoplasms, chronic myelogenous leukaemia, and human immunodeficiency virus (HIV).

Hydroxycarbamide has been used for over 30 years in the SCD arena, with the first clinical application in SCD patients reported in 1984, when Platt and colleagues demonstrated a rapid and dramatic increase in HbF-containing reticulocytes without significant bone marrow toxicity.

Clinical studies

A bioequivalence study has been conducted in healthy adults comparing the product under authorisation with the reference product Hydrea 500 mg capsules from UK and USA. The applicant has conducted this study in order to show bioequivalence between Hydroxycarbamide Nova Laboratories 100 mg oral solution and Hydrea 500 mg capsules and bridge Hydrea-based literature with the product under authorisation.

Table 6: Tabular overview of clinical studies

Type of Study	Study Identifier	Objective of the Study	Study Design and Type of Control	Test Products: Dosage Regimen; Route of Administration	Number of Subjects	Healthy Subjects of Diagnosis of Patients	Duration of Treatment
Bioequivalence study	RD 729/26118	To assess whether hydroxycarbamide 100 mg/mL solution is bioequivalent to the EU RLD and USA RLD hydroxycarbamide 500 mg capsule (Hydrea)	Open-label, randomised, three-period crossover study of test product versus two reference products	Single doses of: 5mL oral hydroxycarbamide 100 mg/mL solution (Test) Reference: Hydrea 500mg capsule (EU RLD) Hydrea 500 mg capsule (USA RLD)	30 enrolled (28 completed)	Healthy male and female subjects between 18 and 50 years of age	Three single doses with at least 3 days between each IMP administration

2.4.2. Pharmacokinetics

Bioequivalence study

In view of this hybrid application to the reference products Hydrea 500 mg capsules (UK) and Hydrea 500 mg capsules (USA), the focus of the clinical program to support the application of Oral Hydroxycarbamide Solution 500 mg/5 ml was to bridge the available clinical data from Hydrea 500 mg capsules (UK and USA) with a comparative three way cross-over PK study of single dose of Oral Hydroxycarbamide Solution 500 mg/5 ml relative to the reference products in healthy volunteers. This bioequivalence study provides information on the comparative PK profiles used to support the appropriate dose of Hydroxycarbamide Oral Solution relative to Hydrea 500 mg capsules that would provide a comparable exposure to hydroxycarbamide compared to the reference product.

The primary objective was to determine whether the test product, hydroxycarbamide oral 500 mg/5 ml solution and the two reference products, Hydrea 500 mg UK, and Hydrea 500 mg USA capsules were bioequivalent. For this purpose, the pharmacokinetic (PK) profile of hydroxycarbamide was compared after administration of a single dose of each of the 3 (test and 2 reference) formulations, under fasting conditions. The secondary objective was to assess the safety and tolerability of the test product, hydroxycarbamide oral 500 mg/5 ml solution.

Methods

Study design

The study design was chosen in accordance with the EMA and the USA Food and Drug Administration (FDA) guidelines on the design, conduct and evaluation of bioavailability and bioequivalence studies. The study was carried out in healthy subjects in the fasted state. Each subject received in random order a single dose of hydroxycarbamide oral solution (500 mg/5 mL) (test IMP), Hydrea 500 mg capsule UK (reference IMP (B)) and Hydrea 500 mg capsule USA (reference IMP (C)), over 3 treatment periods (1 IMP/period).

The guidance required that sample collection should be spaced in such a way that C_{max} and elimination rate constant (k_{el}) can be accurately estimated. It was therefore important that there were enough sampling times clustered around C_{max}. The guidance also required that sampling should continue for at least 3 $t_{1/2}$ of the drug, and that at least 3 to 4 samples should be obtained during the terminal log-linear phase. Hydroxycarbamide reaches C_{max} in approximately 1 - 4 h. In patients with sickle cell disease C_{max} is reached at approximately 1.2 h with an elimination $t_{1/2}$ of approximately 6 - 7 h. Therefore, collecting samples up to 24 h post-dose (day 2) was considered appropriate. The washout period of at least 3 days between doses (longer than the required 5 $t_{1/2}$), allowed complete elimination of hydroxycarbamide between each dose and was deemed sufficiently long to avoid any carry-over effect.

As the primary objective of the study was to compare the bioavailability of 3 formulations of hydroxycarbamide, an open-label design was chosen. As the PK parameters investigated were objective, it was considered by the applicant that there was only minimal risk of introducing bias into the study.

In the open label situation, the potential for bias remains a concern, in particular related to operational bias (e.g. influence on trial conduct/differential follow up of patients) and biased safety/tolerability assessment on the subject's as well as on the investigator's side. However, blinding of study staff is not required in the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **).

The 3-period crossover design permitted within-subject comparison, which resulted in reduced variability of the data obtained.

A washout period of 3 days was considered adequate as this period exceeds more than 5-fold the half-life of hydroxycarbamide (elimination half-life of 6-7 hrs). This period is believed to be long enough to avoid any carry over effect to the second period.

The study was conducted under fasting conditions considering that it is proposed that the product should be taken once daily, preferably in the morning before breakfast and, where necessary, with a glass of water or a very small amount of food.

Monitoring was long enough (24h, approx. 3 $t_{1/2}$) to cover the plasma concentration-time curve and the sampling schedule provides a reliable estimate of the extent of exposure. $AUC_{(0-t)}$ covers at least 93% of $AUC_{(0-\infty)}$. Healthy volunteers were treated with the same dose of hydroxycarbamide (500 mg) administered in three different formulations. This dose represents a single administration that would be used in clinical practice, therefore the dose selection is understood and agreed.

A randomization scheme has been provided and subjects have been randomized to the six possible sequences ABC, ACB, BAC, BCA, CBA and CAB according to a randomization code produced by Simbec using the PROC PLAN procedure of SAS Version 9.3.

Test and reference products

Xromi 100 mg/ml manufactured by Nova Laboratories Ireland Limited (batch No. 1212d004, exp. date; 27 March 2017) has been compared to Hydrea 500 mg manufactured by (Batch No: 6G03422 and Batch No: 5D03762, exp. date March 2018).

Currently hydroxycarbamide is licensed as Hydrea 500 mg capsule in the UK and USA. Following advice from the CHMP at the EMA and the USA FDA, Hydrea 500 mg capsule UK and Hydrea 500 mg capsule USA were chosen as reference IMP (B) and (C), respectively.

IMP	Product Name	Strength*	Form	Batch No.	Expiry
Test	Hydroxycarbamide Oral Solution	500 mg/5 mL	Solution	1212d004	27 Mar 2017
Reference (B)	Hydrea® 500 mg Capsule UK	500 mg	Capsule	6G03422	Mar 2018
Reference (C)	Hydrea® 500 mg Capsule USA	500 mg	Capsule	5D03762	Mar 2018

* The active ingredient in test and reference IMPs was hydroxycarbamide. Reference IMPs are both marketed products.
IMP = investigational product

Population(s) studied

Thirty (30) subjects aged between 18 and 50 years of age were enrolled in the bioequivalence study; 26 male (86.7%) and 4 female (13.3%) subjects. 28 subjects received Test IMP (Hydroxycarbamide Oral solution), 30 subjects received Reference IMP (B) (Hydrea 500 mg capsule UK), 29 subjects received Reference IMP (C) (Hydrea 500 mg capsule USA), and 28 subjects completed the study per protocol and received all treatments.

All demographic and background data were listed. Demographic data were summarised descriptively (age, height, weight and BMI) and by frequency (race and gender). Subject demographics (mean and SD) are also summarised in Table 7.

Table 7: Summary of subject demographics

Parameter	Statistic	Overall (N=30)
Age (yrs)	Mean	34.8
	SD	9.03
Height (cm)	Mean	175.4
	SD	7.42
Weight (kg)	Mean	79.68
	SD	10.973
BMI (kg/m²)	Mean	25.835
	SD	2.6341
Race:		
Caucasian	n (%)	30 (100.0)
Other	n (%)	0 (0.0)
Gender:		
Male	n (%)	26 (86.7)
Female	n (%)	4 (13.3)

% are based on the total number of subjects within the safety set.
BMI = body mass index

Analytical methods

The certificates of analysis of test and reference products demonstrate that the assay values for the test product (102.3%) and reference product (98.9%) are within 5% of each other.

The proposed inclusion and exclusion criterion are acceptable. No paediatric subjects were included in the study and this is agreed for a comparability exercise.

Incurred samples reanalysis for the bioequivalence study (Study n° RD 729/26118) have been performed and the results have been provided by the applicant. Incurred sample reanalysis passed the standard conventional acceptance criteria (at least 67% of ISR samples that have reanalysed concentrations within 20% of their original concentrations when normalized to their means).

The applicant provided the requested Analytical Study Report with information on the method, instrumentation and statistical summaries, to demonstrate that the analytical method used for the determination of Hydroxyurea in human plasma was reproducible and accurate throughout the program of work. No repeat analysis was performed. In addition, an Analytical Study Report - Addendum 1 was also provided which contains representative chromatograms for 20% of the study data.

Sample analysis was performed at Seirian Laboratories, Simbec Research Limited, Merthyr Tydfil, CF48 4DR, United Kingdom. The provided analytical report describes the assay characteristics obtained during analysis of 1566 human plasma samples for Hydroxyurea from the clinical study conducted in healthy volunteers to determine the bioequivalence of the test product, hydroxycarbamide oral 500 mg/5 mL solution, and the two reference products, Hydrea 500 mg UK, and Hydrea 500 mg US capsules.

Hydroxyurea was determined in human plasma, over the concentration range 0.50 to 99.09µg.ml^{-1} (nominal concentrations) by liquid chromatography with mass spectrometry detection (LC-MS), applying a suitable method that has previously been validated by Seirian Laboratories at Simbec Research Ltd.

The study samples were processed in each batch as described in the Bioanalytical Phase Plan and table below.

TABLE 1.1

ANALYTICAL BATCHES SEQUENCE LIST

Batch Number	Date of Extraction	Samples Analysed	Workbook Number	Workbook Pages	Batch Status
1	23 Feb 17	Standards and Quality Controls	017295	11 -16	Acceptable
2	27 Feb 17	Subjects 001 and 002 periods 1, 2 and 3	017295	17-22	Acceptable
3	28 Feb 17	Subjects 003 and 004, Periods 1, 2 and 3	017295	23-28	Acceptable
4	1 Mar 17	Subject 005, Periods 1, 2 and 3 Subjects 006, Periods 1 and 2	017295	29-34	Acceptable
5	2 Mar 17	Subjects 007, Periods 1, 2 and 3 Subject 008, Period 1	017295	35-40	Acceptable
6	6 Mar 17	Subjects 009 and 010, Periods 1, 2 and 3	017295	41-46	Acceptable
7	7 Mar 17	Subjects 011 and 012, Periods 1, 2 and 3	017295	47-52	Acceptable
8	8 Mar 17	Subjects 013 and 014, Periods 1, 2 and 3	017295	53-58	Acceptable
9	9 Mar 17	Subjects 015 and 016, Periods 1, 2 and 3	017295	59-64	Acceptable
10	14 Mar 17	Subjects 017 and 018, Periods 1, 2 and 3	017295	65-70	Acceptable
11	15 Mar 17	Subjects 019 and 020, Periods 1, 2 and 3	017296	11-16	Acceptable
12	16 Mar 17	Subjects 021 and 022, Periods 1, 2 and 3	017296	17-22	Acceptable
13	21 Mar 17	Subjects 023 and 024, Periods 1, 2 and 3	017296	23-28	Acceptable
14	22 Mar 17	Subjects 025 and 026, Periods 1, 2 and 3	017296	29-34	Acceptable
15	23 Mar 17	Subjects 027 and 028, Periods 1, 2 and 3	017296	35-40	Acceptable
16	27 Mar 17	Subjects 029 and 030, Periods 1, 2 and 3	017296	41-46	Acceptable
17	10 Apr 17	ISR	017296	47-52	Acceptable
18	11 Apr 17	ISR	017296	53-58	Acceptable

All samples were transferred to the Bioanalytical Unit in a satisfactory condition and stored at approximately -20°C upon receipt. The original and duplicate samples were stored in separate freezers. Details of sample receipt and storage location are detailed in sample receipt book 017194. All standard and QC samples were stored alongside the test samples at approximately -20°C.

Table 8: Sample storage table

Study ID and Analyte	Longest Storage Period
RD 729/26118H Hydroxyurea	Subjects 001-014 30 January 2017 to 8 March 17 from first sample taken to last sample analysed (37 days at approximately -20°C).
RD 729/26118H Hydroxyurea	Subjects 015-030 1 February 2017 to 27 March 17 from first sample taken to last sample analysed (54 days at approximately -20°C).

Calibration curve characteristics, linearity, precision, accuracy as well as method specificity/sensitivity are provided.

The validation summary table is given in Appendix 3 as follows:

APPENDIX 3
HYDROXYUREA VALIDATION SUMMARY TABLE

Analytical Validation Report Location(s)	RD 729/26118HV Simbec electronic archive drive	
The analytical method was used in the following studies:	RD 729/26118H	
Short description of the method	LC-MS (Isocratic)	
Biological Matrix	Human Plasma (anti-coagulant lithium heparin)	
Analyte Location of product certificate	Hydroxyurea See page 68 of the validation report	
Internal Standard (IS) Location of product certificate	N-Methylurea See page 69 of the validation report	
Calibration concentrations ($\mu g.ml^{-1}$) (nominal)	0.5, 1, 2, 10, 25, 50, 90 & 100	
Lower limit of quantitation (nominally 0.50 $\mu g.ml^{-1}$)	Accuracy – 98.0%, Precision – 4.0%	
QC concentrations ($\mu g.ml^{-1}$) (nominal)	0.5, 1.5, 20, 40, 80 & 400	
Inter Batch (Between-run) accuracy	92.8 – 110.3%	
Inter Batch (Between-run) precision	7.4 – 13.4%	
Intra Batch (Within-run) accuracy	89.5 – 113.0%	
Intra Batch (Within-run) precision	2.3 – 7.3%	
Matrix Effect (nominal values) IS normalised Matrix Factor (IS N MF) CV% of IS normalised MF % of QC's with >85% & <115% (wrt mean of abs value)* % matrix lots with mean <80% or>120% (wrt IS N MF)# Haemolysed Plasma Lipaemic Plasma	Low QC ($1.50\mu g.ml^{-1}$) 0.68 4.9 100% 100% +10.0% +10.0%	High QC ($80\mu g.ml^{-1}$) 0.70 2.4 100% 100% -4.9% -5.0%
Long term stability of the stock solution and working solution (stored at either approximately 4°C or at room temperature (RT))	Hydroxyurea 1000mg.$100ml^{-1}$ in methanol RT for 24hrs +0.8%, 4°C for 43 days +1.3%. Hydroxyurea 100mg.$100ml^{-1}$ in methanol RT for 24hrs +8.5%, 4°C for 43 days -6.3% Internal Standard N-methylurea 100mg.$100ml^{-1}$ in methanol RT for 24hrs -1.4%, 4°C for 43 days +8.1%.	
Short term stability in biological matrix at RT	18hrs, Q2 -4.0%, Q5 -2.7%	
Long term stability in biological matrix Location	Up to 65 days at -20°C, Q2 -12.6%, Q5 -8.8% Up to 289 days at -80°C, Q2 -4.7%, Q5 +7.3% See pages 56 and 57 of the validation report	
Extended Storage Stability	4°C for 5 days, Q2 +4.0%, Q5 -3.2%	
Post-preparative stability	RT for 164hrs, Q2 +2.0%, Q5 -7.5%	
Freeze and thaw stability	3 cycles at -20°C, +6.7% % to -12.7% 3 cycles at -80°C, -1.3% to -10.1%	
Blood stability	Up to 60 minutes, Q2 +4.3%, Q5 -2.3%	
Dilution Integrity	1 in 10 dilution Between accuracy 100.7%, precision 13.4% Within accuracy 111.7%, precision 2.3%	
Recovery	Mean recovery 89.7% Mean recovery relative to IS 97.7%, precision 12.4%	
Selectivity Specificity Sensitivity Selectivity in presence of Paracetamol and Ibuprofen	 No detectable peaks in 6 out of 6 plasmas accuracy 106.0%, precision 4.5% Q2 accuracy 89.3%, precision 12.8% Q5 accuracy 96.0%, precision 1.6% No detectable peaks in 3 out of 3 plasmas	
Carry over	No significant carry over detected for hydroxyurea (7 samples none detected, 2 samples with small, insignificant peaks) No carry over detected for N-methylurea in all 9 samples.	

* - Each calculated matrix effect QC value is compared to the mean of the calculated QC value for the absolute and the % of QC's between >85% and <115% is determined.

- The percentage of matrix lots where the IS normalised MF is <80% or>120% is determined.

Abbreviations

CV	Coefficient of Variation	IS	Internal Standard	abs	absolute
QC	Quality Control	RT	Room Temperature	LLOQ	Lower Limit of Quantitation
wrt	with respect to	hrs	hours		

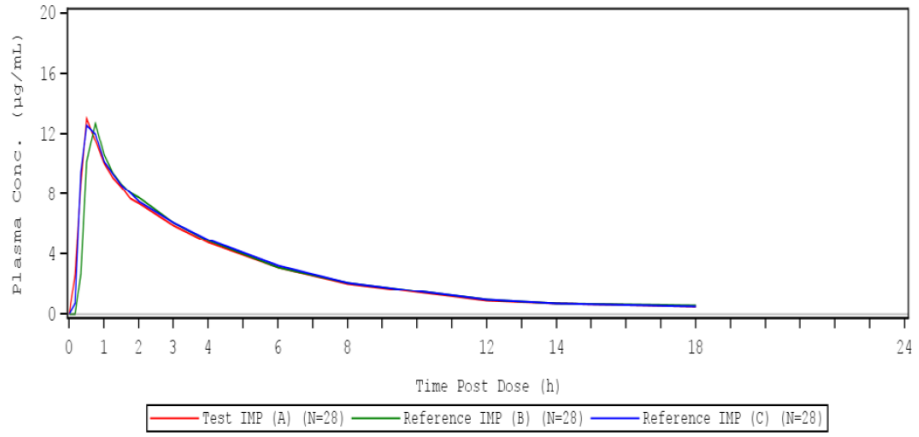
Measured hydroxyurea concentrations from all analysed samples are also provided.

The data provided in the submitted Analytical Study Report regarding the applied bioanalytical method and the representative chromatograms included in the Analytical Study Report - Addendum 1, can be considered adequate. Based on data from a previous study the intra-subject CV% for C_{max} was approximately 22.12%, with a lower variability demonstrated for AUC. Using the method of Diletti a sample size of 22 subjects was considered sufficient to detect a 20% difference between the test and reference IMPs with a power of 80% and alpha of 5%, based upon a test/reference ratio of between 0.95 and 1.05. To allow for a higher CV%, dropouts or subjects who otherwise failed to complete the study, 30 subjects were enrolled in the study.

Pharmacokinetic variables

The PK variables were derived from plasma hydroxycarbamide concentration-time data following administration of each IMP using Phoenix WinNonlin 6.4. Primary PK variables were C_{max}, AUC_{0-t}, and AUC_{0-inf}, and secondary PK variables were T_{max}, k_{el}, $t_{1/2}$, and $AUC_{\%extrap}$. Mean plasma concentration-time profiles for the 28 subjects who completed the study are shown in Figure 2:

Figure 2: Mean Concentration-Time Profiles for Hydroxycarbamide following each IMP – Linear Scale



Test IMP: Hydroxycarbamide oral solution (500 mg/5 mL)
Reference IMP (B): Hydrea® 500 mg capsule UK
Reference IMP (C): Hydrea® 500 mg capsule USA
IMP = investigational medicinal product

Statistical methods

Following logarithmic transformation C_{max}, AUC_{0-t} and AUC_{0-inf} values were subjected to an analysis of variance (ANOVA) including fixed effects for sequence, period, treatment, and subject (sequence). Point estimates and 90% confidence intervals (CIs) were constructed for the contrasts between treatments using the residual mean square error obtained from the ANOVA. The point and interval estimates were back-transformed to give estimates of the ratios of the geometric least square means (LSmeans) and corresponding 90% CI. Estimated geometric means were also presented for each treatment group. Bioequivalence would be achieved if the 90% CI of the geometric mean ratios for C_{max} and AUC fell within 80.00% and 125.00%. An assessment of T_{max} was performed using the Wilcoxon Signed-Rank test.

The plasma PK parameters (C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, k_{el}, $t_{1/2}$ and $AUC_{\%extrap}$) derived for hydroxycarbamide following administration of each IMP are summarised in Table 9 and the geometric LSmean test/reference ratios and corresponding 90% CI for C_{max}, AUC_{0-t}, and AUC_{0-inf} that were calculated are presented in Table 10.

Table 9: Summary of derived PK parameters for Hydroxycarbamide following each IMP

Treatment	Summary Statistic	C_{max} (µg/mL)	AUC_{0-t} (h*µg/mL)	AUC_{0-inf} (h*µg/mL)	$AUC_{\%Extrap}$ (%)	T_{max} (h)	$T_{1/2}$ (h)
Test IMP (N=28)	n	28	28	28	28	28	28
	Mean	14.2	49.1	52.4	6.35	0.611	3.40
	SD	3.00	6.47	6.67	1.43	0.223	0.447
	CV%	21.1	13.2	12.7	22.5	36.5	13.1
	Min	9.37	29.7	32.8	3.61	0.333	2.44
	Median	13.7	50.4	53.7	6.16	0.500	3.43
	Max	19.3	58.2	63.3	9.55	1.25	4.62
	Geo Mean	13.9	48.7	52.0	6.20	0.576	3.37
	Geo CV(%)	21.7	14.5	13.8	22.8	35.7	13.3
Reference IMP (B) (N=28)	n	28	28	28	28	28	28
	Mean	14.7	48.8	52.1	6.45	0.780	3.43
	SD	4.26	6.81	6.80	1.60	0.418	0.480
	CV%	29.0	14.0	13.0	24.9	53.6	14.0
	Min	9.31	30.5	34.4	4.35	0.333	2.52
	Median	14.0	50.1	53.1	6.16	0.750	3.44
	Max	26.2	61.0	63.8	11.3	2	4.95
	Geo Mean	14.2	48.4	51.7	6.27	0.706	3.40
	Geo CV(%)	27.3	14.9	13.8	23.8	44.4	13.8
Reference IMP (C) (N=28)	n	28	28	28	28	28	28
	Mean	16.4	49.9	53.5	7.01	0.662	3.40
	SD	6.20	7.51	7.00	3.37	0.343	0.378
	CV%	37.9	15.1	13.1	48.0	51.9	11.1
	Min	9.80	32.9	37.8	3.62	0.333	2.67
	Median	14.5	50.9	54.8	6.22	0.500	3.44
	Max	33.9	67.2	69.9	20.9	1.50	4.07
	Geo Mean	15.4	49.3	53.1	6.50	0.595	3.38
	Geo CV(%)	35.3	15.8	13.7	38.6	47.8	11.5

Lower limit of quantitation = 0.50 µg/mL.
Test IMP: Hydroxycarbamide oral solution (500 mg/5 mL)
Reference IMP (B): Hydrea® 500 mg capsule UK
Reference IMP (C): Hydrea® 500 mg capsule USA
IMP = investigational medicinal product

Table 10: Summary of statistical analysis of bioequivalence (C_{max}, AUC_{0-t}, and AUC_{0-inf})

Parameter	Reference	Test Geometric LSMean[1]	Reference Geometric LSMean[1]	Geometric LSMean Test/Reference Ratio (90% CI)
C_{max} (µg/mL) (N=28)	Reference IMP (B)	13.86	14.10	98.32 (89.92 - 107.50)
	Reference IMP (C)	13.82	15.33	90.14 (81.45 - 99.75)
AUC_{0-t} (h*µg/mL) (N=28)	Reference IMP (B)	48.85	48.35	101.04 (98.86 - 103.27)
	Reference IMP (C)	48.78	49.40	98.73 (95.74 - 101.82)
AUC_{0-inf} (h*µg/mL) (N=28)	Reference IMP (B)	52.16	51.68	100.93 (98.85 - 103.06)
	Reference IMP (C)	52.08	53.15	97.98 (95.39 - 100.64)

[1]n = 28
Lower limit of quantitation = 0.50 µg/mL.
Results obtained using a fixed effects ANOVA with fixed effects of sequence, period, treatment and subject nested within sequence.
Test IMP: Hydroxycarbamide oral solution (500 mg/5 mL)
Reference IMP (B): Hydrea® 500 mg capsule UK
Reference IMP (C): Hydrea® 500 mg capsule USA
ANOVA = analysis of variance, IMP = investigational medicinal product

The geometric LSmean test/reference ratio 90% CI calculated for C_{max}, AUC_{0-t}, and AUC_{0-inf}, indicate that the test IMP is bioequivalent to reference IMP (B) and reference IMP (C) when administered fasted, with upper and lower limits contained within the acceptance interval of 80.00 – 125.00% (Table 11).

However, the test Geometric LSmean value used for the calculation of the GMR LSmean test/reference ratio and corresponding 90% CI regarding Reference IMP (B) is different from that used regarding Reference IMP (C), for all 3 PK parameters (C_{max}, AUC_{0-t}, and AUC_{0-inf}).

The Applicant stated that Guidance (e.g. EMA Guideline on The Investigation of Bioequivalence, CPMP/EWP/QWP/1401/98 Rev. 1/ Corr ** relevant section: Section 4.1.8) specifies that only data from the relevant treatment comparisons should be included in the statistical model (i.e. only the data from test and reference comparison being made and not the data from the second reference).

As this is the case a separate model was fitted for each of Test (A) vs. Reference (B) and Test (A) vs Reference (C). LSMeans estimate means for a balanced population where the design is unbalanced, hence an adjustment is made based on the data and the factors in the analysis where the design is unbalanced. As the design was unbalanced (e.g. not an equal number of sequences), these estimates are slightly different as the models are different.

Results

A statistically significant reduction in T_{max} was observed for the test IMP when compared to reference IMP (B) (p=0.0467), indicating faster rate of absorption, although there was no statistical difference in T_{max} between the test IMP and reference IMP (C) (Table 11).

Table 11: Summary of the statistical analysis of T_{max}

Parameter	Reference	Test Median[1]	Reference Median[1]	Wilcoxon Signed Rank Test p-value
T_{max} (h) (N=28)	Reference IMP (B)	0.50	0.75	0.0467
	Reference IMP (C)	0.50	0.50	0.5997

[1]n = 28
Test IMP: Hydroxycarbamide oral solution (500 mg/5 mL)
Reference IMP (B): Hydrea® 500 mg capsule UK
Reference IMP (C): Hydrea® 500 mg capsule USA
IMP = investigational medicinal product

Geometric mean $t_{1/2}$ was similar between the test IMP and reference IMP (B) and (C) at 3.37, 3.40 and 3.38 h, respectively, indicating little difference between the 3 formulations.

$AUC_{\%extrap}$ was ≤ 6.5% (geometric mean) for each IMP tested, indicating that AUC_{0-t} covers at least 80% of AUC_{0-inf}. Review of individual values demonstrated that 1 subject (subject 005) had $AUC_{\%extrap}$ of 20.9% following administration of reference IMP (C). This is likely the result of the fact that in this subject measurable plasma concentrations were detected up to 8 h post-dose following reference IMP (C), vs 18 h post-dose for test IMP and reference IMP (B), thus resulting in flat elimination phase and was not considered to significantly affect interpretation of the data.

No subjects were excluded from analysis due to protocol deviations. There were no major protocol deviations and it is unlikely that these deviations affected the interpretation of the study results. There was no concomitant medication reported during the study. Study conduct is acceptable.

Information concerning intra-individual variability as observed during the bioequivalence study (Study n° RD 729/26118) has been adequately reported by the Applicant.

Table 12: Summary of statistical analysis of hydroxycarbamide Cmax, AUC 0-Inf, PK data

Parameter	Reference	Test IMP (A) n	Reference n	Test Geometric LSMean	Reference Geometric LSMean	Geometric LSMean Test/Reference Ratio (90% C.I.)	Within Subject CV% from ANOVA
C_{max} (µg/mL) (N=28)	Reference IMP (B)	28	28	13.86	14.10	98.32 (89.92 - 107.50)	19.7
	Reference IMP (C)	28	28	13.82	15.33	90.14 (81.45 - 99.75)	22.5
AUC_{0-t} (h*µg/mL) (N=28)	Reference IMP (B)	28	28	48.85	48.35	101.04 (98.86 - 103.27)	4.8
	Reference IMP (C)	28	28	48.78	49.40	98.73 (95.74 - 101.82)	6.7

Source Listing: 16.2.5.2; Produced: 24JUL2018 10:32 - Page 1 of 2
Test IMP (A): Single dose of Hydroxycarbamide Oral Solution 500mg/5mL.
Reference IMP (B): Single dose of Hydrea(R) 500mg Capsule (UK).
Reference IMP (C): Single dose of Hydrea(R) 500mg Capsule (US).
Results obtained using a fixed effects ANOVA with fixed effects of sequence, period, treatment and subject nested within sequence.
Lower Limit of Quantitation = 0.50 µg/mL.
Details of how BLQ values were handled can be found in section 9.7.1 of the CSR.

Table 13: Summary of statistical analysis of hydroxycarbamide Cmax, AUC 0-t, AUC 0-Inf, PK data

Parameter	Reference	Test IMP (A) n	Reference n	Test Geometric LSMean	Reference Geometric LSMean	Geometric LSMean Test/Reference Ratio (90% C.I.)	Within Subject CV% from ANOVA
AUC_{0-inf} (h*µg/mL) (N=28)	Reference IMP (B)	28	28	52.16	51.68	100.93 (98.85 - 103.06)	4.6
	Reference IMP (C)	28	28	52.08	53.15	97.98 (95.39 - 100.64)	5.9

Source Listing: 16.2.5.2; Produced: 24JUL2018 10:32 - Page 2 of 2
Test IMP (A): Single dose of Hydroxycarbamide Oral Solution 500mg/5mL.
Reference IMP (B): Single dose of Hydrea(R) 500mg Capsule (UK).
Reference IMP (C): Single dose of Hydrea(R) 500mg Capsule (US).
Results obtained using a fixed effects ANOVA with fixed effects of sequence, period, treatment and subject nested within sequence.
Lower Limit of Quantitation = 0.50 µg/mL.
Details of how BLQ values were handled can be found in section 9.7.1 of the CSR.

From the results of this bioequivalence study, the intra-subject variability (intra-subject CV%) has been provided for all pharmacokinetic parameters. For Cmax, the intra-subject variability was limited (from 19.7% to 22.5%) and this intra-subject variability was low for AUC_{0-t} (from 4.8% to 6.7%) as well as for $AUC_{0-\infty}$ (from 4.6% to 5.9%).

The dissolution profiles of the UK reference product (Hydrea capsules) were performed at three different buffers: 0.1 M HCl (pH 1.3), acetate buffer (pH 4.5), and phosphate buffer (pH 6.8). Hydrea capsules should be considered 'very rapidly' dissolving reference product since more than 85% of the labelled amount is dissolved with 15 min. Therefore, in view of its solubility, Hydrea capsules should be considered an appropriate reference product for the bioequivalence study (Study n° RD 729/26118) which compares this reference product with an oral solution test product (hydroxycarbamide Oral Solution 500 mg/5 mL).

Table 3: **Hydrea 500 mg capsules, Water**

Sample Number	Time Point (Minutes)	Vessel (% released) 1	2	3	4	5	6	Mean	% RSD
1	5	55.4	36.4	48.8	43.2	70.8	51.6	51	23.0
2	10	99.9	83.9	96.7	87.7	101.0	91.2	93	7.4
3	20	102.9	98.6	102.0	98.3	104.3	98.1	101	2.7
4	30	103.2	103.3	104.8	103.7	105.4	98.7	103	2.3
5	45	103.3	102.7	105.0	104.4	104.8	98.8	103	2.3
6	60	103.4	102.6	104.9	104.1	104.5	98.3	103	2.4

Table 4: **Hydrea 500 mg capsules, pH 1.3, 0.1 M HCl**

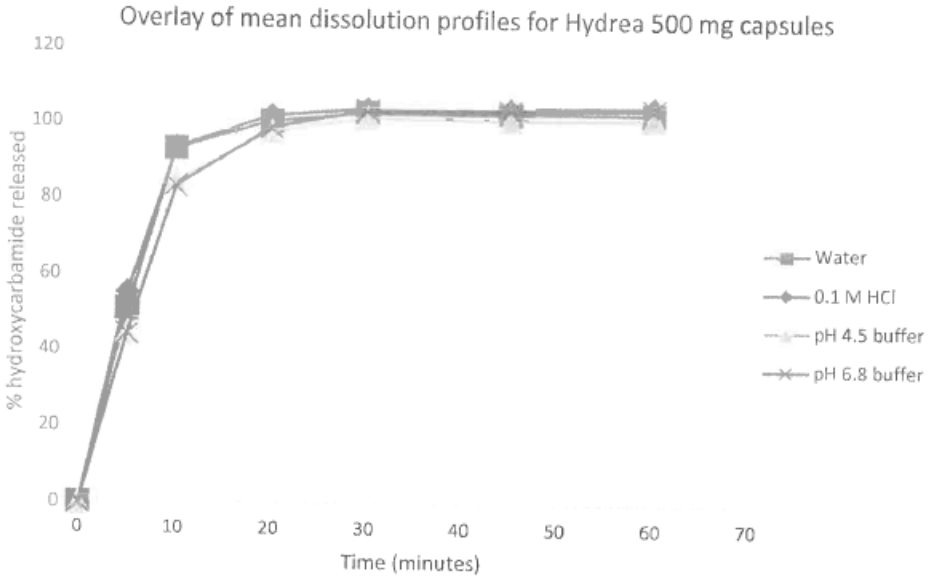
Sample Number	Time Point (Minutes)	Vessel (% released) 1	2	3	4	5	6	Mean	% RSD
1	5	62.0	59.1	54.2	61.7	44.0	51.0	55	12.8
2	10	96.6	93.5	100.3	95.8	87.9	88.8	94	5.1
3	20	105.0	103.6	103.6	103.1	101.0	97.2	102	2.7
4	30	107.1	107.5	103.4	103.4	103.2	100.6	104	2.5
5	45	107.2	107.4	104.1	104.2	103.3	100.8	105	2.4
6	60	108.0	107.1	103.4	103.9	104.8	101.6	105	2.3

Table 5: **Hydrea 500 mg capsules, pH 4.5 acetate buffer**

Sample Number	Time Point (Minutes)	Vessel (% released) 1	2	3	4	5	6	Mean	% RSD
1	5	50.9	37.4	42.0	46.5	42.9	49.0	45	11.1
2	10	83.6	92.3	82.8	88.9	75.0	88.5	85	7.2
3	20	96.7	104.9	97.1	102.0	89.7	98.3	98	5.3
4	30	100.1	105.0	100.1	105.9	100.0	101.0	102	2.6
5	45	99.7	104.3	99.4	105.3	99.1	100.3	101	2.7
6	60	100.9	104.4	99.9	105.6	99.4	100.6	102	2.5

Table 6: Hydrea 500 mg capsules, pH 6.8 phosphate buffer

Sample Number	Time Point (Minutes)	Vessel (% released)						Mean	% RSD
		1	2	3	4	5	6		
1	5	53.7	53.7	37.5	36.8	45.3	39.4	44	17.6
2	10	90.7	90.6	68.1	82.6	89.1	80.8	84	10.4
3	20	103.3	98.6	93.0	98.0	98.6	103.4	99	3.9
4	30	105.8	103.8	101.8	103.8	101.8	105.6	104	1.7
5	45	105.0	104.2	102.3	104.4	101.8	106.3	104	1.6
6	60	105.0	104.5	102.7	104.6	101.7	106.5	104	1.6



Bioavailability

Bioavailability is complete or nearly complete in cancer patients (Rodriguez et al, 1998, Tracewell et al, 1995).

Estepp *et al* (2016) reported on a prospective, open-label trial, investigating the PK of hydroxycarbamide in children (age range 2 – 17 years) following ingestion of an oral liquid (unlicensed, compounded) and/or capsule (Droxia) formulations, using frequent blood sampling. In Arm 1, toddlers (≥2 to ≤5 years) (n=17) received a single dose (mean 22.7 mg/kg) of a standardised liquid formulation of hydroxycarbamide (100 mg/mL). In Arm 2, children (>5 to ≤17 years) (n=22) received each standardised formulation (liquid and capsule) on separate occasions (mean dose 22.1 mg/kg) in a randomized, crossover fashion. Bioequivalence was demonstrated for the two formulations; there were no significant differences in systemic exposure between the two arms (mean C_{max} liquid 37.4 mg/L vs 34 mg/L; mean $AUC_{0\text{-}inf}$ liquid 104.5 vs 111.9 mg×h/L). Between liquid and capsule formulations the largest difference in the PK profiles was a trend towards a shorter time to peak concentration following ingestion of the liquid compared with the capsule, but that difference did not reach statistical significance (0.74 vs 0.97 hours, p=0.14). Mean (SD) half-life was the same for both the liquid and capsule formulations, 2.3 (0.5) hours. A population PK, 1-compartment model was developed and bodyweight was found to influence both the CL/F and V/F, and allometric (0.75) scaling accounted for the weight differences in

CL/F. Measures of systemic exposures (AUC- or CL/F-related) were not altered by formulation, suggesting dosing adjustments are not required when transitioning from one formulation to another.

In order to demonstrate that different formulations have the same bioavailability and are bioequivalent, the applicant based its justification on the fact that Xromi is highly soluble and a permeable compound. In order to justify the high solubility, an *in vitro* solubility test was performed. The highest single dose administrated must be dissolved in 250 mL of buffers within the range of pH 1 to 6.8 at 37°C ± 1°C in order for a compound to be regarded as highly soluble. Hydroxycarbamide highest single dose is 3500 mg i.e. 35 mg/kg (maximum dose in Sickle Cell Disease) with an assumption of a large adult weighing 100 kg). Therefore, a quantity of 4000 mg of hydroxycarbamide was added in 250 mL of buffers with pHs of 1.3, 4.5, and 6.8 and put in a shaking bath. The vessels were rotated at 100 rpm for 24 h at 37°C ± 0.5°C. The results after 24 h showed that hydroxycarbamide had fully dissolved (see table below) confirming that the drug substance is highly soluble.

Buffer	Replicate number	Hydroxycarbamide concentration (mg/250 mL buffer) corrected for lost volume during test	pH
pH 1.3	1	4068	1.04
	2	4092	1.05
pH 4.5 acetate buffer	1	4102	4.84
	2	4159	4.84
pH 6.8 phosphate buffer	1	4007	6.76
	2	3855	6.74

Regarding hydroxycarbamide permeability, the Applicant refers to the Rodriguez *et al* (1998) study, where the absolute bioavailability of the substance was studied. Patients received Hydrea capsules or an IV infusion for 3 weeks and plasma concentration was measured. The mean absolute bioavailability was 108%, confirming that hydroxycarbamide is a highly permeable compound. Figure 3 presents the similarity in PK profiles of oral and IV hydroxycarbamide formulations.

Figure 3: PK profiles of oral and IV hydroxycarbamide formulations

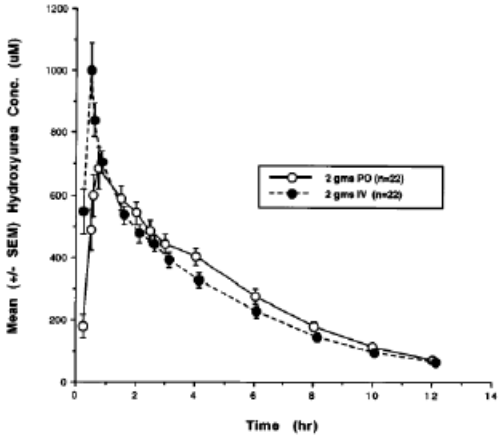


Fig 1. Plasma concentration-versus-time plots for hydroxyurea after both oral (PO) and IV administration. The mean (SE) concentrations as a function of time for all 22 patients are depicted. (–○–), 2 g PO (n = 22); (–●–), 2 g IV (n = 22).

Xromi does not contain any excipients that will affect the rate or extent of absorption of the drug. The bioequivalence study performed showed that hydroxycarbamide is rapidly absorbed (Tmax 0.5-0.75 h), something that was also observed in the Estepp et al 2016 study.

Because hydroxycarbamide is a highly soluble and highly permeable compound, the Applicant argues that data from non-Hydrea literature may be used for the justification of the efficacy and safety of Xromi 100 mg/ml oral solution. The Applicant identified the different formulations used in the main and supportive studies submitted. These were Droxia, Siklos, gelatin capsules filled with hydroxycarbamide drug substance, and compounded oral solutions.

Therefore, to allow bridging of the non-Hydrea cited literature data to the reference Hydrea 500 mg capsule, and therefore Xromi (the proposed oral solution), dissolution studies have been conducted.

Quality attributes and Dissolution profiles of hydroxycarbamide formulations identified in the treatment of SCD

With regards to the quality attributes of hydroxycarbamide formulations, disintegration and dissolution were considered the most relevant parameters for consideration in bridging literature data to Hydrea 500 mg capsules.

Dissolution testing has been performed on the following comparator products using dissolution media at pH 1.3, pH 4.5 and pH 6.8 (Table 14):

Table 14: Hydroxycarbamide formulations and brands tested in dissolution studies

Product	Excipients	Disintegration Time	Manufacturer	Batch number	Expiry date
Hydrea 500 mg capsules[1]	Citric acid, anhydrous Lactose monohydrate Magnesium stearate Sodium phosphate.	4 minutes 25 seconds	Bristol-Myers Squibb	5D03762	Mar 2018
Siklos 1000 mg tablets	Sodium stearyl fumarate Silicified microcrystalline cellulose Basic butylated methacrylate copolymer	30 seconds	Addmedica	80367C	Mar 2021
Siklos 100 mg tablets	Sodium stearyl fumarate Silicified microcrystalline cellulose Basic butylated methacrylate copolymer	8 seconds	Addmedica	71412E	Nov 2020
Droxia	Citric acid Lactose Magnesium stearate Sodium phosphate	3 minutes 55 seconds	Bristol Myers Squibb	8G04472	Jun 2021
Hydroxycarbamide drug substance[2]	None	4 minutes 10 seconds	Euticals	IA1800669A	Apr 2021
Hydroxycarbamide drug substance[2]	None	4 minutes 25 seconds	Olon	1720003160	Oct 2022

[1] Batch used in bioequivalence study

[2] 500 mg of Hydroxycarbamide drug substance was filled into a size 0 capsule. These studies mimic the formulations used in the MSH study (Charache *et al* 1995) and Ferster *et al* 1996.

Figure 1

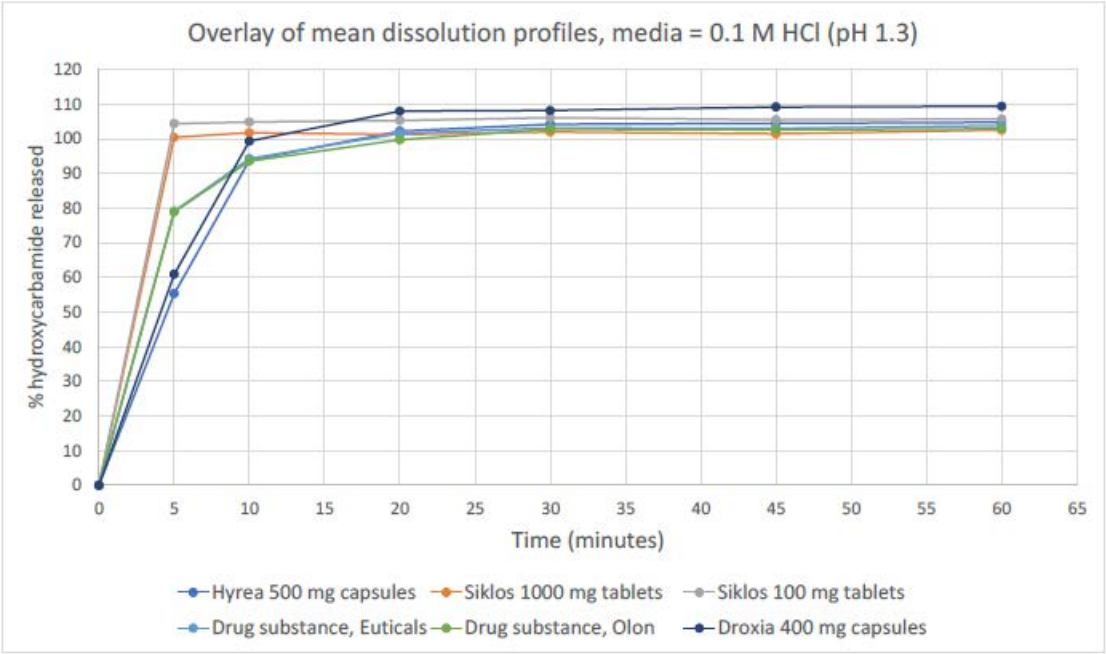


Figure 2

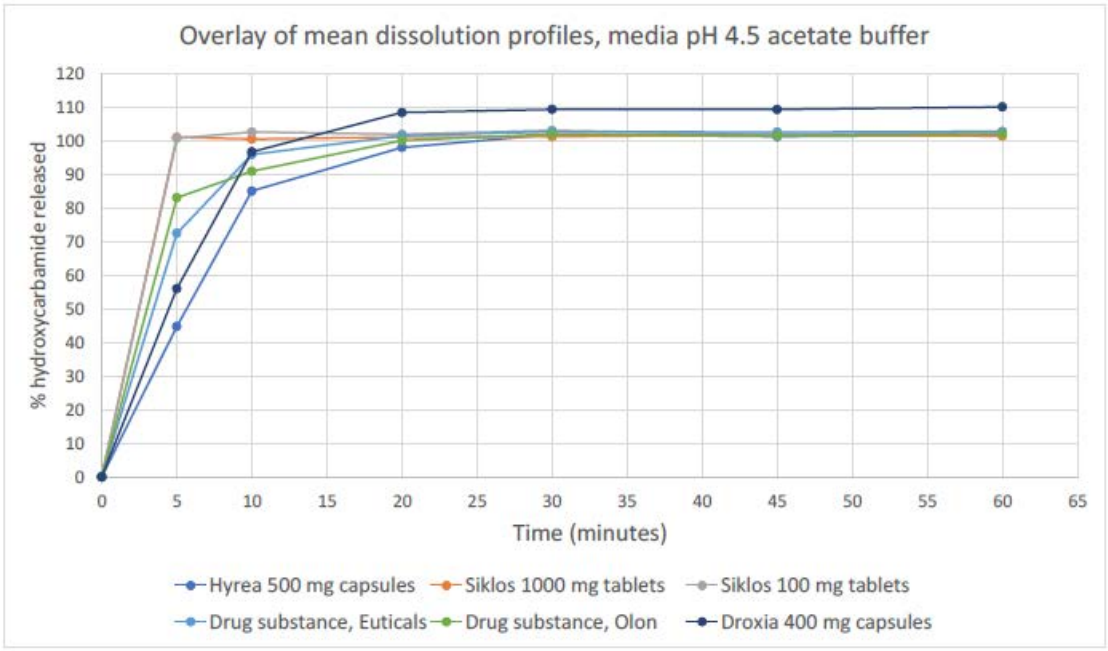
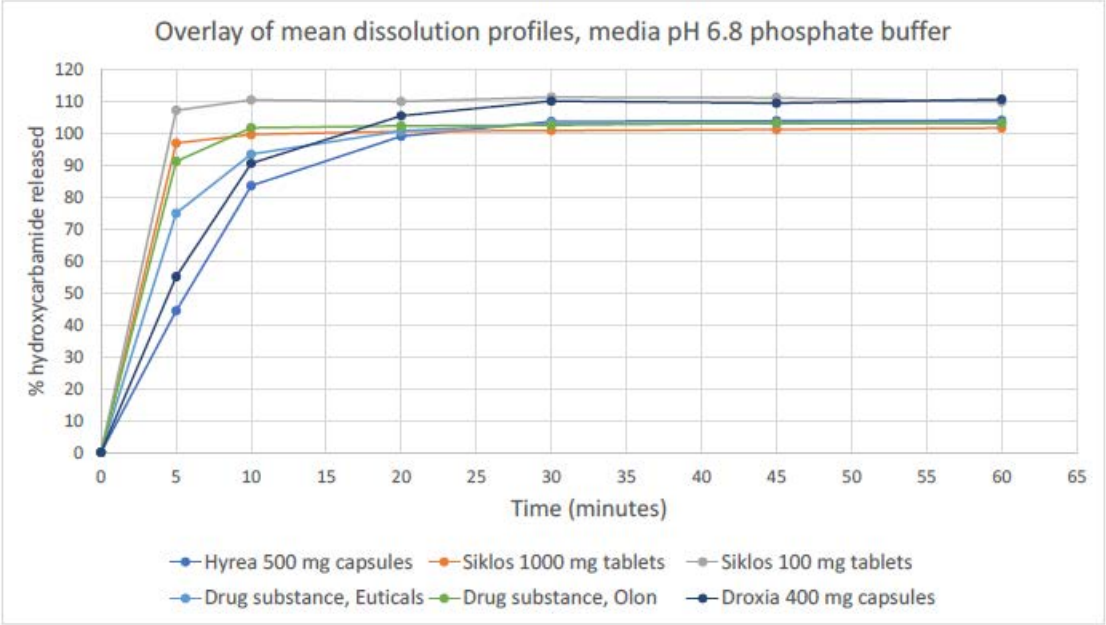


Figure 3



The dissolution profiles for Hydrea and the comparator formulations in three different media, covering the physiological pH range, show that dissolution from all formulations was rapid with the release of more than 85% of the labelled amount within 15 min (see Figure 1, Figure 2 and Figure 3). Any small differences in profiles are linked to the disintegration times e.g. Siklos disintegrates very rapidly with almost complete dissolution in 5 minutes. In fact all the formulations are considered 'very rapidly' dissolving since at least 85% of labelled hydroxycarbamide has dissolved in 15 minutes. In these cases, similarity factor and difference factor calculations become unnecessary. (*'Where more than 85% of the drug is dissolved within 15 minutes, dissolution profiles may be accepted as similar without further mathematical evaluation.: Guideline on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **).*

Comparison of excipients in Hydrea and non-Hydrea formulations

In addition to demonstrating similarity of *in vitro* dissolution profiles, an additional characteristic of formulations that must be taken into account to be certain about expected *in vivo* similarity of the formulations is the excipients. Thus, the excipients should not affect the gastrointestinal transit, absorption (dissolution, solubility, permeability) or *in vivo* stability of the active substance.

The applicant does not believe that any of the excipients in Siklos or Droxia (the non-Hydrea formulations cited in clinical studies extrapolated to Hydrea) are likely to affects any of these processes *in vivo*.

Excipients listed in Siklos 100 mg/1000 mg tablets

All the excipients listed are commonly used in tablet formulations and can be found in many approved tablet products in the EU:

Sodium stearyl fumarate

Used as a tablet lubricant, and there is no data in the literature of incompatability with hydroxycarbamide (indeed since sodium stearyl fumarate is supplied in a purer form than alternatives such as stearic acid and magnesium stearate, chemical incompatibility is improved, Li *et al* 2014), and no known effects on absorption and bioavailability.

Silicified microcrystalline cellulose

Used as a filler/binder in the formulation of tablets, and there is no evidence in the literature of incompatability with hydroxycarbamide, and no known effects on absorption and bioavailability.

Basic butylated methacrylate copolymer (film coating agents)

Basic butylated methacrylate copolymer (BBMC) is used for film (protective) coating of tablets. It is a high molecular weight substance and is virtually not absorbed from the gastrointestinal tract after oral administration. BBMC becomes water-soluble by forming salts with acids; thus is soluble in gastric juice. Although there is some limited *in vitro* data that BBMC may affect membrane permeation of some low permeability compounds (apical to basal), such in vitro experiments are very difficult to extrapolate to the in vivo milieu, where the presence of mucous protein in the intestine is likely to attenuate any potential permeation effects (Grube *et al* 2008). Moreover, hydroxycarbamide is a highly soluble and highly permeable compound (with more or less 100% bioavailability) and therefore is unaffected by permeability enhancers.

Excipients listed in Droxia capsules and Hydrea capsule

Droxia 400 mg:

citric acid

lactose

magnesium stearate

sodium phosphate

gelatin capsule: titanium dioxide

colorants: 200 mg capsule (FD&C Blue No. 1 and FD&C Green No. 3), 300mg capsule (D&C Red No. 28, D&C Red No. 33, and FD&C Blue No. 1), 400mg capsule (D&C Red No. 28, D&C Red No. 33, and D&C Yellow No. 10).

Hydrea 500 mg capsules:

citric acid anhydrous

lactose monohydrate

magnesium stearate

sodium phosphate

gelatin capsule: titanium dioxide, sodium laurilsulfate (lubricant)

colorants: erythrosine, indigotine, yellow iron oxide, opacode S-1-8100

The excipients blended with the active ingredient appear to be identical in both Droxia and Hydrea capsules. Although the chemical colourants of the gelatin capsule differ, they are approved in the EU/US and there is no reason to believe these colourants affect bioavailability.

As it was shown that hydroxycarbamide is a highly soluble and highly permeable drug, the discussed quality attributes of non-Hydrea formulations (dissolution tests and excipient description) mean that bridging can be done and non-Hydrea literature can be used to justify product's efficacy/safety.

Influence of age

In adult SCA patients with normal renal function (n=7), the mean AUC_{0-inf} after a single 15 mg/kg dose of hydroxycarbamide was 82.5 mg×h/L (Yan et al 2005). In a population PK study of children (median age 10, range 5 -17 years) who received either a MTD dose (25-35 mg/kg) or initial starting dose (15-20 mg/kg), the mean AUC0-inf was 101 and 105 mg×h/L, respectively (Wiczling et al 2014). De Montalembert et al (2006) compared hydroxycarbamide tablet pharmacokinetics in adults vs. children (aged 4 to 19 years) receiving equivalent weight normalized doses (mean 20.9 vs 21.9 mg/kg respectively). There was no significant difference in systemic exposure (Cmax; 24.5 vs 26.5 mg/L) or AUC_{0-inf} (115.8 vs 128.4 mg×h/L).

Rogers et al (2005) in an abstract reported limited PK data from an initial cohort of children (mean age 14.5 months, range 12 – 18 months) recruited into the BABY HUG trial. The first dose PKs were obtained 0, 1, 2, and 4 hours after administration of 20 mg/kg of an oral solution of hydroxycarbamide in 22 consecutive patients. The reported Cmax (19.81±5.8 mg/L), and AUC (68.82±11.5 mg×hr/L) are estimated to be lower than equivalent doses administered to older children and adults in other studies, suggesting perhaps age-related differences in drug clearance (Rogers et al 2005).

In the most recently reported study, Dong et al (2015), both bodyweight and Cystatin C (biomarker of renal function) were identified as influential covariates of clearance, but not age. Although a relationship between systemic exposure and response (effect on HbF, bone marrow suppression and clinical outcomes) has not been demonstrated for hydroxycarbamide, based on the data from children recruited into the HUSTLE trial, the authors suggested that a target AUC_{0-inf} of 115 mg×h/L may be utilized to optimize dosing. However, they caution applying this target AUC to patients less than 2 years of age, on account of immature renal and hepatic function.

Influence of weight

The PK of hydroxycarbamide in children has been characterised through data collected from the Hydroxyurea Study of Long-Term Effects (HUSTLE, NCT00305175). A primary objective of this study was to determine the first-dose PK parameters for children commencing hydroxycarbamide treatment, using a fixed oral dose of 20 mg/kg. Subsequently, children underwent standardized dose escalation to MTD. A total of 712 hydroxycarbamide plasma concentrations from 96 patients after initial treatment (Day 1) were available for PK analysis. Among them, 63 patients had paired PK profiles after they had reached MTD. The age range of children recruited was 1.2 to 16.5 years. All participating subjects were given a single oral 20 mg/kg hydroxycarbamide dose (majority oral liquid). Patients then followed a dose escalation protocol with periodic monitoring of toxicity until individual MTD (range 14.2 to 35.5 mg/kg/d). Plasma samples were collected Day 1 and at MTD at the following times: pre-dose, and at 20 min, 40 min, 1, 2, 4, 6 and 8 h after oral hydroxycarbamide administration. A population PK model was developed, which revealed bodyweight on clearance (V_{max}) and V_d and Cystacin C (a measure of renal function) on clearance (V_{max}), as the most significant covariates (Dong *et al* 2015).

As mentioned above, De Montalembert *et al* (2006) showed that there was no significant difference in systemic exposure (Cmax; 24.5 vs 26.5 mg/L) or AUC_{0-inf} (115.8 vs 128.4 mg×h/L) in adults vs children (aged 4 to 19 years) receiving equivalent weight normalized doses (mean 20.9 vs 21.9 mg/kg respectively).

Distribution

The volume of distribution following oral dosing of hydroxycarbamide is approximately equal to total body water: adult values of 0.48–0.90 L/kg have been reported, whilst in children a population estimate of 0.7 L/kg has been reported (Rodriguez *et al* 1998, Tracewell *et al* 1995, Gwilt *et al* 2003; Yan *et al* 2005, Wiczling *et al* 2014).

Elimination

Hydroxycarbamide is rapidly cleared from the circulation in cancer patients; with a mean half-life of approximately 3 h in adults (Rodriguez et al, 1998). Similarly, in children aged 9.6 ± 4.8 years, with SCA a mean half-life of 1.7 hours has been reported (Ware et al 2011). Hydroxycarbamide is cleared by both metabolism in the liver and unchanged through the kidney. In adults, urinary recovery of unchanged drug is reported to be approximately 40% of the oral dose when renal function is normal (Rodriguez et al, 1998, Newman et al, 1997). In children, the fraction of hydroxycarbamide excreted into the urine was estimated to be about 50% (Wiczling et al, 2014). The total body clearance of hydroxycarbamide in adult patients with Sickle Cell disease is 0.17 L/h/kg (Paule et al 2011) and the respective value in children was similar, 0.22 L/h/kg (Wiczling et al, 2014).

Excretion

Hydroxycarbamide is known to be significantly cleared by the kidney (up to 50% excreted unchanged in urine) (Estepp et al 2016). In adults, urinary recovery of unchanged drug is reported to be approximately 40% of the oral dose when renal function is normal (Rodriguez et al 1998, Newman et al 1997). In children, the fraction of hydroxycarbamide excreted into the urine was estimated to be about 50% (Wiczling et al 2014).

Metabolism

Recently, Kovacic et al (2011) described the biotransformation pathway(s) for hydroxycarbamide as well as the metabolites have been characterised. It appears that nitroxyl (HNO), the corresponding carboxylic acid and nitric oxide (NO) are metabolites: The first portion involves oxidation of hydroxycarbamide to a carbamoyl nitroso. Both horseradish peroxidase and catalase participate catalytically (catalase is found in the liver and erythrocytes too). The next transformation consists of hydrolysis of the intermediate to HNO and the corresponding carboxylic acid. NO is also generated (Kovacic et al 2011). Indeed NO is considered to play an important role in the treatment of SCD. Urea has also been postulated as one metabolite of hydroxycarbamide (Adamson et al 1965).

In a rodent study, Andrae (1984) reported the involvement of cytochrome P450 dependant monooxygenases in the formation of HU genotoxic metabolites. This study was however performed on liver microsomes from rodents using high concentrations (25000 µM) of hydroxycarbamide not reflective of systemic concentrations observed in SCD (10 – 500 µM). Sassi et al, 2010, reported that hydroxycarbamide at 30, 100 and 300 µM is not metabolised in vitro by cytochrome P450s of human liver microsomes, nor is it a P-glycoprotein substrate. Hence, no interaction is to be expected in case of concomitant administration with substances being substrates of cytochromes P450 or P-glycoprotein (Sassi *et al,* 2010).

Dose proportionality

No data were presented by the applicant. There is relevant data in the literature showing that there is a nonlinear relationship between plasma hydroxyurea concentrations and dose for high doses, whereas for lower doses there is a linear relationship (Yan et al 2005).

Interactions

Hydroxycarbamide is not metabolised *in vitro* by cytochrome P450s of human liver microsomes, nor is it a P-glycoprotein substrate (Sassi *et al* 2009). Hence, no interaction is to be expected in case of concomitant administration with substances being substrates of cytochromes P450 or P-glycoprotein.

Intra- and inter-individual variability

There is substantial interpatient variability in hydroxycarbamide pharmacokinetics. Cmax has been reported with a range of ≈20-40 µg/mL across different trials. Also, in Ware *et al* 2011 reference, substantial interpatient variability was observed for 87 children for all PK parameters. This interpatient PK variability may contribute to the observed response (HbF% levels) and dosing (MTD) variability.

Impaired renal function

Impaired renal function is a relatively frequent and serious complication in patients with SCD. These patients commonly develop proteinuria, which may progress to the nephrotic syndrome and end-stage renal disease (ESRD). As renal excretion is a pathway of elimination (normally approximately 50% of unchanged parent compound is recovered in urine), consideration should be given to decreasing the dose of hydroxycarbamide in patients with renal impairment.

In an open single-dose study in adult patients with Sickle Cell disease (Yan *et al,* 2005) the influence of renal function on pharmacokinetics of hydroxycarbamide was assessed. Patients with normal renal function (creatinine clearance CLCr > 80 ml/min, n=7), mild (CLCr 60–80 ml/min, n=2), moderate (CLCr30 - 60 ml/min, n=3), or severe (CLCr < 30 ml/min, n=3) renal impairment, and End Stage Renal Disease (ESRD, n = 3), with 2 of them on maintenance haemodialysis. Except for patients with ESRD, all the patients received a 15-mg/kg single oral dose of hydroxycarbamide (Droxia capsules). Patients with ESRD received a 15 mg/kg oral dose of hydroxycarbamide on 2 occasions. Blood and urine samples were collected for the assessment of hydroxycarbamide pharmacokinetics. As the degree of renal impairment worsened, the systemic exposure to hydroxycarbamide increased, and the urinary recovery decreased. The mean AUC0-∞ was increased by 88%, 70%, 62%, 80%, and 152% for patients with mild, moderate, severe renal function impairment, and ESRD with and without hemodialysis, respectively, compared to patients with normal renal function. The changes in $CL_{total/F}$, CL_{renal}, and $t_{1/2}$ were also generally in keeping with this trend (i.e. higher exposure, lower clearance, and longer half-life). On the basis of the altered pharmacokinetic parameters in renally impaired patients, the authors proposed an initial dosing regimen of hydroxycarbamide (7.5 mg/kg/day) for SCD patients with CLCr < 60 mL/min. This dosing strategy is anticipated to provide a safe dose for SCD patients with renal impairment. However, since systemic exposure is likely to be unpredictable in patients with moderate to severe renal impairment (CLCr < 30 ml/min), the use of hydroxycarbamide in such patients should be avoided.

Impaired hepatic function

No studies have investigated the pharmacokinetics of hydroxycarbamide in hepatically impaired patients, but bearing in mind hepatic elimination is a significant component of overall elimination, perhaps up to 60%, it is prudent to assume that clearance of hydroxycarbamide will be reduced in patients with severe liver dysfunction and therefore careful monitoring of response is required. Since systemic exposure is likely to be unpredictable in patients with severe hepatic impairment, the use of hydroxycarbamide in such patients should be avoided.

2.4.3. Pharmacodynamics

It has long been recognised that raised HbF levels can ameliorate the clinical effects of SCD (Perrine 1978; Platt 1994). HbF levels are high at birth and decrease over the first year of life and hence clinical manifestations are often delayed until the HbF levels decrease. In addition, individuals who inherit high levels of HbF display a milder disease phenotype. This is because the HbF interferes with the polymer formation of the sickle haemoglobin within the red blood cell and because HbF reduces HbS concentration, but more importantly, it cannot enter the deoxy sickle haemoglobin polymer phase (Akinsheye et al 2011). This polymerisation is the underlying pathology in SCD. The more HbF there is, the greater the inhibition. Post-natal, physiological decline of HbF reduces these protective effects. The spleen is affected earliest, typically in the first 1-2 years of life, but the kidney, brain, and other organs also begin to reflect sickle-related injury in early childhood from chronic and repeated sickling events. Hydroxycarbamide was first shown to raise HbF levels in SCD in the 1980s (Platt 1984; Veith 1985).

Whilst disease manifestations are not always clinically evident, the abnormal sickled erythrocytes circulate continuously and cause repeated injury due to hypoxia/reperfusion events related to sickling. For these reasons, early intervention with disease-modifying treatments are warranted in children with SCA, even in the absence of obvious clinical signs and symptoms. Therapies designed to increase HbF levels are particularly attractive, since they offer direct intracellular protection against *in vivo* HbS polymerisation and sickling. HbF induction can therefore help prevent acute clinical complications of SCA such as pain and stroke, while also ameliorating the long-term complications related to chronic organ injury (McGann *et al* 2015).

Hydroxycarbamide was chosen for studies of sickle cell disease because of its oral efficacy and low toxic effects, although other beneficial effects have subsequently emerged. Hydroxycarbamide was approved for the treatment of adults with SCA by the Food and Drug Administration (FDA) in 1997, and for both children (>2 years) and adults by the European Medicines Agency (EMA) in 2006.

Information on pharmacodynamics is derived from the published literature, as no PD endpoint was investigated in the bioequivalence study. The applicant is referring to Letvin *et al* 1984, Platt *et al* 1984, Charache *et al* 1992, and Lavelle *et al* 2001 on the primary pharmacology of hydroxycarbamide.

Mechanism of action

Hydroxycarbamide's mode of action is complex and can generally be categorised into two overlapping pathways: effects on HbF production and improved blood flow through reduced intercellular adhesion.

The principal and most well understood mechanism of action of hydroxycarbamide *in vivo* is the reversible inhibition of ribonucleotide reductase (RR), a critical enzyme that converts ribonucleosides into deoxyribonucleosides, which are required for the synthesis and repair of DNA. Potent inhibition of RR leads to decreased intracellular pools of deoxyribonucleotide triphosphates and impedes progression of cellular division through S phase. Temporary arrest of haematopoiesis through once-daily hydroxycarbamide results in altered erythroid kinetics upon recovery, inducing the so called "stress erythropoiesis." Enhanced HbF production from intermittent mild marrow toxicity is believed to stem from the steady shifting of marrow physiology to the stressed state. The marrow responds to the repetitive pharmacological injury of daily use by enhanced erythropoiesis and increased HbF production. Paradoxically, the net effect of marrow toxicity is induced HbF and stabilization of cellular haemoglobin solubility. HbF inhibits intracellular HbS polymerization and prevents the sickling process within erythrocytes. These effects lead to decreased levels of RBC membrane damage and haemolysis (Green *et al,* 2014). RBCs with more HbF are larger (higher MCV) and more deformable (better rheology) (McGann *et al,* 2015). Hydroxycarbamide appears to influence RBC–endothelial interactions. Decreased expression of RBCs, white blood cells, and endothelial integrins and other adhesion molecules probably improves microvascular blood flow and reduces proinflammatory cell–cell interactions. Hydroxycarbamide may also

reduce cellular adhesion in general and/or adhesion provoked by infection or inflammation (Penkert *et al* 2017). Integrins and other cell surface glycoproteins regulate neutrophil migration and RBC flow through endothelial interactions. In a murine model for SCD and pneumococcal pneumonia and sepsis, hydroxycarbamide provided some protection by decreasing the recruitment of neutrophils into infected lungs. Mice genetically engineered to lack E-selectin were not protected by hydroxycarbamide, strengthening the view that hydroxycarbamide effects may also be HbF-independent. Hydroxycarbamide also decreases the degree of chronic inflammation (as seen by the decrease in white cells and platelets) and may also stimulate nitric oxide (NO) production as an NO donor or through stimulation of intermediates, facilitating vasodilation (Green *et al,* 2014; McGann *et al,* 2015).

Primary pharmacology

In the 1980s, to determine whether hydroxycarbamide, a cytotoxic/cytostatic drug that does not influence DNA methylation, might stimulate HbF synthesis, two juvenile *Cynomolgus* monkeys were phlebotomised to induce anaemia and reticulocytosis and then treated with hydroxycarbamide. Immediately after phlebotomy was initiated, there was a rise in the level of F cells (erythrocytes with measurable HbF), which stabilised at an average value of 13% in one animal and 20% in the other during a two-month control period. Foetal haemoglobin gradually rose from undetectable values before bleeding to 3% in one animal and 5% in the other. Sixty-two days after initiation of phlebotomy, hydroxycarbamide (50 mg/kg/day for five days) induced only a small and transient increase in F cells and HbF. Two weeks later, however, a similar course (100 mg/kg/day) resulted in a prompt and dramatic increase in both indexes. These results strongly suggested that hydroxycarbamide (an S-phase–specific cytotoxic) increases HbF by a mechanism that does not involve inhibition of DNA methylation (Letvin *et al* 1984).

The effect of treatment with a combination of erythropoietin (EPO), stem cell factor (SCF) and hydroxycarbamide on HbF levels, F-cell numbers, and globin chain synthesis was evaluated in a baboon model (Lavelle et al 2001). The combination of SCF+EPO+hydroxycarbamide resulted in an additional two-fold increase in HbF, whereas F-cells and F-reticulocytes increased only 25% compared to the SCF + EPO regimen.

The first clinical application of hydroxycarbamide for patients with SCA was reported in 1984, when Platt and colleagues demonstrated a rapid and dramatic increase in HbF-containing reticulocytes without significant bone marrow toxicity (Platt *et al* 1984). Many cytotoxic drugs increase HbF concentrations, which is beneficial in patients with sickle cell disease.

Over the last 30 years, following a series of 'proof of principle' experiments, phase 1-3 studies in adults and children with SCA treated with hydroxycarbamide at maximum tolerated dose (MTD), and which demonstrated significant dose-dependent increases in Hb and HbF along with concurrent reduction in total WBC, neutrophils, and reticulocytes and reduction in haemolysis, hydroxycarbamide has become established as a disease modifying therapy (McGann *et al* 2011a).

The effects of hydroxycarbamide are dose dependent, and many clinical trials have demonstrated that the laboratory and clinical benefits of hydroxycarbamide are optimized when escalated to MTD. Charache et al 1992 studied the hydroxycarbamide dose-response curve in 49 patients and reported an increase in %HbF and %F cells with increasing doses, approaching an asymptote at around 35 mg/kg/day. Mean MTD was 22.1 mg/kg (range 15-35 mg/kg/day). MTD is defined as a stable and tolerated dose (mg/kg/day) that achieves a target range of mild marrow suppression, most commonly determined by the absolute neutrophil count (ANC) but also by the absolute reticulocyte count (ARC).

At MTD, almost all patients with SCA have a significant increase in the total Hb concentration and the mean corpuscular volume (MCV), but the laboratory benefits of hydroxycarbamide are most often defined by the amount of HbF achieved. All children with good adherence will respond to hydroxycarbamide, but

there is substantial interpatient variability in both the MTD itself and the %HbF levels achieved. For example, some patients tolerate an MTD as high as 35 mg/kg/day before reaching appropriate myelosuppression, while others can only tolerate a dose of 15 mg/kg/day. Similarly, some patients achieve HbF levels greater than 40%, while others are never able to reach 20%. These observations suggest important individual pharmacokinetics, pharmacodynamics, and pharmacogenomics differences that contribute to the phenotypic variability in both the dosing and response to hydroxycarbamide therapy (McGann and Ware 2015).

Secondary pharmacology

Hydroxycarbamide is also used in solid tumours, polycythaemia vera, chronic myelogenous leukaemia and psoriasis. The mechanism of its cytoxic and antineoplastic effect is through interference with the synthesis of DNA without interfering with the synthesis of RNA or protein.

The principal and most well understood mechanism of action of hydroxycarbamide *in vivo* is the reversible inhibition of ribonucleotide reductase (RR), a critical enzyme that converts ribonucleosides into deoxyribonucleosides, which are required for the synthesis and repair of DNA. Potent inhibition of RR leads to decreased intracellular pools of deoxyribonucleotide triphosphates. The drug is an S-phase inhibitor and may cause cells to arrest at the G1—S border, decrease the rate of cell progression into the S phase, and/or cause cells to accumulate in the S phase as a result of inhibiting DNA synthesis (AHFS Hydroxycarbamide monograph).

Animal studies indicate that the cytotoxic effects of hydroxycarbamide are limited to those tissues with high rates of cellular proliferation and the effects are evident only in those cells that are actively synthesizing DNA. Hence it is predictable that myelosuppression, gastrointestinal and cutaneous effects are commonly observed following chronic hydroxycarbamide therapy (AHFS Hydroxycarbamide monograph).

Neutropenia is the first and most common manifestation of haematological suppression, while thrombocytopenia/anaemia occurs less frequently and is rarely seen without a preceding neutropenia. Recovery from myelosuppression is usually rapid when therapy is discontinued (typically 1 week of therapy cessation) (Strouse *et al* 2012). Treatment can then be re-initiated at a lower dose.

Although hydroxycarbamide-induced myelosuppression usually is put to therapeutic use in patients with polycythaemia vera, dose-dependent myelotoxicity can occur during therapy with the drug in such patients (Parasuraman *et al* 2016). The risk of clinical toxicity generally can be minimised by adequate monitoring and titration of dosage (e.g., by reducing hydroxycarbamide dosage and increasing the use of supplemental phlebotomy if necessary). Despite good long-term haematologic control, thrombotic episodes can occur in patients receiving hydroxycarbamide for polycythaemia vera. However, some evidence indicates that the risk of thrombotic complications is reduced overall compared with phlebotomy therapy alone in patients with this disease, at least during for the first several years of such therapy (AHSF hydroxycarbamide monograph). Hydroxycarbamide is also thought to work in psoriasis by inhibiting rapidly dividing skin cells (Ranjan *et al* 2007).

Self-limiting megaloblastic erythropoiesis is often seen soon after the initiation of hydroxycarbamide therapy and becomes less pronounced as therapy continues (AHSF hydroxycarbamide monograph). The morphologic change resembles pernicious anaemia but is not related to vitamin B12 or folic acid deficiency and is not necessarily accompanied by anaemia. Hydroxycarbamide-induced macrocytosis may mask incidental folic acid deficiency, and hence prophylactic administration of folic acid is recommended (AHSF hydroxycarbamide monograph). Haemolysis and decreased serum iron values have also been reported. Hydroxycarbamide may delay plasma iron clearance and reduce the rate of iron utilisation by the erythrocytes, but it does not appear to alter the red blood cell survival time (AHSF hydroxycarbamide monograph).

Patients with previous radiotherapy or chemotherapy have an increased risk for myelosuppression; monitoring is recommended and dose reduction or discontinuation may be required.

Pharmacodynamic interactions with other medicinal products or substances

Potentially fatal pancreatitis and severe peripheral neuropathy have been reported in HIV patients who received hydroxycarbamide in combination with antiretroviral drugs, particularly didanosine plus tavudine. Median decline in CD4 cells of approximately 100/mm^3 is also reported with concomitant use. (Bloch *et al* 2016, Halvir *et al* 2001, Rutschmann *et al* 1998, Rutschmann *et al* 2000, Swindells *et al* 2005).

Also concurrent use of hydroxycarbamide and other myelosupressive products or radiation therapy may increase bone marrow depression, gastrointestinal disturbance and mucositis. Erythema caused by radiation may be aggravated by hydroxycarbamide (Lerner *et al* 1977).

Cutaneous vasculitis toxicities including ulcerations and gangrene are more reported in patients with myeloproliferative disorder if hydroxycarbamide is used concomitantly with interferon therapy or used in patient with prior exposure to interferon therapy. (Hydrea Label).

Concomitant use of hydroxycarbamide with a live virus vaccine may potentiate the replication of the vaccine virus and/or increase the adverse drug reaction of the vaccine virus, because normal defence mechanisms may be suppressed by hydroxycarbamide therapy. Compared with placebo, hydroxycarbamide treatment resulted in significantly lower total lymphocyte, CD4, and memory T-cell counts (Lederman *et al* 2014). It may result in severe infections and hence concomitant therapy is to be avoided (https://www.gov.uk/drug-safety-update/live-attenuated-vaccines-avoid-use-in-those-who-areclinically-immunosuppressed). In addition, cytotoxics suppress antibody levels against vaccinepreventable diseases, reducing vaccine efficacy and subdued response to (re-)vaccination in children is a possibility (Tilburg *et al* 2006).

Genetic differences in PD response

SCD is a term that includes different genotypes (HbSS, HbSC, $HbS\beta^0$, HbSβ+ account for most SCD in Europe). Pharmacogenomics differences contribute to the phenotypic variability in both the dosing and response to hydroxycarbamide therapy. Ware et al 2011 (described as key study by the Applicant during the Scientific Advice phase) report SNPs that could affect PD response. It would have been very informative if the Applicant had discussed this section in more detail, explaining if and how genetic differences affect PD response. However, this is not expected to affect the intended clinical use of the product or the target population.

Relationship between plasma concentration and effect

Charache *et al* (1992) showed that the effects of hydroxycarbamide are dose dependent. They studied the hydroxycarbamide dose-response curve in 49 patients and reported an increase in %HbF and %F cells with increasing doses, approaching an asymptote at around 35 mg/kg/day. Mean MTD was 22.1 mg/kg (range 15-35 mg/kg/day). Subsequently many clinical trials have demonstrated that the laboratory and clinical benefits of hydroxycarbamide are optimised when escalated to MTD.

In a recent report from the Hydroxyurea Study of Long-Term Effects (HUSTLE), a prospective observational study (NCT00305175) with a primary goal of describing the long-term effects of hydroxycarbamide therapy in children with SCA, in children receiving hydroxycarbamide therapy escalated to MTD, higher % HbF levels conferred greater protection against hospitalisation for severe vaso-occlusive pain VOC or ACS. In 230 children, providing 610 patient-years of follow up, the mean attained HbF% at MTD was >20% for up to 4 years of follow-up. When HbF% values were ≤20%, children had twice the odds of hospitalisation for any reason (P<0.0001), including VOC (P<0.01) and ACS

(P<0.01), and more than four times the odds of admission for fever (P<0.001). Thirty day readmission rates were not affected by HbF%. Neutropenia (ANC<1000×10^6/L) was rare (2.3% of all laboratory monitoring), transient, and benign. Therefore, attaining HbF >20% was associated with fewer hospitalisations without significant toxicity. These data support the use of hydroxycarbamide in children, and suggest that the preferred dosing strategy is one that targets an HbF endpoint >20% (Estepp *et al* 2017).

Although a target therapeutic concentration (related to HbF, bone marrow suppression or clinical outcomes) has not been demonstrated for hydroxycarbamide, based on the data from children recruited into the HUSTLE trial, the authors suggested that a target $AUC_{0\text{-}inf}$ of 115 mg×h/L may be utilised to optimise dosing.

2.4.4. Clinical efficacy

Dose-response studies and main clinical studies

Despite the robust laboratory and clinical benefits from hydroxycarbamide treatment for SCA, substantial phenotypic variation is observed; for example, the %HbF achieved in young patients at hydroxycarbamide MTD ranges from a low of 10%-15% to a high that occasionally exceeds 40% HbF. Additional phenotypic variability is observed in the MTD itself; some patients tolerate hydroxycarbamide at a dose of only 10-15 mg/kg/d before developing dose-limiting myelosuppression (typically ANC and ARC), whereas others tolerate 30-35 mg/kg/d without excessive myelosuppression. In many cases, a lower MTD limits the efficacy of hydroxycarbamide but not always; some patients have remarkable HbF responses despite a relatively low MTD. Most of this phenotypic variability observed with hydroxycarbamide treatment in SCA remains unexplained at this time, although clinically relevant differences in pharmacokinetics, pharmacodynamics, and pharmacogenomics of hydroxycarbamide have been postulated (Ware *et al* 2011).

Individualisation of hydroxycarbamide dosing

Presently, dosing in adults is individualised to the MTD (to a max of 35 mg/kg/day), through careful incremental dose increases, cautious monitoring of haematology and the HbF response. In children, too, hydroxycarbamide is initiated at a relatively low dose (15 -20 mg/kg) and slowly escalated to MTD (maximum 35 mg/kg) to reach a goal of mild myelosuppression. Using this dose escalation strategy, MTD is reached in approximately 6-12 months.

This individualised dosing approach has been reported in the pivotal RCTs and observations studies in adults and children (>2 years): MSH (pivotal RCT study of hydroxycarbamide in adults with SCA), Paediatric Belgian Trial, SWiTCH, TWiTCH.

The pivotal RCT in children < 2 years (BABY HUG) and the first phase of the HUSOFT study utilised a fixed dose of 20 mg/kg without escalation, demonstrating efficacy and safety, and a positive risk-benefit profile. In HUSTLE, the initial dose was 20 mg/kg/day and then a dose escalation protocol was followed with periodic monitoring of toxicity until individual MTD was reached. The findings from these studies (benefit in hematologic results and the relative lack of toxicity) closely resemble those seen in the adult MSH study and studies in older children.

Hence, although PK/PD studies have not been conducted, the efficacy/safety data to date suggest that the therapeutic dose range (margin) is not significantly different across the age range: infants, older children and adults. Figure 4 shows the range of doses tested in the pivotal trials, and overlaid are the

hypothetical PK-PD curves for efficacy and safety. It should be emphasized that evidence beyond a dose of 35 mg/kg is not available.

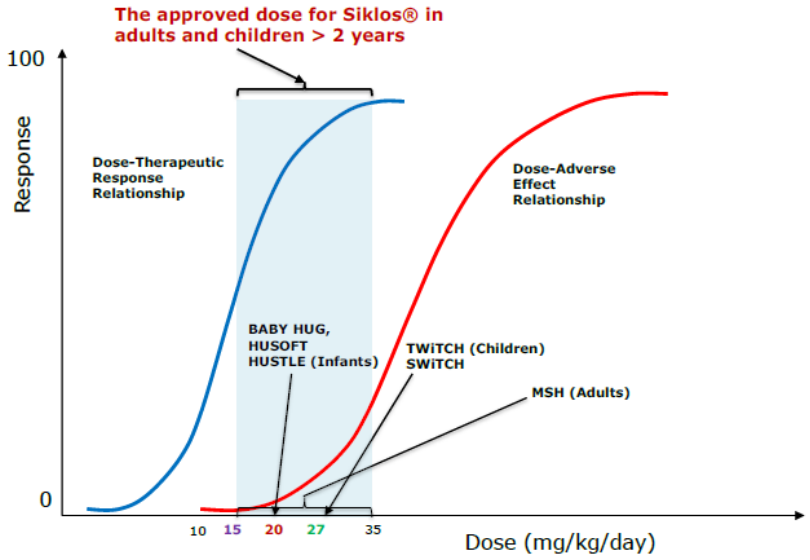


Figure 4: Hydroxycarbamide in SCA: The Assumed Therapeutic Margin (and hypothetical dose response curves), Approved Dose in the EU and Pivotal Doses Tested in Clinical Trials in Infants, Children and Adults

Main studies

Three studies (384 adults and children with HbSS or HbSβ⁰thal) compared hydroxycarbamide to placebo, recruiting individuals with severe disease (Charache *et al* 1995; Belgian Study 1996; Jain 2012). There were statistically significant improvements in terms of pain alteration (using measures such as pain crisis frequency, duration, intensity, hospital admissions and opioid use), measures of foetal haemoglobin and neutrophil counts and fewer occurrences of ACS and blood transfusions in the hydroxycarbamide groups. There were no consistent statistically significant differences in terms of quality of life and adverse events (including serious or life-threatening events). Seven deaths occurred during the studies, but the rates by treatment group were not statistically significantly different. In the TWiTCH study, hydroxycarbamide was compared with transfusion in children with abnormal TCD velocities who had received transfusion for at least one year and had no evidence of vasculopathy on magnetic resonance angiography (MRA) (TWiTCH 2015). Hydroxycarbamide was as effective as transfusion in preventing further stroke and final TCD velocity was lower in the hydroxycarbamide group. Table 15 summarises the four main studies.

Table 15: Main studies of Hydroxycarbamide in children and adults

Author, year	Location, Recruitment Date	Inclusion / Exclusion criteria	Intervention	Planned duration of treatment	Patient groups Intervention (N)	Age, mean (range) years	Genotype/ haplotype (%)	Methodological quality	Hydroxycarbamide Formulation	Main findings (efficacy and side effects)
MSH: Charache, 1995	North America Jan. 1992– Apr. 1993	Inclusion: Age >18 yr; SCA; HbSα°-thal, pain >3/yr. Exclusion: HbS β°-thal; HbSβ°-thal; HbSC; transfusion dependent; pregnancy; Op; SA; CTA; stroke in last 6 yr; HIV; HC; not willing to use contraception; blood counts that could not be distinguished from marrow suppression; >15% HbA if recent transfusion; breastfeeding.	HC: Initial dose of 15 mg/kg/d, increased by 5 mg/kg/d every 12 weeks, unless marrow depression (ANC < 2,000 per cubic millimeter, a retic or platelet < 80,000 per cubic millimeter, or a Hb< 4.5 g/dl) was present. If present, treatment was stopped until blood counts recovered, then resumed at a dose that was 2.5 mg per kilogram lower than the dose associated with marrow depression, starting a new 12-week cycle. Placebo: Adjusted by the data coordinating centre in a similar manner in order to maintain blinding.	2 yr	299 (HC: 152)	30 yrs (18-59	HbSS, HbSα+ thalassaemia	Low risk (patients, care givers, data collectors all blinded; trial discontinued early)	Capsules (prepared in pharmacy)	Compared to placebo, HC group significantly decreased VOC, ACS, pain crises, transfusions (p<0.01). Compared to placebo, HC group significantly increased HbF
Belgian Trial: Ferster, 1996	Europe, June -1992– Dec. 1993	Inclusion: SCA, HbSα°-thal; 3/yr pain episodes, stroke; acute chest syndrome (ACS); splenic sequestration. Exclusion: HbS β°-thal; HbSβ°-thal	HC: 20 mg/kg/d—Increased by 5 mg/kg/d after 2 mo, if no response increased to 25 mg/kg. Placebo: Not described	HC: 6 mo Placebo: 6 mo (Cross-over)	25	Median 9 yr (2-22)	HbSS, HbSS, HbSα+ thalassaemia	Moderate risk (patients blinded, but care givers, data collectors not blinded)	Capsules (prepared in pharmacy)	Significant increases in HbF and MCV occurred during the HC treatment period. Sixteen of 22 patients (73%) experienced a complete disappearance of events requiring hospitalization (p<0.05). The number of days of hospitalization as well as the number of hospitalizations for patients on HC, as compared with those for the patients

Author, year	Location, Recruitment Date	Inclusion / Exclusion criteria	Intervention	Planned duration of treatment	Patient groups Intervention (N)	Age, mean (range) years	Genotype/ haplotype (%)	Methodological quality	Hydroxycarbamide Formulation	Main findings (efficacy and side effects)
										receiving placebo, were significantly reduced (p<0.01)
Jain, 2012	India, NR	Children with severe sickle cell anaemia (SCA) (more than 3 hospitalizations per year for vaso-occlusive crisis (VOC) or 3 transfusions per year) Exclusion: seropositivity for HIV or any chronic illness that could enhance HU toxicity	HC 10 mg/kg/day or placebo	18 mo	60 (HC:30)	12 (5–18)	NR	Moderate risk of bias Double-blinded (unclear who was blinded), unclear allocation concealment and loss to follow up	Capsules	Compared to placebo, HC group had significantly decreased VOC frequency, increased haemoglobin and haemoglobin F percentage (p<0.001 for all 3 outcomes) Side effects: No serious events, no leukopoenia, neutropenia, renal or hepatic toxicity
Ware *et al*, 2015 TWiTCH	United States, Canada; Sept 20, 2011 to April 17, 2013,	abnormal TCD velocities (≥200 cm/s) and if they had received at least 12 months of chronic transfusions Exclusion: documented clinical stroke, transient ischaemic attack or severe vasculopathy	randomly assigned 1:1 to continue standard transfusions (standard group) or hydroxycarbamide (alternative group)	24 months	121 (60 HC 61 transfusions)	4-12 years	HbSS (98%)	Low risk	Hydrea, Droxia, Compounded liquid	hydroxycarbamide at MTD was non-inferior and possibly superior to chronic transfusions for maintaining TCD velocities (p<0.05). Sickle cell related serious adverse events were more common in the hydroxycarbamide arm than the transfusion arm (23 to 15), but none was related to study treatment or study procedures.

HC= Hydroxycarbamide; § Change from baseline, mean±SD where applicable.

‖ Patients, P=.002, red blood cell (RBC) units transfused, 423 P=.002

§§ p<.001

¶¶ p=.016, days hospitalized, 3.6, p=.0027, hospitalizations/yr

Multicentre Study of Hydroxycarbamide in Sickle Cell Anaemia (MSH study, Charache *et al* 1995)

The MSH study was a randomized, double-blind, placebo-controlled trial involving 299 adults with SCA who had experienced three or more VOCs in the previous year. The clinical end point of three or more documented VOCs was chosen because of earlier data documenting that people who experience pain at that frequency had markedly lower survival rates. The trial was conducted to test the hypothesis that hydroxyurea could substantially reduce the frequency of painful crises (often called vaso-occlusive crises, VOCs) in adults with SCA. Because of the beneficial effects observed, the trial was stopped before the planned 24 months of treatment were completed for all patients.

Methods

Study Participants

The study group was composed of four central units located in Baltimore (a central office, a data coordinating center, a treatment distribution center, and a core laboratory) and a consortium of 21 clinics in the United States and Canada. Most of the study clinics were in the eastern United States, with the largest in Chicago. Several drew on patients from rural areas, in some cases using satellite facilities to increase patient participation in the study. All clinics were associated with academic medical centers. There were similar numbers of men (148) and women (151), and 95% of them identified themselves as black. More than half were 18-29 years of age. Almost all had completed high school. Public medical assistance plans provided medical care for most MSH patients. For the group as a whole, during the 28-day run-in period, medical contacts were reported on 11% of the days (Charache et al 1995b).

To be eligible, the patients had to be at least 18 years old and had to have sickle cell anemia; patients known to have sickle cell–b-thalassemia and sickle cell–b°-thalassemia were excluded, but those with sickle cell–a-thalassemia were not. If patients had received transfusions, hemolysates of their red cells could not contain more than 15% hemoglobin A at the time treatment was initiated. The patients had to have reported at least three crises to the study physician in the year before entry into the study; for purposes of eligibility, documentation of crises was not necessary. There was no upper limit on the number of crises per year.

Other reasons for exclusion from the study included pregnancy; known narcotic addiction or regular consumption of more than 30 oxycodone capsules (or the equivalent) every two weeks; participation in a long-term program of transfusion; concurrent treatment with another potential antisickling agent; pretreatment blood counts that could not be distinguished from levels considered to indicate marrow depression; a history of stroke during the preceding six years; prior hydroxyurea therapy; and the presence of antibody to the HIV.

Treatments

After an initial four-week run-in period, during which only folic acid tablets were dispensed, the patients were randomly assigned to a treatment group. Hydroxyurea (provided in powder form by Bristol-Myers Squibb) and placebo (Starch 1500) were encapsulated by John Hopkins Manufacturing Pharmacy into 200-mg and 500-mg capsules and dispensed to the clinics by the treatment distribution center. Patients assigned to hydroxyurea received an initial dose of 15 mg per kilogram of body weight per day, and the dose was increased by 5 mg per kilogram per day every 12 weeks, unless marrow depression (indicated by a neutrophil count below 2000 per cubic millimeter, a reticulocyte or platelet count below 80,000 per cubic millimeter, or a hemoglobin level below 4.5 g per deciliter) was present. If marrow depression occurred, treatment was stopped until blood counts recovered; it was then resumed at a dose that was 2.5 mg per kilogram lower than the dose associated with marrow depression, starting a new 12-week

cycle. The dose of placebo was adjusted by the data coordinating center in a similar manner in order to maintain blinding. All patients were given 1 mg of folic acid per day. At each follow-up visit, the capsules were counted and patients were asked whether they had had any adverse effects.

The patients were seen every two weeks, and blood samples were shipped to Baltimore and analysed at Johns Hopkins Hospital. Fetal hemoglobin and red cells containing fetal hemoglobin (F cells) were assayed, dense cells were counted, and a-globin and b-globin DNA were analyzed (at the Veterans Affairs Medical Center, Jackson, Miss.) according to previously described methods.

Objectives

The Multicenter Study of Hydroxyurea in Sickle Cell Anemia (MSH) was designed to determine if treatment with orally administered HU, in maximal tolerated doses, could decrease by at least 50% the frequency of painful crises in patients with moderate to severe SS disease. A parallel (rather than a crossover) design was chosen not only because it might lead to a faster answer, but because of uncertainties over the appropriate duration of a “washout” period between treatments. Secondary objectives included an examination of the relationship of HbF levels to the occurrence of crises and an evaluation of the effect of treatment on the quality of patients’ lives (Charache *et al* 1995b).

Outcomes / endpoints

A painful crisis was defined as a visit to a medical facility that lasted more than four hours for acute sickling-related pain (hereinafter referred to as a medical contact), which was treated with a parenterally administered narcotic (except for a few facilities in which only orally administered narcotics were used); the definition is similar to that used in a previous study. The measurement of the length of the visit included all time spent after registration at the medical facility, including the time spent waiting to be seen by a physician. The occurrence of chest syndrome (chest-wall pain in association with findings of a new pulmonary infiltrate on chest X-ray films and fever), priapism, and hepatic sequestration (a sudden increase in liver size associated with pain in the right upper quadrant, a decrease in the hemoglobin concentration of at least 2 g per deciliter, and more abnormal results of liver-function tests not due to biliary tract disease) was considered a crisis; the occurrence of hematuria and exacerbations of chronic pain was not.

Because painful crises are both the most distressing acute feature of SCD and the presumed cause of later organ damage, the MSH selected a reduction in the frequency of crises as the subject of the primary analysis. Preexisting organ damage cannot be reversed and it did not seem likely that advancing vascular compromise could be reversed in a relatively short trial. Therefore, the primary analysis does not count such complications as pulmonary, cardiac, or renal failure; ankle ulcers; and aseptic necrosis of bone.

Not all patients respond to therapy; treatment (and some form of monitoring for bone marrow depression) must continue lifelong if benefits are to be maintained; the risks of teratogenicity, mutagenicity, and carcinogenicity are not clearly defined; and the cost of treatment, while less than the cost of illness, is not trivial. It was considered that hydroxyurea treatment would have to make a large change in crisis frequency to be considered worthwhile. A difference of 50% in crisis attack rate between the two treatment arms was specified as a clinically important difference which the trial should detect if hydroxyurea is to be considered effective (Charache et al 1995b).

Sample size

Study size was based on power calculations for a test of differences in normally distributed means. It was assumed that (1) at most 10% of patients will be lost to observation for crises and the losses would be comparable in the two treatment arms; (2) in addition to those lost, 10% of patients assigned to receive

hydroxyurea will not take study treatment, and these patients will experience the same crisis rates as placebo patients; (3) the effect of hydroxyurea on crises will increase linearly during the first year of study participation (during dose titration), and the full effect will be reached at 1 year after initiation of therapy for all patients. Because the normal scores test always has the same or greater power than the t-test, power calculations for a t-test offer conservative and convenient estimates of study power.

A stringent criterion (a 50% overall reduction in crisis attack rate) was chosen for efficacy. Data from a previous study (Cooperative Study of Sickle Cell Disease) suggested that increasing HbF to 15 % might be associated with a 50% reduction, and a mean final HbF level of 15% was achieved in a preliminary study with hydroxyurea. Unpublished data from that study suggested that among adult patients with at least three crises in a given year, the mean number of crises (± 1 SD) would be 6.3 (± 6.6). Assuming the standard deviation for crises over 2 years will remain approximately equal to the mean and that a 25 % reduction will be present in both groups because of a placebo effect, the mean number (and SD) of crises during the 2-year follow-up was estimated to be 9.4 in the placebo group. Assuming that the effect of treatment increases until MTD is reached among patients assigned to hydroxyurea and that there is a 50% reduction in crises once MTD is attained, their mean number of crises over 2 years was estimated as 5.9. If 10% of patients did not take study treatment, the mean in the hydroxyurea group would be 6.2 and the SD would be 6.4. Assuming in addition that 10% of patients would be lost at random to follow up, at a=0.05 enrolling 150 patients into each treatment group will give the study approximately 90% power to detect a 50% reduction in crisis rate after MTD is achieved. If the reduction in crisis rate is only 40%, with the same number of patients power would be approximately 0.72; if 20% of the patients do not take study treatment and the reduction in crisis rate is 50%, power would be 0.87. The recruited population (299 patients) is expected to be satisfactory for the analyses planned (Charache et al 1995b).

Randomisation

Not available.

Blinding (masking)

Neither the patients nor the investigators and staff members at the clinical sites were aware of the patients' treatment assignments. Because knowledge of repeated measurements of a patient's MCV or fetal hemoglobin level might make the treatment assignments apparent to staff members, such measurements were made at the central laboratory. Clinic staff members agreed not to look at the results of tests requested by other clinicians at their institutions. Treatment assignments could be revealed if knowledge of the assignment would alter a patient's subsequent medical care (e.g., if a patient or a patient's partner became pregnant). In such cases, the study treatments were stopped, but the researchers continued to follow the patients and to count their crises.

Statistical methods

All patients were included in the primary analysis according to their original treatment assignments. Treatment groups were compared on the basis of annual crisis rates. The primary end-point analysis was to be a two-sided comparison at an overall alpha level of 0.05.

Annual rates were computed by dividing the number of crises by the number of years elapsed (e.g., 6 crises in 1.9 year=3.16 crises per year). To test the effect of treatment on the crisis rate, the patients were ranked according to the number of crises they had had per year for observed periods of up to two years. Death was considered the worst outcome, followed by a stroke (defined as a documented new neurologic deficit lasting more than 24 hours, confirmed by a neurologist) or the institution of long-term transfusion therapy (more than four months); outcomes for all other patients were ranked according to

the individual crisis rate. These ranks were used to compare the two treatment groups (Van der Waerden's test). A rank statistic was planned for the primary analysis because it was expected to have more power to detect differences and to be less influenced by extreme values than a t-test of the means.

Four interim analyses were planned to be conducted every six months after enrolment began. To take into account multiple examinations of the data, a P value of less than 0.001 was specified for the differences between groups to reject the null hypothesis at each of the planned interim analyses, and a P value of less than 0.046 was required at the final analysis.

In secondary analyses, for discrete variables, chi-square tests were used to compare the frequency of specific characteristics. For continuous data, mean values were compared by analysis of variance and linear regression. Cumulative event rates were estimated by the product-limit (Kaplan–Meier) method, and the log-rank statistic was used to compare the distributions of events over time. An interaction term, testing whether the effect of hydroxyurea changed with time, was assessed by Cox proportional-hazards models. To adjust for multiple tests of the data in secondary analyses, two-sided tests with P values between 0.01 and 0.001 were considered to provide some evidence of significant differences between groups, and tests with P values below 0.001 were considered to provide strong evidence of such differences.

Formulations

Hydroxyurea (provided in powder form by Bristol-Myers Squibb) and placebo (Starch 1500) were encapsulated by Johns Hopkins Manufacturing Pharmacy into 200-mg and 500-mg capsules and dispensed to the clinics by the treatment distribution center.

A board appointed by the NHLBI approved the protocol, reviewed each clinic's consent form, provided advice, and oversaw patient safety and the progress of the study. The board was composed of four hematologists, two biostatisticians, an ethicist, and an educator–patient advocate. It was empowered to recommend the discontinuation of the study and did so when interim analyses showed hydroxyurea to be effective.

Results

Participant flow

The published paper did not provide details on the participant flow. From the 299 patients enrolled in the trial, 279 (93%) were being seen regularly for follow-up visits at the clinics and 5034 medical contacts (occurring during two years of follow-up).

Recruitment

The treatment started on January 28, 1992. As of June 30, 1994 (10 months before the planned end of the study), 279 of the 299 patients who were enrolled (93 percent) were being seen regularly for follow-up visits at the clinics and 5034 medical contacts (occurring during two years of follow-up).

Baseline data

There were no significant differences between the two groups of patients with respect to sex, age, race or ethnic group, number of a-globin genes, or b-globin haplotype, and blood counts in the two groups were similar before treatment was begun. After treatment had begun, one patient in the hydroxyl urea group was discovered to have sickle-cell–b+-thalassemia (a small amount of hemoglobin A was present). Table 16 presents the baseline patient characteristics.

Table 16: Characteristics of the patients at base line, according to treatment group

Characteristic	Hydroxyurea (N = 152)	Placebo (N = 147)
No. of crises in the year before study entry (% of patients)		
3–5	44	44
6–9	26	21
≥10	30	35
Complications of sickle cell anemia (% of patients)		
Chest syndrome	66	65
Ankle ulcer	31	33
Aseptic necrosis of bone	20	22
Hemoglobin (g/dl)	8.4	8.5
Mean corpuscular volume (μm^3)	94	93
White cells ($10^{-3}/mm^3$)	12.6	12.3
Platelets ($10^{-3}/mm^3$)	468	457
Reticulocytes ($10^{-3}/mm^3$)	327	325
Fetal hemoglobin (%)	5.0	5.2
F cells (%)	33	33
Dense cells (%)	14.3	14.0

Numbers analysed

299 participants (152 in the hydroxyurea group, 147 in the placebo group).

Outcomes and estimation

As of June 30, 1994, 279 of the 299 patients who were enrolled (93%) were being seen regularly for follow-up visits at the clinics and 5034 medical contacts (occurring during two years of follow-up) had been classified. The ranks of the crisis rates differed in the two treatment groups, with median rates of 2.5 crises per year in the hydroxyurea group and 4.5 crises per year in the placebo group — a 44% difference ($P<0.001$). When only crises severe enough to cause hospitalization were considered, the median annual rates were 1.0 and 2.4, respectively ($P<0.001$). Moreover, the two groups did differ with respect to the number of patients in whom chest syndrome developed (25 in the hydroxyurea group vs 51 in the placebo group, $P<0.001$), the number of patients who received transfusions (48 vs 73, $P=0.001$), and the number of units of blood transfused (336 vs 586, $P=0.004$ by Van der Waerden's test). The median time to the first vaso-occlusive crisis was longer in patients treated with hydroxyurea than in those given placebo (3.0 vs 1.5 months, $P=0.01$), as was the time to the second crisis (8.8 vs 4.6 months, $P<0.001$) (Figure 5). There was no evidence to suggest that the effect of hydroxyurea changed during two years of treatment ($P=0.77$ for the analysis of the time to the first crisis and $P=0.86$ for the analysis of the time to the second crisis).

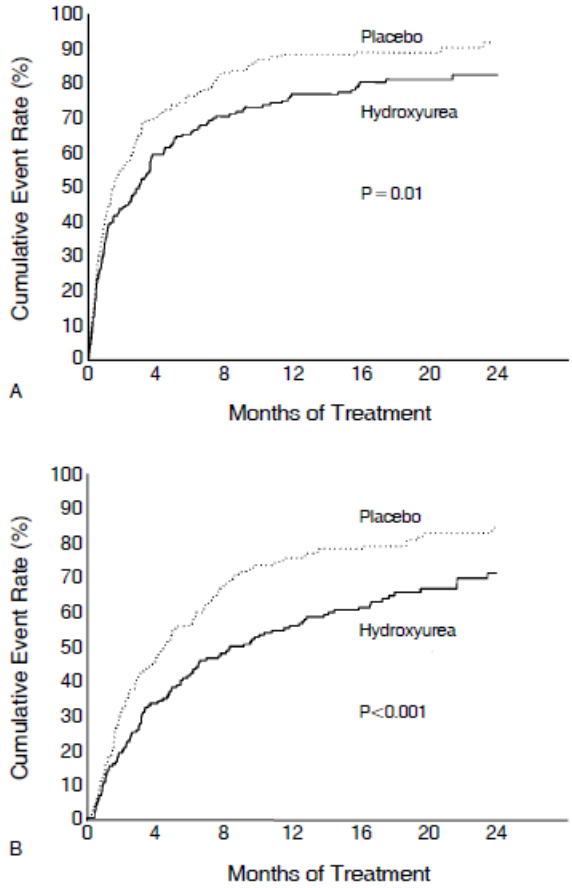


Figure 5: Median Time from the Initiation of Treatment to the First (Panel A) and Second (Panel B) Painful Crises, According to Treatment Group. Painful crises occurred later in patients receiving hydroxyurea than in those receiving placebo, and the effect was evident in less than six months.

After the study ended (January 1995), examination of the doses of hydroxyurea revealed that after six months of treatment, only 33% of the patients in the hydroxyurea group were receiving the maximal tolerated dose or had been receiving a higher dose that was subsequently reduced. By the time the study ended, 51% of the patients treated with hydroxyurea were receiving the maximal tolerated doses, and doses for the remainder of patients were nearly maximal. The daily doses of hydroxyurea ranged from 0 mg per kilogram in the 2% of patients who could not tolerate hydroxyurea to 35 mg per kilogram— the maximal prescribed dose — in 21% of patients. Capsule counts suggested that about 75% of the patients took more than 80% of their capsules. During the dose titration, blood counts consistent with a finding of marrow depression were observed at least once in 35% of the patients who received placebo. Hemoglobin levels, MCVs, fetal hemoglobin levels, and proportions of F cells were higher in the hydroxyurea group than in the placebo group at the time the study ended, and white-cell, platelet, reticulocyte, and dense-cell counts were lower. Differences between the groups in the MCV and proportion of F cells appeared within 8 weeks of the initiation of the study, reached a peak at about 40 weeks, and then declined (Figure 6).

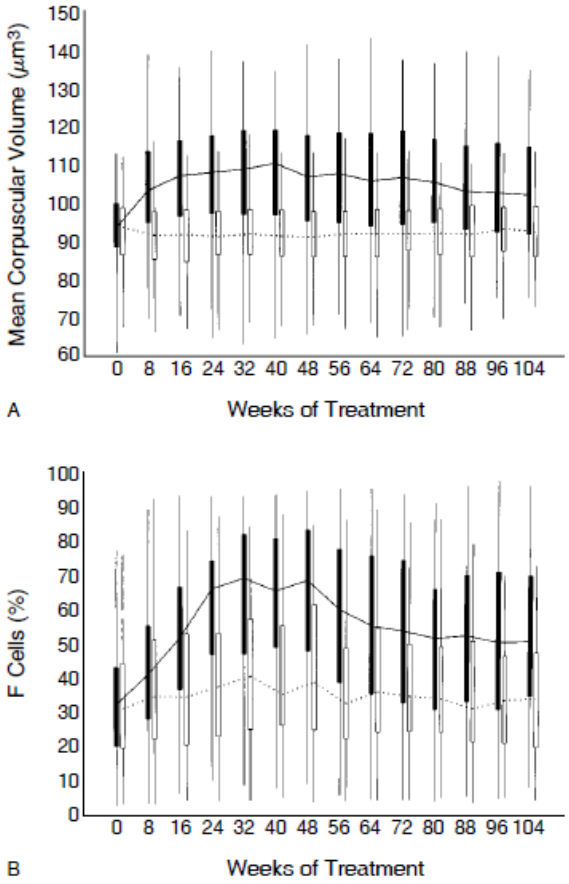


Figure 6: Measurements of MCV (Panel A) and F Cells (Panel B) in Patients Who Received Hydroxyurea (Solid Bars) or Placebo (Open Bars). At each point, the median value, the 25th and 75th percentiles (upper and lower limits of boxes), and the maximal and minimal values (vertical lines) are shown.

Differences in the effect on mortality and stroke outcomes were not statistically significant. Over 2 years of treatment, the benefit of hydroxycarbamide on quality of life was limited to people who maintained increased HbF levels. These restricted benefits occurred in social function, pain recall, and general health perception. The trial had low risk of bias but was stopped early for benefit, which may have exaggerated the observed benefit. In 1998, based on the results of this trial, the FDA approved hydroxycarbamide for the treatment of clinically severe SCA in adults.

A 9-year follow up analysis of MSH participants indicated a reduction in mortality for the group of people who took hydroxycarbamide compared to those who did not take the medication. When the cohort was followed for up to 9 years, people taking hydroxycarbamide had 40% reduced mortality (analysis according to cumulative hydroxycarbamide exposure, not the original randomization). Survival was related to HbF levels and frequency of vaso-occlusive crises. More recently, extension of the follow up analysis to 17.5 years for nonrandomized people indicated continued safety and benefit of hydroxycarbamide, including reduced mortality. After a follow up of 17.5 years, 43.1% of patients had died (n=129; 4.4 per 100-patient years). Eighty seven percent of patients who died were never exposed to hydroxycarbamide or took it for less than five years, suggesting that long-term use of hydroxycarbamide reduces mortality (Steinberg et al 2010).

Low fixed dose hydroxycarbamide in children with sickle cell disease (Jain et al 2012)

This was a randomised, double blind, placebo controlled study conducted in a tertiary hospital in India. The study was conducted in children with sickle cell anaemia (proportion with each genotype not stated) between the ages of 5 and 18 years with three or more blood transfusions or vaso-occlusive crises requiring hospitalization per year despite high HbF. The primary endpoint was the decrease in vaso-occlusive crises per patient per year. Secondary outcomes were a decrease in frequency of blood transfusions and hospitalisations and an increase in HbF levels.

Methods

Study Participants

This is a double blind randomized controlled study that was conducted at a tertiary care hospital in Nagpur City located in Central India. Permission was obtained from the institutional ethics committee and informed con sent was obtained from the parents of all participants. Children with severe sickle cell anemia ranging in age from 5 to 18 years were included in the study. The mean age of patients in the HU group and placebo group were 12.73 ± 4.4 and 11.73 ± 4.08, respectively. Severe sickle cell anemia was defined as frequent vaso-occlusive crises requiring hospitalization (>3 per year) and/or frequent blood transfusion requirement (>3 per year). Exclusion criteria were seropositivity for human immunodeficiency virus or any chronic illness that could potentially enhance HU toxicity. All patients were confirmed to have sickle cell anemia by hemoglobin (Hb) analysis with high performance liquid chromatography (HPLC). Eligible subjects were randomized using randomization tables to receive either HU or placebo therapy. The laboratory technician and the clinician who assessed these patients were not aware of the treatment arm.

Treatments

Children in the HU group received HU at a fixed dose of 10 mg/kg/day orally as a single dose for 18 months. Patients in the placebo group received powdered glucose capsules having the same appearance as the HU capsules. Subjects were supplied with enough drugs for 1 month. The patients were asked to attend regular monthly follow-up for evaluation with detailed history and physical examination. They were also monitored with complete blood count and reticulocyte count at monthly intervals, with HbF, renal and liver function tests at 3 monthly intervals. Compliance was assessed by counting the total number of capsules remaining at the next follow-up visit. All patients were advised to take folic acid (5 mg/day) and increased fluid intake a long with avoiding extreme temperatures. After completion of the study period all participants were unblinded and HU therapy was offered to all participants.

Objectives

This study was under taken to evaluate the efficacy and toxicity of fixed low dose HU therapy in Indian sickle cell anemia patients.

Outcomes/endpoints

The primary outcome of the study was a decrease in the frequency of vaso-occlusive crises per patient per year. Secondary outcomes were a decrease in frequency of blood transfusions and hospitalizations and an increase in HbF levels. Hematological toxicities were defined as reticulocyte count <80,000/μL with simultaneous Hb value <9.0 g/dL, platelet count <80,000/μL, total leucocyte count <4000/μL, 20.0% decrease in Hb concentration from entry or previous value or Hb concentration <5.0 g/dL. Hepatic toxicity

was defined as alanine aminotransferase (ALT) two times above the baseline value. Renal toxicity was defined as serum creatinine level of> 1.0 mg/dL or a 50.0% increase from baseline. General toxicities including hair loss, skin and nail changes, and gastrointestinal disturbances were noted. A major adverse event was defined as death or any other life-threatening clinical event likely to interfere, either temporarily or permanently with the subject's ability to continue or tolerate HU therapy.

Sample size

Sixty patients (32 females and 28 males) with sickle cell anemia were enrolled for the study with 30 patients (16 females and 14 males) in each group.

Randomisation

Not available.

Blinding (masking)

The trial was double blind.

Statistical methods

The study had sufficient statistical power (90.0%) to detect a mean difference in frequency of vaso-occlusive crises of 1.9 per patient per year with a standard deviation of 0.5, assuming an a error of 0.05. The statistical analysis was done using SPSS version 16. Paired t test was used to compare the baseline findings with those after HU therapy, while independent t test was used to compare the HU treated group with the placebo treated group at baseline and at 18 months. A p value of <0.05 was considered statistically significant.

Formulations

Capsules were used, but it was not specified if they were Hydrea, Droxia, or custom-made.

Results

Participant flow

This information was not available in the literature.

Baseline data

The HU treated group and placebo group did not differ with respect to age, gender distribution or any of the factors listed in Tables 17 and 18.

Outcomes and estimation

Hematological parameters at baseline and after 18 months of intervention in the HU and placebo groups are summarized in Table 17. Hemoglobin levels and HbF percentages increased significantly in the HU treated group compared to baseline of HU group and 18 months of placebo group. There was significant decrease in the reticulocyte count and serum billirubin in the HU group which suggested decreased hemolysis with HU therapy. White blood cell count decreased significantly in the HU treated group although no patients had leucopenia. There were no significant differences in red cell count, platelet count, serum creatinine, ALT and aspartate transaminase (AST) levels in either group.

Table 17: Comparison of the Hematological Parameters at Baseline and After 18 Months of Intervention of the Hydroxyurea and Placebo Groups

	Hydroxyurea		Placebo			
Parameters	Baseline	18 Months	Baseline	18 Months	*p* Value[a]	*p* Value[b]
Hb (g/dL)	8.10 ± 0.68	9.29 ± 0.55	8.21 ± 0.68	7.90 ± 0.58	0.27	<0.001
Hb F (%)	19.80 ± 6.90	24.00 ± 5.90	19.21 ± 6.37	18.92 ± 5.77	0.11	<0.001
Reticulocytes (10^5/mm^3)	1.83 ± 0.96	1.15 ± 0.10	1.73 ± 0.49	1.81 ± 0.67	0.17	<0.001
Leucocytes (10^3/mm^3)	7.36 ± 6.03	6.54 ± 5.54	7.26 ± 4.91	7.38 ± 2.85	0.21	<0.001
Platelets (10^3/mm^3)	1.78 ± 0.26	2.01 ± 0.18	1.91 ± 0.21	2.06 ± 0.26	0.20	0.28
RBC (10^6/mm^3)	2.89 ± 0.57	1.98 ± 0.22	1.84 ± 0.47	3.11 ± 0.20	0.31	0.05
Total bilirubin (mg/dL)	2.32 ± 1.42	1.10 ± 0.42	2.27 ± 1.28	2.71 ± 0.93	0.31	<0.001

[a] *p* Value is for comparison between the HU and placebo groups at baseline.
[b] *p* Value if for comparison between the HU and placebo groups at 18 months.

Table 18 summarises the number of sickle cell anemia related clinical events reported during the study period. With HU therapy, event rates per patient per year for vaso-occlusive crises, blood transfusions and hospitalizations decreased by 95.0, 94.6 and 93.1%, respectively. When compared with the placebo group, patients treated with HU therapy had 94.0, 93.4 and 89.5% lesser vaso-occlusive crises, blood transfusions, and hospitalizations, respectively. Even among hospitalized patients, the duration of hospitalization was less in the HU group (3. 1 ± 1.2 days) than their counterparts in the placebo group (7.1 ± 2.1 days).

Table 18: Comparison of the Number of Clinical Events Before and After Intervention in the Hydroxyurea and Placebo Groups

	Hydroxyurea		Placebo			
Number of events/patient/year	Before	After 18 Months	Before	After 18 Months	*p* Value[a]	*p* Value[b]
Vasoocclusive crises	12.13 ± 8.56	0.60 ± 1.37	11.46 ± 3.01	10.2 ± 3.24	0.10	<0.001
Blood transfusions	2.43 ± 0.69	0.13 ± 0.43	2.13 ± 0.98	1.98 ± 0.82	0.25	<0.001
Hospitalizations	10.13 ± 6.56	0.70 ± 1.28	9.56 ± 2.91	9.59 ± 2.94		<0.001

[a] *p* Value is for comparison between the HU and placebo groups at baseline.
[b] *p* Value if for comparison between the HU and placebo groups at 18 months.

Ferster *et al* 1996

This was a placebo-controlled, randomized, crossover study. It was conducted in a single centre in Belgium and involved 25 children and young adults (age range: 2 to 22 years) with HbSS genotype and severe clinical manifestations (defined as >3 vaso-occlusive crises in the year before study entry and/or with previous history of stroke, acute chest pain, recurrent crises without a free interval, or splenic sequestration) with the primary end-point of number of hospitalizations and number of days in hospital.

Methods

Study Participants

Twenty-five children and young adults severely affected by SCA were selected to receive HU. They all originate from Central African countries (24 from Zaire and 1 from Cameroun). There were 13 girls and 12 boys. Ages ranged from 2 to 22 years, with a median of 9 years. These 25 patients were initially selected for the study on the basis of severe clinical disease. To be eligible for the study, the patients had to have homozygous SCA. Patients with sickle cell β-thalassemia were excluded, but patients with α-gene deletion were included. To enter the study, patients had to have reported more than 3 vaso-occlusive crises in the year before entry into the study and/or have a previous history of stroke, acute chest syndrome,

recurrent crises without free interval, or splenic sequestration. Transfusion therapy is clearly recommended in preventing recurrent stroke. Because many of our SCA patients develop alloimmunization, this therapy may become ineffective in them. For this reason, patients with a previous history of stroke were also eligible for the trial, but only if appropriate transfusion therapy could not be conducted (severe alloimmunization in 1 patient and refusal in another). The study was approved by both hospital ethics committees and oral informed consent was obtained from the patient and/or the parents or tutors. Patients and/or parents or tutors were made aware of the potential risk of teratogenesis and mutagenesis associated with chronic exposure to HU. Teenage and adult patients were counselled regarding contraception and pregnancy. The characteristics of the 25 patients are presented in Table19.

Table 19: Characteristics of the 25 patients in the Ferster *et al* 1996 study

Age (yr)	
Median	9
Range	2-22
No. of crises in the year before study	
<3	6
3-5	15
6-9	0
≥10	4
No. of hospitalizations in the year before study	
None	4
1-3	14
4-5	2
≥6	5
Complications of sickle cell anemia	
Chest syndrome	8
Osteomyelitis/osteonecrosis	5
Stroke	2
Splenic sequestration	3

Treatments

HU was administered at an initial dosage of 20 mg/kg every day. If no change in HbF level had occurred after 2 months (increase <2%), the doses of HU were increased up to 25 mg/kg/d. In case of bone marrow toxicity defined by a white blood cell count (WBC) less than 3×10^9/L and/or a platelet count less than 80×10^9/L, the initial dosage was reduced by 50%. All patients received 1 mg of folic acid each day. Children under 6 years of age received oral penicillin in addition. All patients were seen monthly in the outpatient clinic.

Outcomes/endpoints

The end points of interest in this study were the number of hospitalizations and the number of days in hospital. The number of days of pain was also initially considered as an endpoint of the trial. Patients or their parents were asked to fill out a card to report their painful episodes. However, during the course of the trial, it became clear that this information could not be reliably obtained from a majority of the patients, and this endpoint was dropped from all analyses.

Sample size

25 patients.

Randomisation

Not available.

Blinding (masking)

By drawing consecutive sealed envelopes, patients were randomly allocated to one of the following treatment sequences: either HU first for a period of 6 months, followed by placebo for 6 months, or placebo first, followed by HU for an additional 6 months. The study was run single-blind (the patient was unaware of the treatment received, but the physician had knowledge of the treatment). The drug or the placebo was provided monthly for each patient by the hospital pharmacy. Both were indistinguishable. The trial was run single-blind rather than double-blind because of the logistic difficulty of blinding the attending physician to the treatment received. Indeed, for the blinding to be effective, the attending physician should have been denied access to the pharmacy records and to the laboratory results in the hospital database.

Statistical methods

The paired Student's t-test was used to determine the level of significance of differences between biologic parameters before and after HU treatment. Correlations were computed using the Pearson's correlation test. The standard analysis of the 2-treatment 2-period cross-over trial was adopted. The Wilcoxon rank-sum test was used to assess the significance of the effects of treatment, period, and carry-over. Two-tailed P values less than .05 were considered to be statistically significant.

Formulations

No specific details are given on the formulation used.

Results

Participant flow

This information was not available in the literature.

Baseline data

Not available.

Numbers analysed

Twenty-five patients entered the study. Twenty-four patients fulfilled the initial inclusion criteria. One patient was included although he experienced only 2 vaso-occlusive crises during the 12 months preceding the study. Three patients were excluded after 4 to 5 months because they failed to attend the monthly evaluation visits. Because these patients could not be evaluated at the end of their first time period and during their second time period, they could not contribute to the treatment comparisons and were therefore excluded from all analyses. There were thus 22 patients who could be analysed (median age, 8 years).

Outcomes and estimation

The mean initial hemoglobin level was 8.1 g/dL (range, 6.7 to 9.3 g/dL). After 6 months of HU treatment, the mean hemoglobin level increased to 8.5 g/dL (range, 7 .2 to 10 g/dL). An increase of more than 1 g/dL was observed in 9 of 22 evaluable patients (Table 20). The difference was not significant (P = .068).

The mean initial mean corpuscular volume (MCV) was 85.2 fL (range, 63 to 98.3 fL). After 6 months of HU therapy, it increased to 95.5 fL (range, 68 to 112 fL). This change was highly significant (P < .001; Table 20). The mean corpuscular hemoglobin concentration (MCHC) did not change significantly (P = .069). The mean initial HbF value was 4.7% (range, 0.1% to 20.2%). After 6 months of HU therapy, it increased up to 15% (range, 1.1 % to 38%). This difference was highly significant (P < .001). A threefold increase of the initial HbF value was observed in 11 patients; a twofold increase was observed in 7 patients. The increase of HbF correlated significantly with the increase of MCV. In some patients, the MCV increased weeks before any increase in the HbF level.

After 6 months of HU treatment, the reticulocyte count significantly decreased from 149% ±: 54% to 103% ±: 49% (P < 0.001). The absolute neutrophil count remained within the normal range in all patients; however, it decreased significantly in patients treated with HU (Table 20). Only 2 children developed transient mild thrombocytopenia between 100×10^9/L and 140×10^9/L, which did not require a reduction of the initial HU doses. Five patients reached the final dose of 25 mg/kg/d. No other biologic side effect was observed. No patient required a dose reduction for thrombocytopenia or leukopenia.

Table 20: Mean hematologic values before and after 6 Months of treatment with HU

	Before HU Therapy (mean ± SD)	After HU Therapy (mean ± SD)	P*
Hb (g/dL)	8.1 ± 0.75	8.5 ± 0.83	NS
MCV (fL)	85.2 ± 9.74	95.5 ± 11.57	<.001
MCHC (%)	33.0 ± 2.08	32.3 ± 1.12	NS
Platelets ($\times10^9$/L)	443.2 ± 189.1	386.7 ± 144.6	NS
WBC ($\times10^9$L)	12.47 ± 4.58	8.90 ± 2.51	<.001
HbF (%)	4.65 ± 4.81	15.34 ± 11.3	<.001
Reticulocytes (‰)	148.6 ± 53.8	102.7 ± 48.5	<.001

Abbreviations: SD, standard deviation; NS, not significant.

* Paired Student's *t*-test.

No correlation was found between HbF increase and the initial HbF level, WBC count, or platelet count. In addition to the significant changes in the MCV and HbF level, most patients responded favorably clinically. In 16 patients, a complete disappearance of vaso-occlusive events requiring hospitalisation occurred within the first month of HU treatment. Among these 16 patients, 3 were severely disabled: 1 with a previous history of recurrent strokes, 1 with severe chronic bone pain, and 1 with 208 days in hospital during the year preceding entry into the trial.

No relevant change in the number of crises requiring hospitalization occurred in 6 patients. Of interest, in these 6 patients, the HbF level only slightly increased from 1.7% (range, 0.1% to 4.7%) to 3.2% (range, 1.1% to 7%). Three of them required a dose increase up to 25 mg/kg according to the initial protocol, whereas only 2 of the 16 responders needed a dose increase. Two of the three patients who were excluded from analysis remained highly symptomatic (recurrent acute chest syndrome [ACS] or/and high hospitalization rate for crises). The third patient, who had a history of previous stroke, died 2.5 years after inclusion from suspected intracranial haemorrhage.

Overall, the number of hospitalisations was reduced when patients were on HU therapy as compared with placebo. The statistical tests (Table 21) indicated a very significant effect of treatment and no significant period effect or carry-over effect from the first to the second 6-month period. Combining the results of

both periods, 16 patients of 22 (73%) did not require any hospitalization for painful episodes when treated with HU as compared with only 3 of 22 (14%) when treated with placebo.

The number of days in hospital was also significantly lower when patients were on HU therapy (range, 0 to 19 days) than when they were on placebo (range, 0 to 104 days). During the first 6-month period, the mean number of days in hospital was 5.3 days for HU, as compared with 15.2 days for placebo; during the second 6-month period, these figures were, respectively, 1.8 days for HU and 8.2 days for placebo. The treatment difference was statistically significant, but the downward trend from the first to the second period was not (Table 21).

No pregnancies occurred in the female patients.

Table 21: *P* Values of the statistical tests performed on the number of hospitalizations and the number of days in hospital

	Treatment Effect	Period Effect	Carry-Over Effect
No. of hospitalizations	*P* = .0016	*P* = .47	*P* = .80
No. of days in hospital	*P* = .0027	*P* = .15	*P* = .64

TWiTCH Study (Ware *et al* 2015)

Hydroxycarbamide was shown to be inferior to transfusion in the Stroke with Transfusions Changing to Hydroxycarbamide (SWiTCH) trial based on a composite end point (Ware *et al* 2012). More recently though, the TCD with Transfusions Changing to Hydroxycarbamide (TWiTCH) clinical trial was terminated early in agreement with the recommendations of the Data and Safety Monitoring Board (DSMB). TWiTCH (NCT01425307) was a NHLBI-funded Phase III multicentre randomized clinical trial comparing 24-months of standard treatment (transfusions) to alternative treatment (hydroxycarbamide) in children with SCA and abnormal TCD velocities. All eligible children had received at least 12 months of transfusions and did not have severe vasculopathy. TWiTCH had a non-inferiority trial design; the primary study endpoint was the 24-month TCD velocity obtained from a linear mixed model, controlling for baseline (enrolment) values, with a non-inferiority margin of 15 cm/s.

Methods

Study Participants

Patients from 26 paediatric hospitals and health centres in the USA and Canada who were aged 4–16 years and had sickle cell anaemia and abnormal TCD velocities (≥200 cm/s) were eligible to participate if they had received at least 12 months of chronic transfusions. Local investigators had discretion with respect to enrolment within their institution. All patients who met the inclusion and exclusion criteria were eligible. Documented clinical stroke, transient ischaemic attack, or severe vasculopathy were exclusion criteria. After local Institutional Review Board approval and written informed consent had been obtained from patients' parents or guardians (with patient assent where required by local Institutional Review Boards), eligible participants were screened for original abnormal TCD verification and baseline brain magnetic resonance imaging (MRI) and angiography, TCD examination with non-imaging instruments that were identical at every centre (SonaraTek, Middleton, WI, USA), liver iron concentration (LIC) assessed by FerriScan R2-MRI (Resonance Health, Claremont, WA, Australia), abdominal ultra-sonography and MRI, neurocognitive testing, and quality of life assessments. Participants with baseline grade 4 or higher severe brain MRA vasculopathy, defined as moderate stenosis in more than two arterial segments or severe stenosis or occlusion in at least one segment, or with inadequate TCD

velocities from poor blood flow or bone windows were deemed screening failures and removed from the study. Children with normal, conditional, or persistent abnormal screening TCD velocities while receiving chronic transfusions remained eligible for randomisation.

Between Sept 20, 2011, and April 17, 2013, 159 patients consented and enrolled in TWiTCH, including 38 who were ineligible for treatment: 19 had severe vasculopathy, ten withdrew from the study, seven failed screening TCD, three failed original TCD verification, and one had an inadequate brain MRA (two participants failed for two reasons). Therefore, 121 participants were randomly assigned to treatment, forming the intention-to treat population. 61 patients were assigned to receive standard transfusions and 60 patients were assigned to hydroxycarbamide treatment.

Treatments

Participants assigned to receive standard treatment continued to receive transfusions once per month to maintain HbS at 30% or lower, with local discretion with respect to transfusion type (simple, partial exchange, or erythrocytapheresis). For these children, deferasirox was recommended to manage iron overload; children already receiving chelation therapy maintained their current dose, whereas those starting chelation therapy received deferasirox tablets at 10–40 mg/kg per day, with the dose dependent on LIC at screening. Participants assigned to receive oral hydroxycarbamide initiated treatment at 20 mg/kg per day (capsules or liquid formulation) with escalation to maximum tolerated dose (MTD), which we defined as the dose at which moderate marrow suppression of neutrophils and reticulocytes was achieved, as previously reported. Transfusions were slowly weaned in accordance with a standard protocol over 4–9 months to protect against stroke during hydroxycarbamide dose escalation. After MTD had been established and transfusions were discontinued, patients receiving hydroxycarbamide underwent serial phlebotomy to manage iron overload. Every 4 weeks until the end of the 24 month treatment period, 10 mL/kg (maximum 500 mL) venous blood was removed during 30–60 min in accordance with a standardised protocol. Smaller phlebotomy volumes (5 mL/kg) were removed if patients had haemoglobin concentrations of 80–85 g/L, and phlebotomy was not done if haemoglobin concentration was less than 80 g/L. The treatment period was 24 months after randomisation, with a 6 month visit after completing exit studies.

TCD velocities were measured every 4 weeks in triplicate during screening, at 12 week intervals during the 24 month treatment period, and again in triplicate at the end of the 24 month treatment period. TCD examinations were done just before transfusions or phlebotomy, and all were read centrally by observers masked to treatment assignment and previous TCD results. Additional assessments included brain MRI and MRA at study entry and exit; liver MRI for iron burden at study entry, midpoint, and exit; neurocognitive testing at study entry and exit; and quality of life at study entry, midpoint, and exit.

All new potential stroke events were assessed with careful neurological evaluation and brain MRI/MRA examinations, then adjudicated centrally by a panel of expert reviewers. Independent and then consensus opinions were obtained from neurologists and neuroradiologists masked to study treatment. Participants with possible or likely stroke based on new neurological signs or symptoms, but without corresponding radio logical changes, were scored as transient ischaemic attack. Brain MRI/MRA examinations at study exit allowed us to confirm that no strokes had been missed by the adjudication process.

Protocol-defined rescue transfusions were given to participants in either treatment group if they were identified as having perceived increased stroke risk: reasons included failure to suppress HbS percentage in the standard group, and excessive toxic effects or failure to achieve marrow suppression targets in the alternative group. A protocol-defined alert algorithm was used to identify participants whose TCD velocities varied substantially from baseline and might confer increased stroke risk, and these children received additional assessments and closer therapeutic monitoring during the course of the study.

Objectives

TWiTCH was a multicentre, open-label, phase 3, non-inferiority trial, in which we compared alternative treatment (hydroxycarbamide) with standard treatment (transfusions) for the maintenance of TCD velocities as a surrogate for stroke risk. TCD velocities were chose for investigation on the basis of evidence showing that high velocities confer an increased stroke risk.

Outcomes/endpoints

The primary study endpoint was the maximum TCD time-averaged mean velocity on the index side, which was defined as the cerebral hemisphere with the higher mean arterial velocity at baseline assessment. Secondary endpoints were TCD velocity on the non-index side, new stroke or non-stroke neurological events, new brain MRI/MRA lesions, hepatic iron overload, sickle-related events, neuropsychological status, quality of life, growth, and treatment-related complications. Secondary endpoints such as neurocognitive status, quality of life, and growth and development will be addressed in future manuscripts. AEs were assessed in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and analysed according to the Medical Dictionary for Regulatory Activities (MedDRA) version 18.0.

Blinding (masking)

Treatment was open-label, but the principal investigator was masked to all treatment-related results, and local investigators were masked to TCD results.

Statistical methods

The primary endpoint was the 24 month TCD velocity, which was calculated from a general linear mixed model using all TCD velocities captured throughout the trial. This endpoint was calculated based on the intention-to-treat population, with two planned interim analyses after 33% and 67% of participants had completed exit studies. The stopping guidelines for non-inferiority used the Lan-DeMets version of the O'Brien-Fleming group sequential method. A one-sided non-inferiority margin of 15 cm/s was used in the analysis, which represents the biological variation of TCD examinations. It was estimated that a sample size of 148 children would be needed, assuming that 20% dropped out during screening, yielding 118 participants assigned to treatment. Assuming another 15% dropped out after treatment allocation, 100 participants (50 per group) would complete the 24 month treatment period. This study design provided at least 90% power to test the non-inferiority hypothesis, assuming a difference of 5 cm/s (alternative higher than standard) and a standard deviation of 24 cm/s. Comparisons of continuous variables with t tests and comparisons of categorical variables with χ^2 analyses were done. Analyses in the intention-to-treat population were performed, except for a planned per-protocol analysis of TCD velocities, which excluded participants who exited the study early. Also, a sensitivity analysis that adjusted for baseline age was done. All analyses were performed with Stata 14.0 or SAS 9.4.

Formulations

Capsules (Droxia and Hydrea) or liquid formulation (20 mg/kg per day).

Recruitment

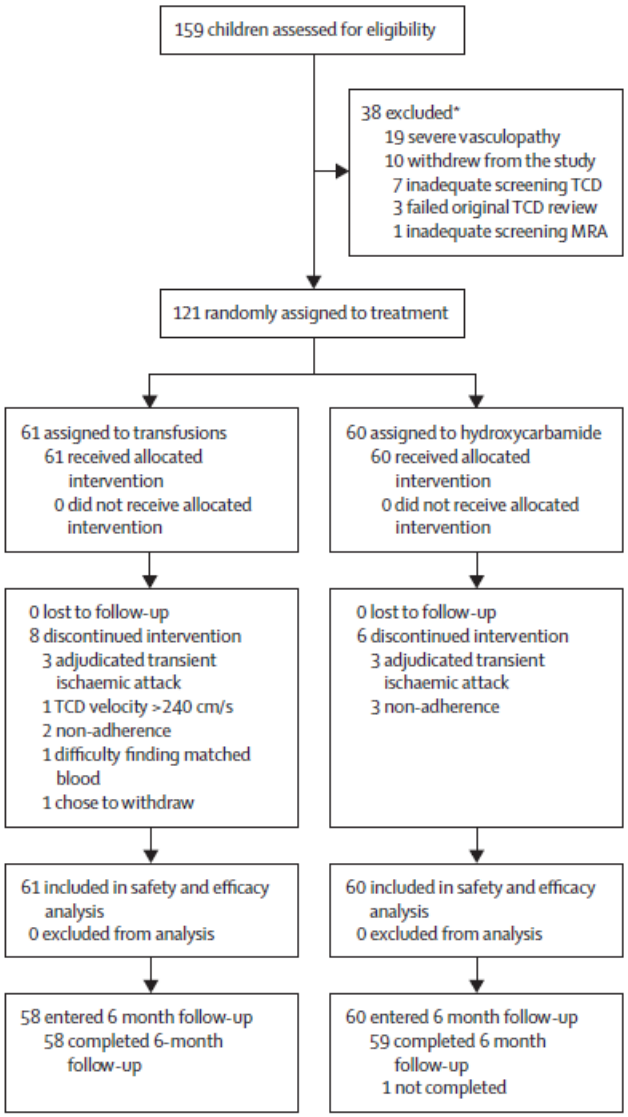
Between Sept 20, 2011, and April 17, 2013, 121 participants were randomly assigned to treatment. The Ware et al paper does not provide the date of the follow-up period, but mentions that it lasted 6 months.

Conduct of the study

The NHLBI–appointed Data and Safety Monitoring Board periodically reviewed all enrolment, safety, toxicity, and efficacy data, including all new stroke events and interim analyses. The SWiTCH principal investigator was masked to all treatment-specific results, including laboratory tests and clinical events.

Results

Participant flow



Baseline data

Most baseline demo graphic, clinical, and laboratory characteristics were similar between treatment groups, except for higher white blood cell and absolute neutrophil counts and higher bilirubin concentrations in the alternative group (Table 22). Despite the exclusion criterion of clinically overt stroke, more than a third of the participants in both treatment groups had evidence of previous silent cerebral infarctions.

Table 22: Enrolment characteristics of intention-to-treat population

	Standard (n=61)	Alternative (n=60)
HbSS genotype	59 (97%)	60 (100%)
Male	19 (31%)	29 (48%)
Age at study enrolment (years)	9·5 (2·6)	9·7 (3·2)
TCD history		
Age at index abnormal TCD (years)	5·7 (2·0)	5·0 (1·8)
Average index TCD value (cm/s)	226 (25)	220 (17)
Average entry TCD value (cm/s)	145 (21)	145 (26)
Brain MRI/MRA		
Silent cerebral infarction	25 (41%)	22 (37%)
Mild to moderate vasculopathy*	6 (10%)	4 (7%)
Transfusion history		
Duration (years)	3·8 (1·8)	4·5 (2·8)
Simple transfusions	36 (59%)	39 (65%)
Red blood cell alloantibodies	9 (15%)	11 (18%)
Red blood cell autoantibodies	12 (20%)	10 (17%)
Iron overload status		
Liver iron (mg Fe per g dry weight liver)	8·5 (7·5)	11·3 (9·5)
Serum ferritin (ng/mL)	2713 (2207)	3080 (2347)
Current chelation usage	51 (84%)	48 (80%)
Laboratory parameters		
Haemoglobin (g/L)	93 (8)	92 (8)
Mean corpuscular volume (fL)	86 (4)	86 (4)
Haemoglobin A (%)	67·8 (11·4)	67·4 (10·4)
Sickle haemoglobin (%)	26·5 (10·3)	27·6 (9·9)
Fetal haemoglobin (%)†	10·3 (6·5)	8·8 (5·5)
Absolute reticulocyte count (×10^9 cells per L)	319 (124)	358 (144)
White blood cells (×10^9 cells per L)	12·2 (4·4)	13·9 (3·5)
Absolute neutrophil count (×10^9 cells per L)	6·9 (2·7)	8·0 (2·8)
Alanine aminotransferase (U/L)	46 (34)	53 (52)
Creatinine (mg/L)	4·6 (2·1)	4·4 (1·6)
Total bilirubin (mg/L)	23·9 (10·5)	29·4 (17·9)

Data are mean (SD) or n (%) unless indicated otherwise. TCD=transcranial doppler. MRA=magnetic resonance angiography. *Grades 1–3 as described previously.[13] †Because of the large amount of HbA present, HbF was calculated as (HbF) / (HbF + HbS) as described previously.[14]

Numbers analysed

121 participants were randomly assigned to treatment, forming the intention to treat population. 61 patients were assigned to receive standard transfusions and 60 patients were assigned to hydroxycarbamide treatment.

Outcomes and estimation

Participants assigned to standard treatment mainly received simple transfusions (57% of procedures), with some partial exchange transfusions (31% of procedures), or automated erythrocytapheresis (12% of procedures). In patients receiving standard transfusions, haemoglobin concentration remained steady at about 90 g/L with average HbS remaining less than 30% throughout the study treatment period (Figure 7). Chelation was provided to children receiving transfusions who had hepatic iron overload, usually deferasirox starting at an average dose of 25.7 mg/kg per day (SD 6.0). In the alternative group, all 60 participants started at 20 mg/kg per day followed by dose escalation. MTD was reached in 57 (95%)

of 60 children, who then discontinued transfusions as per protocol; two patients did not reach MTD because of medication non-adherence leading to study withdrawal and one patient had an early adjudicated transient ischaemic attack during the overlap period while on both hydroxycarbamide and transfusions. The median time to MTD was 27 weeks (IQR 24–32); medianhydroxycarbamide MTD was 27.4 mg/kg/day (IQR 24.0–30.1). In children receiving hydroxycarbamide, haemoglobin concentrations remained stable at about 90 g/L, and we saw the expected substantial haematological changes in MCV, percentage of HbF, white blood cell count, neutrophils, platelets, and reticulocytes. Marrow suppression targets were achieved, with median absolute neutrophil count at MTD of 3.4×10^{9} cells per L (IQR 2.4–4.1) and final average neutrophil count of 3.6×10^{9} cells per L (Table 23). HbF responses included a median MTD value of 26.2% (IQR 22.4–31.2) and final average HbF of 24.4%.

Baseline TCD velocities were similar in the two treatment groups, but the final average velocity of the alternative group was slightly lower than that of the standard group (Figure 8). After full enrolment and when 37% of the participants had exited the study, the first scheduled interim analysis showed that the stopping boundary had been passed and non-inferiority was shown. After 50% of participants had exited, repeat analyses supported these findings and the study was terminated by NHLBI. Remaining participants then completed all exit studies before discontinuing protocol-directed study treatment. In total, the standard group included 42 participants who completed study treatment, 11 who had truncated treatment, and eight who exited early; the alternative group included 41 participants who completed treatment, 13 who had truncated treatment, and six who exited early. The final model-based TCD velocity in participants who received transfusions was 143 cm/s (95% CI 140–146), whereas this was 138 cm/s (135–142) in those who received hydroxycarbamide, with a difference of 4·54 (0.10–8.98). Non-inferiority ($p=8.82\times10^{-16}$) and post-hoc superiority (p=0.023) were met. The planned per-protocol analysis that excluded study participants who exited the study early showed almost identical findings, with a difference of 5·06 (0.56–9.57), non-inferiority p value of 1.05×10^{-15}, and a post-hoc superiority p value of 0.015. An age adjusted sensitivity analysis gave almost identical findings (not shown). TCD velocities for all participants were mostly in the normal range at both study entry and exit (Figure 8). No child in either treatment group reverted from normal to abnormal TCD velocities. Table 23 includes additional laboratory values at study exit.

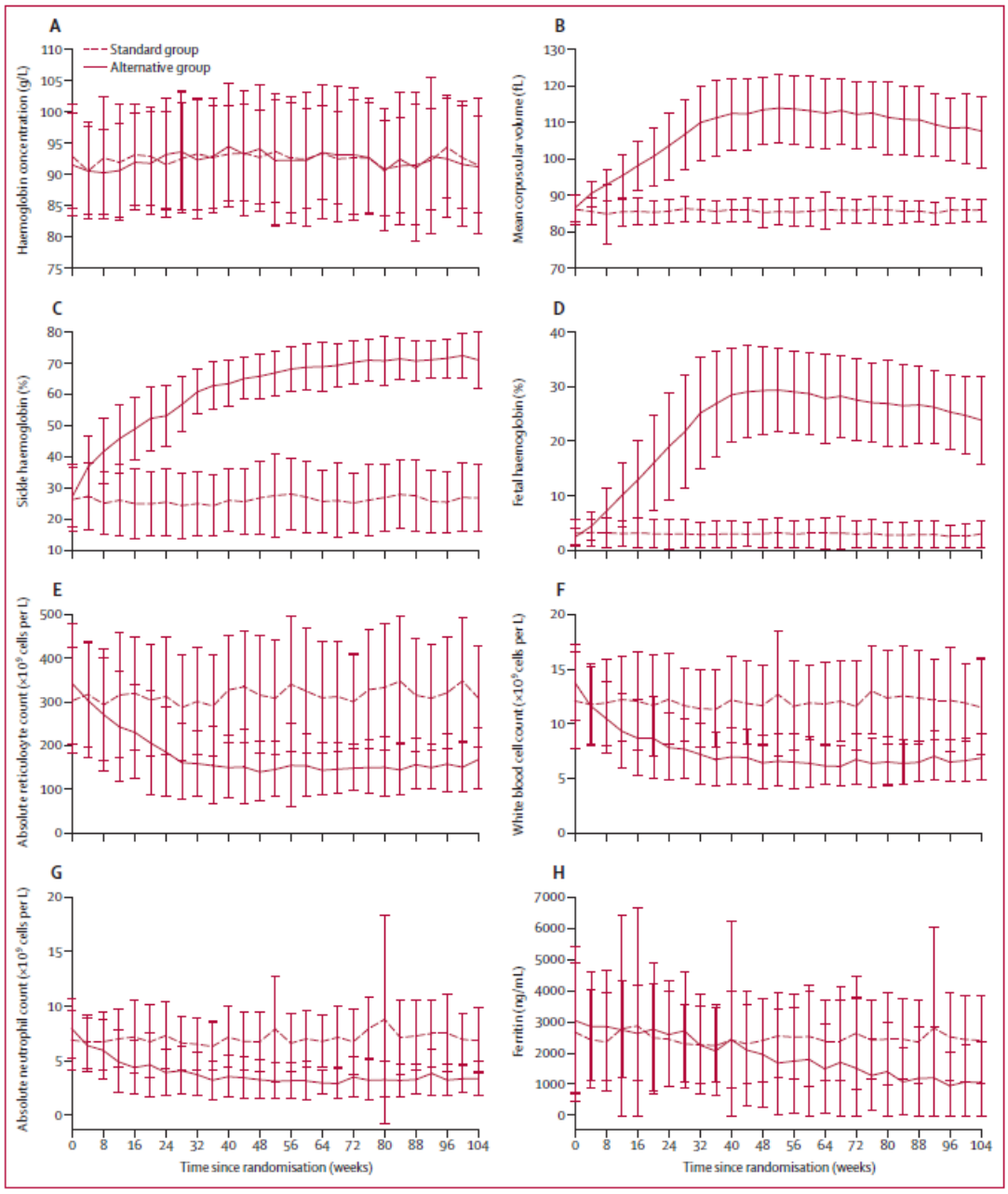


Figure 7: Laboratory parameters of the intention-to-treat population

Results are shown for haemoglobin concentration (A); MCV (B); sickle haemoglobin (C); fetal haemoglobin (D); white blood cell count (E); ANC (F); ARC (G); and serum ferritin (H). Complete blood counts and reticulocytes were measured locally, and haemoglobin electrophoresis and ferritin were analysed centrally. Data are shown as mean plus or minus 1 standard deviation. All parameters are significantly different at exit ($p<0.0001$) between treatment groups except haemoglobin concentration ($p=0.800$).

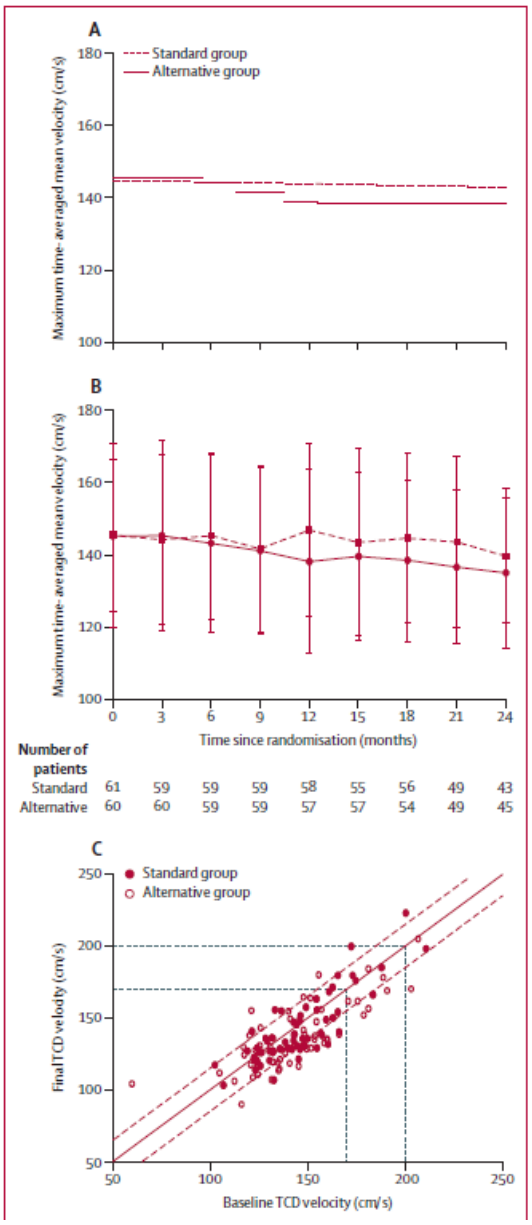


Figure 8: Primary endpoint analysis of TCD velocities

TCD data are shown by use of mixed model statistical analysis (A); the curves are significantly different using the non-inferiority comparison ($p=8.82\times10^{-16}$) and also by post-hoc analysis for superiority ($p=0.023$). Actual TCD velocity data in each group are shown as mean plus or minus 1 standard deviation (B), including the number of participants assessed at each timepoint. Baseline (enrolment) and final (exit) maximum time-averaged mean TCD velocities for each participant (C); the lines at 170 cm/s and 200 cm/s denote the normal and abnormal TCD boundaries, respectively.

Table 23: Laboratory parameters of intention-to-treat population at final assessment

	Final assessment			Change from baseline		
	Standard (n=61)	Alternative (n=60)	p value*	Standard (n=61)	Alternative (n=60)	p value†
Haemoglobin (g/L)	92 (7)	91 (11)	0·800	−1 (10)	−2 (12)	0·828
Mean corpuscular volume (fL)	86 (3)	107 (10)	<0·0001	0 (4)	21 (10)	<0·0001
Fetal haemoglobin (%)	10·3 (7·3)	24·4 (7·9)	<0·0001	−0·1 (5·0)	15·5 (7·1)	<0·0001
Sickle haemoglobin (%)	27·6 (10·1)	70·7 (9·6)	<0·0001	1·2 (10·7)	43·0 (15·5)	<0·0001
Absolute reticulocyte count (x10⁹ cells per L)	329 (112)	181 (86)	<0·0001	9 (158)	−169 (157)	<0·0001
White blood cells (x10⁹ cells per L)	11·8 (4·0)	7·2 (2·2)	<0·0001	−0·4 (2·9)	−6·6 (3·9)	<0·0001
Absolute neutrophil count (x10⁹ cells per L)	6·9 (2·6)	3·6 (1·6)	<0·0001	0·0 (2·6)	−4·4 (3·2)	<0·0001
Platelets (x10⁹ cells per L)	378 (112)	333 (133)	0·050	−3 (86)	−73 (135)	0·001
Total bilirubin (mg/L)	27·2 (14·3)	16·1 (12·7)	<0·0001	3·3 (9·0)	−13·3 (12·7)	<0·0001
Liver iron concentration (mg Fe per g dry weight liver)‡	11·3 (10·1)	9·5 (8·7)	0·323	2·4 (8·7)	−1·9 (4·2)	0·0011
Serum ferritin (ng/mL)	2674 (1717)	1276 (1228)	<0·0001	−38 (2095)	−1805 (1651)	<0·0001
Lactate dehydrogenase (U/L)	547 (191)	475 (196)	0·044	−6 (215)	−140 (231)	0·001

Data are mean (SD) unless indicated otherwise. Values were recorded at final assessment, which was after completing study treatment for 83 participants, after truncated treatment for 24 participants, and at early termination for other reasons for 14 participants. Treatment group differences for laboratory parameters were assessed with t tests. *p values based on final assessment. †p values based on change from baseline to final assessment. ‡Values for liver iron concentration represent 52 participants in the transfusion group and 58 in the hydroxycarbamide group.

Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 24: Summary of efficacy for MSH trial

Title: MSH		
Study identifier	Not available	
Design	Randomized, double-blind, placebo-controlled trial in 21 study clinics in the United States and Canada involving 299 adults with SCA who had experienced three or more VOCs in the previous year.	
	Duration of main phase:	January 28, 1992 - June 30, 1994
Hypothesis	Hydroxyurea could substantially reduce the frequency of painful crises (often called vaso-occlusive crises) in adults with sickle cell anemia.	
Treatments groups	Hydroxyurea	Patients received an initial dose of 15 mg per kilogram of body weight per day, and the dose was increased by 5 mg per kilogram per day every 12 weeks, unless marrow depression was present. Duration: 104 weeks 152 patients
	Placebo	The dose of placebo was adjusted in a similar manner in order to maintain blinding. Duration: 104 weeks 147 patients

Endpoints and definitions	Primary endpoint	Frequency of painful crises (often called vaso-occlusive crises), time to the first and second vaso-occlusive crisis, number of patients in whom chest syndrome developed	A painful crisis was defined as a visit to a medical facility that lasted more than four hours for acute sickling-related pain, which was treated with a parenterally administered narcotic. The occurrence of chest syndrome (chest-wall pain in association with findings of a new pulmonary infiltrate on chest x-ray films and fever) was considered a crisis.

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	Hydroxyurea	Placebo	
	Number of subject	152	147	
	Crises per year (median)	2.5	4.5	
	Crises per year severe enough to cause hospitalization (median)	1.0	2.4	
	Months to first vaso-occlusive crisis	3.0	1.5	
	Months to second vaso-occlusive crisis	8.8	4.6	
	Number of patients in whom chest syndrome developed	25	51	

Effect estimate per comparison	Primary endpoint	Comparison groups	Hydroxyurea vs placebo
		Crises per year difference	44%
		P-value	P<0.001
	Primary endpoint	Comparison groups	Hydroxyurea vs placebo
		Crises per year severe enough to cause hospitalization difference	58%
		P-value	P<0.001
	Primary endpoint	Comparison groups	Hydroxyurea vs placebo
		Months to first vaso-occlusive crisis	3.0 vs 1.5
		P-value	P=0.01

	Primary endpoint	Comparison groups	Hydroxyurea vs placebo
		Months to second vaso-occlusive crisis	8.8 vs 4.6
		P-value	P<0.001
	Primary endpoint	Comparison groups	Hydroxyurea vs placebo
		Number of patients in whom chest syndrome developed	25 vs 51
		P-value	P<0.001

Although no secondary endpoints are provided in the Charache *et al* paper, time to the first and second vaso-occlusive crisis, number of patients in whom chest syndrome developed, number of patients who received transfusions, and number of units of blood transfused appear on the Results section of the paper. The first two are considered as primary endpoints by the assessor. Elongation of the time between the first and second crisis can be considered as a description of reduced frequency. ACS is considered in the paper to be a crisis. Therefore, these two results were added to Table 24. The other two parameters are not added, since they are not connected with the primary endpoint nor are they secondary endpoints.

Table 25: Summary of efficacy for Jain *et al* 2012trial

Title: Jain et al 2012			
Study identifier	Not available		
Design	Randomised, double blind, placebo controlled study conducted in a tertiary hospital in India. The study was conducted in 60 children with sickle cell anaemia (proportion with each genotype not stated) between the ages of 5 and 18 years with three or more blood transfusions or vaso-occlusive crises requiring hospitalization per year despite high HbF.		
	Duration of main phase:	18 months	
Hypothesis	Hydroxyurea could decrease the frequency of vaso-occlusive crises per patient per year and the frequency of blood transfusions and hospitalizations and increase HbF levels.		
Treatments groups	Hydroxyurea		Patients received HU at a fixed dose of 10 mg/kg/day orally as a single dose. Duration: 18 months 30 patients
	Placebo		Patients in the placebo group received powdered glucose capsules having the same appearance as the HU capsules. Duration: 18 months 30 patients
Endpoints and definitions	Primary endpoint	Frequency of vaso-occlusive crises per patient per year	

	Secondary endpoints	Frequency of blood transfusions, frequency of hospitalizations, HbF levels.	

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	Hydroxyurea	Placebo	P-value
	Number of subject	30	30	-
	Frequency of vaso-occlusive crises per patient per year	0.60 ± 1.37	10.2 ± 3.24	P<0.001
	Frequency of blood transfusions	0.13 ± 0.13	1.98 ± 0.82	P<0.001
	Frequency of hospitalizations	0.70 ± 1.28	9.59 ± 2.94	P<0.001
	HbF levels (%)	24.00 ± 5.90	18.92 ± 5.77	P<0.001

Table 26: Summary of efficacy for Ferster *et al* 1996 trial

Title: Ferster et al 1996		
Study identifier	Not available	
Design	Placebo-controlled, randomized, crossover study. It was conducted in a single centre in Belgium and involved 25 children and young adults (age range: 2 to 22 years) with HbSS genotype and severe clinical manifestations (defined as >3 vaso-occlusive crises in the year before study entry and/or with previous history of stroke, acute chest pain, recurrent crises without a free interval, or splenic sequestration) with the primary end-point of number of hospitalizations and number of days in hospital.	
	Duration of main phase:	12 months
Hypothesis	Hydroxyurea could reduce the number of hospitalisations and the number of days in hospital.	

Treatments groups	Hydroxyurea		HU was administered at an initial dosage of 20 mg/kg every day. If no change in HbF level had occurred after 2 months (increase <2%), the doses of HU were increased up to 25 mg/kg/d. In case of bone marrow toxicity defined by a white blood cell count (WBC) less than 3×10^9/L and/or a platelet count less than 80×10^9/L, the initial dosage was reduced by 50%. All patients received 1 mg of folic acid each day. Children under 6 years of age received oral penicillin in addition. Duration: 6 months 22 patients
	Placebo		The dose of placebo was adjusted in a similar manner in order to maintain blinding. Duration: 6 months 22 patients
Endpoints and definitions	Primary endpoint	Number of hospitalizations, number of days in hospital	

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	Hydroxyurea	Placebo	P-value
	Number of subject	22	22	
	Number of hospitalizations			0.0016
	Number of days in hospital	mean: 5.3 (first 6-month period), 1.8 (second 6-month period) range: 0-19	mean: 15.2 (first 6-month period), 8.2 (second 6-month period) range: 0-104	0.0027

Table 27: Summary of efficacy for TWiTCH trial

Title: TWiTCH			
Study identifier	NCT01425307		
Design	TWiTCH was a multicentre, open-label, phase 3, non-inferiority trial, in which we compared alternative treatment (hydroxycarbamide) with standard treatment (transfusions) for the maintenance of TCD velocities as a surrogate for stroke risk.		
	Duration of main phase:		Sept 20, 2011- April 17, 2013
	Duration of Run-in phase:		not applicable
	Duration of Extension phase:		not applicable
Hypothesis	Non-inferiority		
Treatments groups	Standard transfusions		Participants assigned to receive standard treatment continued to receive transfusions once per month to maintain HbS at 30% or lower. For these children, deferasirox was recommended to manage iron overload. Duration: The treatment period was 24 months after randomisation, with a 6 month visit after completing exit studies. 61 patients
	Hydroxycarbamide		Participants assigned to receive oral hydroxycarbamide initiated treatment at 20 mg/kg per day (capsules or liquid formulation) with escalation to maximum tolerated dose (MTD), which we defined as the dose at which moderate marrow suppression of neutrophils and reticulocytes was achieved, as previously reported. Transfusions were slowly weaned in accordance with a standard protocol over 4–9 months to protect against stroke during hydroxycarbamide dose escalation. After MTD had been established and transfusions were discontinued, patients receiving hydroxycarbamide underwent serial phlebotomy to manage iron overload. Duration: The treatment period was 24 months after randomisation, with a 6 month visit after completing exit studies. 60 patients
Endpoints and definitions	Primary endpoint	Maximum TCD time-averaged mean velocity on the index side	Defined as the cerebral hemisphere with the higher mean arterial velocity at baseline assessment.

	Secondary endpoints	TCD velocity on the non-index side, new stroke or non-stroke neurological events, new brain MRI/MRA lesions, hepatic iron overload, sickle-related events, neuropsychological status, quality of life, growth, and treatment-related complications.	Neurocognitive status, quality of life, and growth and development will be addressed in future manuscripts.

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat			
Descriptive statistics and estimate variability	Treatment group	Standard transfusions	Hydroxycarbamide	
	Number of subject	61	60	
	Final model-based TCD velocity	143 cm/s	138 cm/s	
	95% CI	140–146 cm/s	135-142 cm/s	
	LIC (average)	8.5 mg Fe per g dry weight liver at baseline, 11.3 mg Fe per g dry weight liver at study exit (p=0.052)	11.3 mg Fe per g dry weight liver to 9.5 mg Fe per g dry weight liver (p=0.001)	

Effect estimate per comparison	Primary endpoint	Comparison groups	Standard transfusions vs hydroxycarbamide
		Final model-based TCD velocity difference	4.54 cm/s
		95% CI	0.10–8.98 cm/s
		P-value	P=8.82×10^{-16} (Non-inferiority) P=0.023 (post-hoc superiority)
	Secondary endpoint	Comparison groups	Standard transfusions vs hydroxycarbamide
		LIC difference	More in hydroxycarbamide group, –4.3 mg Fe per g dry weight liver
		95% CI	–6.1 to –2.5 mg Fe per g dry weight liver
		P-value	P=0.001

Supportive studies

The Applicant has submitted 19 supportive studies that were conducted with adults and children. Table 28 presents a summary of these studies.

Table 28: Supporting Efficacy Studies of Hydroxycarbamide in Children and Adults

Authors/location	Patient population / study design	Genotype / inclusion criteria	N	Follow-up period Mean/median (range)	Formulation	Clinical outcomes					
						VOC	ACS	Transfusion	Stroke	Hospital visits	Comments
Wang et al, 2011 (BABY HUG), US	Infants (< 2 years) RCT, double blind	HbSS, HbSβ⁰ thal	193 (HC:96)	24 months	Oral liquid (compounded)	↓, p<0.002	↓, p<0.02	↓, p=0.03	No change, p=0.31	↓, p=0.05	↓ dactylitis (p<0.001) No difference in splenic or renal function (p>0.2)
Ware et al, 2012 (SWiTCH), US	Children (previous stroke, and ≥18 months of transfusions with documented iron overload, RCT	HbSS	133 (67: HC/Phlebotomy and 66:transfusion and chelation)	30 months	NR	↑, p<0.005	↑, p<0.05	Not applicable	7/67 strokes in hydroxycarbamide/phlebotomy group compared with 0/66 in transfusions/chelation group	NR	Rate difference within the non inferiority margin but liver iron content values were not superior on hydroxycarbamide/phlebotomy so the study was stopped
Hankins et al, 2015 (SCATE), US, Jamaica, Brazil	Children (2-7 – 9.8 years) RCT The aim of the study was to prevent conversion from conditional to abnormal time averaged mean velocity (TAMV) and subsequent stroke.	HbSS, HbSβ⁰ thal, HbSD	38 enrolled, 22 randomised (11:HC, 11: observation)	30 months, but the study was terminated after 15 months follow up due to slow accrual and the unlikelihood of meeting the study's enrolment target (100) and primary endpoint.	Hydrea/Droxia, oral liquid (compounded)				1 participant in the HC group and 5 participants in the observation group converted (not statistically significant) but there was a significantly lower TAMV in the HC group compared to the observation group, mean difference -25.70 (95% CI -45.38 to -6.02) (P = 0.01) . No strokes occurred during the study.		Significant increase in HbF and a significant decrease in ANC at last follow-up in the HC group compared to observation. Also statistically significant increase in Hb and MCV in the HC group compared to the observation group. Other values were not significant (WBC, ANC, Platelets, ARC, HbF, weight and height)
Ali *et al*, 2011/Jamaica	Children/retrospective	42 SCA, 1 HBSC; neuroimaging documentation of stroke of dx of clinical stroke	43 (HC:10)	HC: 4.6 y; no HC: 4.2y	NR	NR	NR	NR	HC: 2/100 pt yrs; no HC or transfusion: 29/100 pt yrs	NR	Chronic transfusion program was not available
Gilmore *et al*, 2011/UK	Children &adults/retrospective	61 SCA, 1 HbSD; majority-recurrent VOC; VOC with ACS	62 (HC:62)	3 y (1-9)	NR	↓, p=0.02 at 2yr	↓, p=0.007 at 7 yr	↓, p=0.002 at 7y	NR	↓ Inpatient annual days, p<0.001 at 7y	Registry data
Italia *et al*, 2009/India	Children and adults/prospective	54 HbSS, 23 HbSβthal; Arab-Indian haplotype; 5+ VOC/y, + CVA in life, >2 ACS/life, or (AVN of femur + any of the above	77 (HC:77)	2y	Capsules (Cytodrox, Cipla Ltd, Mumbai)	↓	↓	↓	↓	↓	High baseline HbF level; reduction in clinical events score stratified according to initial severity (p<0.0001)
Lobo *et al*, 2013/ Brazil	Children/retrospective	SCD; indications for HC: recurrent VOC; ,1 ACS; Hb ,6 g/dL, CVA	1760 (HC: 267 [SCA: 243; HbSB1thal: 10; HbSC: 10; HbSD: 4])	7 y (3-17) HC: 2 y (0.1-6.5)	Capsules Oral liquid (compounded) in those unable to swallow capsule	NR	HC:1 no HC: 17	NR	HC: 0; no HC: 4	↓50% P<0..001 ED visits ↓ 35% P<0.001	↓mortality; 1 in HC group; 37 in no-HC group
Nzouakou *et al*, 2011/France	Adults and adolescents/retrospective	SCA; ≥ VOC hospitalizations/y; recurrent ACS; recurrent	123 (HC: 123)	4.9 y (0.44-13.5)	NR	↓, p<0.0001	NR	15/31 pts stopped transfusion	NR	↓ (data on 64 pts); p<0.0001)	

		priapism; switch from txn program									
Yates *et al*, 2013	Children,retrospective	HbSC	15 (HC)	9.6 years (range: 3.8–14.9)	NR	↓, 0.91 events /yr p<0.01	↓, 0.23 events /yr p<0.004	NR	NR	NR	Significant increase in MCV and HbF
Rigano *et al*, 2013	Adults/retrospective	HbSβ⁰ thal:34; HbS β⁺:67; 2-3 VOC previous y; hx of ACS	104 (HC:104)	11y	NR	↓, p<0.001	NR	NR	6.7% overt stroke	↓, p<0.0001	30% with new/progressed silent cerebral infarction
Sharef *et al*, 2013/ Oman	Children/retrospective, prospective	SCD (not specified);≥3 VOC hospitalisations/yr or 1 episode of ACS	142 (HC:142)	4 y (1.5-10)	Capsule Syrup (compounded) in those unable to swallow capsule	NR	↓	NR	NR	↓, p<0.0001	
Steinberg *et al*, 2010 (MSH)/North America	Adults/prospective	SCA; MSH RCT cohort; analysis by HC use, not original trial assignment	129/299 of original cohort deceased	Up to 17 yrs 7 months	Hydrea	NR	NR	NR	NS	NR	HC at least 5 y compared with <5y had ↓ mortality
Voskaridou *et al*, 2010 (LaSHS)/Greece	Adults/prospective	HbSS: 34, HbSβ⁰ thal: 131, HbSβ⁺ thal: 165; ≥ 3 VOC/previous yr; jaundice; or complications (CVA or ACS in past 5 y)	330 (HC: 131)	HC: 8y (0.1-17) No HC: 5y (0.1 – 18)	NR	↓; p<0.001	↓, p=0.016	↓, p<0.001	HC: 5, no HC: 10	↓, p<0.001	10 yr survival: 86% for HC; 65% for non-HC, p<0.001
Lê *et al*, 2015/Belgium	Adults and children/retrospective and prospective	HbSS + HbSβ⁰: 423 HBSC: 36 HbSβ⁺: 7	HC:185 CT:24 HSCT: 90 No DMT: 170	13.7 (2.1-44) 12.2 (2.6-27) 16.9 (5.1-38) 7.6 (1.0-53) (median age at follow up)	NR	NR	NR	NR	NR	NR	HC showed significant benefit on pts outcome with lower mortality rate and higher 15 yr survival rates compared to HSCT or no DMT: (0.14,0.36 and 0.38 per 100 pt yrs) and (99.4%, 93.8% 95.4%) respectively.
Lebensburger et al 2015	Children/retrospective Age 9.4±4.5 years	HbSC (23) HbSβ⁺ (7)	30	≥2 years	NR	NR	NR	NR	NR	↓ (1.6 vs 0.4 hospitalizations/year) P<0.001	
Luchtman-Jones et al 2016	Children, retrospective	HbSC	HC: 133 pts	12 months	NR	NR	NR	NR	NR	38% reduction in clinic, ED, and hospital-based pain events after 12 months of HC therapy.	
Quarmyne *et al* 2017	Children and adolescents	HbSS + HbSβ⁰: Exclusion: concurrent transfusion, BMT or had on HC in the last 3 years	134 (Median age 7.5 yers)	2 years (78% of study population)	NR	NR	NR	NR	NR	↓: Rate ratio 0.42 (0-7 yrs) and 0.67 (>7 yrs) p<0.0001	2 year rates of hospitalisation and inpatient days were half the rate prior to initiation of HC
Rigano *et al* 2017 / Italy	Children and adults / retrospective Mean(SD) 24.5 ± 15 years, Median (range) 24.0 (1.0–67.0)	HbSS (46.6%) HbSβ⁰ (28.1%), HbSβ⁺ (22.1%), HbS/C(3.2%)	652	7 years (<1 – 29 years)	NR	−34.1 %, p < 0.001)	−29.3 %, p < 0.001	NR	NR	−53.2%, p < 0.001	observed benefit in decreased incidence of ACS, VOC, hospitalisation observed universally across all genotypes.
Phillips et al 2017	Children, retrospective	34 HbSS, 2HbS/β0	37	NR	Hydrea capsules	NR	NR	NR	NR	↓, p<0.001 by χ^2 test; 24-month follow up).	Well tolerated and, some mild transient cytopenias. Increasing dose ≥26

		thalassaemia , 1HbSD			Oral Liquid						mg/kg/day has a significant positive effect on HbF; 29.2% vs. 20.4%, P = 0.0151), MCV (94.4 vs. 86.5, P = 0.0183) and reticulocyte count (99.66 × 109/l vs. 164.3 × 109/l, P = 0.0059). Good adherence to therapy was a significant factor in reducing hospitalisations.

↓, decreased; AVN, avascular necrosis; CVA, stroke; dx, diagnosis, hx, history, NS, not significant; p-y, patient-years; pts, patients; txn, transfusion.

HC= Hydroxycarbamide
DMT, Disease modifying therapy
CT: chronic transfusion
HSCT: haematopoietic stem cell transplantation
NR: Not reported

Extrapolation of Efficacy Data in the Main studies to other SCD genotypes

The vast majority of the supporting efficacy data is derived from patients with the HbSS and HbSβº genotypes (two of the most common genotypes). However, a number of recent observational studies have reported data that provide important supporting evidence for the benefits of hydroxycarbamide in other SCD genotypes.

Italia et al (2009) investigated the efficacy and safety of hydroxycarbamide in an Indian population with severe manifestations where the Sβ gene is linked to the Arab–Indian haplotype and where the phenotype is associated with higher HbF levels. Seventy-seven patients (29 adult sickle homozygous, 25 paediatric sickle homozygous, 23 adult sickle β-thalassaemia) were recruited: most of the sickle (Sβ) chromosomes (104/108) of group I and II were linked to the Arab–Indian haplotype, while 4 βs chromosomes were linked to atypical haplotypes. The Sβ chromosomes among the group III patients were linked to the Arab–Indian haplotype in all except 1, which showed another haplotype. Patients with the Arab–Indian haplotype showed a mean increase in HbF of 39% and mean decrease in clinical score of 44% while patients with atypical haplotypes showed a mean increase in HbF of 52% and mean decrease in clinical score by 42%.

Voskaridou *et al (*2010) reported a prospective clinical study of hydroxycarbamide therapy in Greek patients with a 17-year follow up analysis (Laikon Study of Hydroxycarbamide in Sickle Cell Syndromes LaSHS trial, Voskaridou et al 2010). A total of 330 patients (136 men/194 women; median age, 42 years; range, 20-76 years), were enrolled. Thirty four patients had HbSS, and 296 were compound heterozygotes for HbS and -thalassaemia: 131 patients with HbSβ0-thalassaemia and 165 patients with HbSβ+-thalassaemia. This prospective trial showed a dramatic reduction (> 95%) in the median annual rate of painful crisis and not differentiated by genotype. These results are comparable with those of the Multicentre Study of Hydroxycarbamide (MSH) study, in which the administration of hydroxycarbamide was given for a much shorter median period (< 24 months). The significant reduction of the painful crises, the incidence of transfusion requirements, the hospital admissions, and the incidence of chest syndrome that were observed in this study supports the notion that hydroxycarbamide is of benefit for all SCD patients and use should be extended to SCD syndromes. The Kaplan-Meier method was used to estimate overall survival probabilities. The probability of 10-year survival was 86% for hydroxycarbamide patients, whereas it was only 65% for patients who were conventionally treated. The study also confirmed that the beneficial effect of hydroxycarbamide on survival was mainly in HbSS and HbSβ0-thal patients, whereas this effect on HbSβ+-thal was present but statistically insignificant.

Rigano *et al* (2017) conducted a nation-wide cohort study of SCD in Italy, evaluating the impact of hydroxycarbamide on a total of 652 paediatric and adult patients from 33 Reference Centres for SCD (mean age 24.5 ± 15 years, 51.4% males). Hydroxycarbamide median treatment duration was 7 years (range: < 1 year to 29 years) at a mean therapeutic dose of 18 ± 4.7 mg/kg/day. The analysis compared

endpoints pre- and on-hydroxycarbamide. Hydroxycarbamide treatment was associated with a significant increase in mean total and HbF and a significant decrease in mean HbS, WBC and platelet count and LDH levels. Hydroxycarbamide was associated with a significant reduction in the incidence of ACS (−29.3%, p < 0.001), vaso-occlusive crisis (−34.1%, p < 0.001), hospitalization (−53.2%, p < 0.001), and bone necrosis (−6.9%, p < 0.001). New silent cerebral infarction (SCI) occurred during treatment (+42.4%, p < 0.001) but not stroke (+0.5%, p =0.572). These observations were generally consistent upon stratification for age, descent (Caucasian or African), genotype (HbSS, HbSβ0 or HbSβ+) and duration of treatment (< or ≥10 years). There were no new safety concerns observed compared to those commonly reported in the literature.

Lê *et al* (2015) recently reported the survival of patients with SCD in Belgium and the effects of disease-modifying therapies. These investigators took advantage of a national registry, created in 2008, that includes patients of 8 centres. Data from between 2008 and 2012 provide over 5,000 patient-years (PY) of observation from 469 children and adults with SCD. Four hundred and twenty three of these patients had the HbSS, HbSβ0 genotype, 36 patients had the HbSC genotype and 7 patients had the HbSβ+ genotype. Data were registered from neonatal screening or from diagnosis (first contact) until last follow-up or death. Data included diagnosis, demography, and outcome data. The global mortality rate was low (0.25/100 PY), although 13 patients died (2.8%) and was similar between children, adolescents (10–18 years), and young adults (P=0.76). Out of the cohort, 185 patients received hydroxycarbamide at last follow-up (median duration of treatment: 10.3 years), 90 underwent HSCT, 24 were chronically transfused, and 170 had never had any disease modifying therapy (DMT). Hydroxycarbamide showed significant benefit on patients' outcomes as reflected by a lower mortality rate compared to transplanted individuals or people without DMT (0.14, 0.36, and 0.38 per 100 PY, respectively) and by higher Kaplan–Meier estimates of 15 year survival (99.4%) compared to HSCT (93.8%; P=0.01) or no DMT groups (95.4%; P=0.04). In summary, SCD mortality in Belgium was low with no increase observed in young adults. However, patients treated with hydroxycarbamide demonstrate a significant benefit in survival when compared to those without DMT or transplanted. There were no deaths in HbSC or HbSβ+ group.

Lebensburger *et al* (2015) conducted a retrospective review of 30 patients with HbSC (n=23) and SB+ thalassaemia (n=7), who had matched data for pre- and post-hydroxycarbamide hospitalization. The mean age for starting hydroxycarbamide in this cohort was 9.4±4.5 years without a difference in starting age by genotype (9.2 years in HbSC vs 9.9 in HbSβ+ thalassaemia). There was a significant reduction in hospitalizations on hydroxycarbamide over 2 years as compared to the 2 years prior to hydroxycarbamide initiation (mean total hospitalizations/year prehydroxycarbamide and post-hydroxycarbamide: 1.6 vs 0.4, respectively, P<0.001; also mean pain hospitalizations/year pre-hydroxycarbamide and post-hydroxycarbamide: 1.5 vs 0.3, respectively, P<0.001). Patients demonstrated haematologic changes including an increase in HbF (%) pre– and post-hydroxycarbamide (4.5% to 7.7%, P=0.002), MCV (74 to 86 fL, P<0.0001), and decrease in ANC (5.0 to 3.2×10^9/L, P=0.007). Patients with higher doses of hydroxycarbamide demonstrated the greatest reduction in hospitalizations but this was unrelated to ANC. No differences were noted after 1 year in Hb, absolute reticulocyte count, LDH, bilirubin, or AST.

Yates *et al* (2013) report the long term response to hydroxycarbamide in children with clinically severe HbSC. Fifteen patients (nine female, six male) were treated with hydroxycarbamide. Patients were followed for a median of 6 years (range: 3.1–13.2) prior to initiation of hydroxycarbamide. The median age at hydroxycarbamide initiation was 9.6 years (range: 3.8–14.9). Treatment was indicated because of frequent pain episodes (n =8), recurrent ACS (n = 1), or both pain and ACS (n = 6). Most patients started hydroxycarbamide at a dose of 15–20 mg/kg/day, but four were started on a lower dose. Dose initiation and adjustment were determined by individual providers, but most patients had dose escalation similar to HbSS patients. The median follow-up on treatment was 4.8 years (range: 1.5–7.0) with a total follow-up of 72 patient years. The median maximum dose of hydroxycarbamide was 24 mg/kg/day (range: 15.6–

29.9). After initiation of hydroxycarbamide therapy, the median decrease in ACS was 0.23 events per year (P = 0.0004); pain crises were reduced by a median of 0.91 events per year (P = 0.01). The median increase in MCV and HbF were 16.2 fl (P = 0.0001) and 5.1% (P = 0.0001), respectively. There were significant decreases in the median platelet count (82.4×10^9/L, P =0.003), WBC (2.1×10^9/L, P = 0.0001), ANC (1800/mm^3, P = 0.01), and ARC (40×10^9/L, P = 0.008). Additionally, there were increases in creatinine (0.07 mg/dl, P = 0.04) and bilirubin (0.18 mg/dl, P = 0.008) and a decrease in aspartate aminotransferase (4.3 U/L, P= 0.007). There were no significant changes in Hb, mean corpuscular Hb concentration, ALT and LDH. Five patients experienced thrombocytopenia, not associated with palpable splenomegaly. Hydroxycarbamide was discontinued in one patient after 3.6 years because of recurrent thrombocytopenia (nadir 33×10^9/L) even after multiple dose reductions to a final level of 10 mg/kg/day. One patient with splenomegaly (spleen 6 cm below costal margin) at onset of treatment experienced an acute splenic sequestration crisis (drop in Hb from 9.6 to 6.9 g/dl) after 19 months of therapy; this patient subsequently underwent splenectomy. Four patients had transient neutropenia that was not complicated by serious infection and resolved after temporary suspension or dose reduction of hydroxycarbamide. No hepatic or renal toxicity was noted.

Luchtman-Jones *et al* (2016) report on a large cohort of 133 adult and paediatric HbSC patients from 23 sites across the USA. Data were collected retrospectively: in addition to laboratory data, the number of vaso-occlusive pain events at baseline, and then after 6 and 12 months of hydroxycarbamide therapy was collected. The severity of pain was categorised on the basis of whether the patient was managed at home, sought treatment in the clinic or the emergency department (ED), or required hospital admission. The median age at initiation of treatment (estimated as the interval between birth year and year of treatment initiation) was 15 years, with a range of <1 to 33 years. Over half of the patients were teenagers between 11 and 18 years of age; 15 patients were adults when hydroxycarbamide was initiated. Pain or vaso-occlusive crisis was the most common reason for initiation of hydroxycarbamide treatment, accounting for 119 (89%) patients. Acute chest syndrome was the next most common clinical indication, reported in 30 patients (23%), usually in conjunction with recurrent vaso-occlusive pain (22 of 30 patients). Rarely, other reasons for starting hydroxycarbamide were given, such as priapism (3) and stroke (1). The mean starting dose of hydroxycarbamide for the entire cohort was 17.866.3 mg/kg/day, with a median value of 19.8 mg/kg/day. After 6 and 12 months of treatment, the median hydroxycarbamide dose remained stable at 19.9 mg/kg/day and 20.0 mg/kg/day, respectively. However, at the 12-month time point, the actual dose was broadly distributed: 20% were taking low-dose (<15.0 mg/kg/day), 27% were prescribed 15.0–19.9 mg/kg/day, 33% received 20.0–24.9 mg/kg/day, and 20% received >25.0 mg/kg/day.

Compared to the 12 months before hydroxycarbamide treatment, pain visits (to the clinic or emergency department (ED)) dropped by 35% (P<0.0002) for the entire cohort, while hospitalizations for pain management were reduced by 47% (P<0.0001). Pain events that were treated in the clinic, ED, or hospital setting combined were reduced by 38% (P<0.0001).

Compared to baseline, the Hb concentration remained relatively stable (P<0.0001), while the HbF was mildly increased, <3% (P<0.0001), MCV was higher (P<0.0001), and the ANC (P=0.002) and ARC (P=0.03) were lower after 6 and 12 months of treatment. All of these changes in haematologic parameters were statistically significant for the entire cohort and were not strongly influenced by gender, age, or hydroxycarbamide dose. However, greater marrow suppression was noted at higher doses.

The most common toxicities were cytopenias: isolated neutropenia (13 patients, 11%), isolated thrombocytopenia (7 patients, 5%), and combined neutropenia and thrombocytopenia (4 patients, 3%). Additional toxicities included low ARC (1), elevated hepatic transaminase in conjunction with an illness (1), and high haemoglobin concentration associated with increased pain (1).

2.4.5. Discussion on clinical efficacy

Design and conduct of clinical studies

There are 4 main studies in this application: MSH, Jain *et al* 2012, Ferster *et al* 1996, and TWiTCH. They justify the proposed indication, since the first three studies justify the prevention of recurrent painful vaso-occlusive crises including acute chest syndrome in adults, adolescents and children older than 2 years suffering from Sickle Cell Disease and the TWiTCH study intends to support the replacement of regular blood transfusions for primary stroke prevention (also of vaso-occlusive nature) in children over the age of 2 years with abnormal transcranial Doppler velocities. The supportive studies submitted strengthen the results of the main studies and in the case of the first indication play the role of extrapolating the indication to SCD patients and not only SCA.

Efficacy data and additional analyses

Hydroxycarbamide Nova Laboratories may be used to replace regular blood transfusions for primary stroke prevention in children over the age of 2 years with abnormal transcranial Doppler velocities (time averaged maximum mean velocity, TAMMV > 200 cm/s), provided they have received at least 1 year of regular blood transfusions, their transcranial Doppler velocities have normalised (TAMMV < 170 cm/s), and they have no magnetic resonance imaging / angiography defined severe vasculopathy. Blood transfusions should continue until the maximum tolerated dose of Hydroxycarbamide Nova Laboratories has been achieved. Monitor transcranial Doppler velocities every 3 months and reinstate blood transfusions immediately in the event of reversion back to abnormal transcranial Doppler velocities.

The applicant justified the two proposed indications as follows:

a) Justification for prevention of complications of SCD, including recurrent painful VOC and ACS

According to the applicant, large volume of data on the efficacy and safety of oral hydroxycarbamide in reducing the complications of SCD in the adult and paediatric population is compelling. The MSH study was a landmark study and made hydroxycarbamide the first drug of proven benefit in the prevention of major problems caused by sickle cell disease: vaso-occlusive pain crisis and acute chest syndrome (Charache et al, 1995). Importantly, no significant adverse effects were noted and long-term effects monitored in the MSH follow-up study confirmed that the drug was safe and may decrease mortality (Steinberg et al 2010). The findings from the MSH study in adults have been replicated in children with SCD through a couple of smaller randomised trials (Jain et al 2012, Ferster et al 1996). In addition, a double-blind, placebo-controlled, randomised controlled trial in infants of mean age 13.6 months (range 9-18 months), treated for between 18-24 months, meaning that in all participants, a large proportion of treatment phase continued when the child was > 2 years, provides valuable supporting data for the proposed indication. The study showed that oral hydroxycarbamide reduced pain crises and acute chest syndrome and this effect was maintained throughout the 24 months of study (Wang et al 2011).

According to the applicant, a number of large, prospective and retrospective, observational and real-life studies support the laboratory and clinical findings of the adult and paediatric randomised, controlled studies and provide important longer term safety data, including in non-SCA genotypes, and which provide reassurance to patients and prescribers of the benefits of oral hydroxycarbamide as a long term disease modifying therapy in the management of SCD. Oral hydroxycarbamide has been for many years been incorporated into adult and paediatric clinical practice for the treatment of symptomatic sickle cell disease sufferers with recurrent acute pain crises associated with VOC and hospitalization due to acute chest syndrome. Indeed, the use of oral hydroxycarbamide to prevent recurrent VOC and acute chest syndrome has been included into national guidelines such as the 'Evidence Based Management of Sickle Cell-Disease: Expert Panel Report IUS National Heart, Lung and Blood Institute, 2014) and more recently Guidelines for the use of hydroxycarbamide in children and adults with sickle cell disease: A British

Society for Haematology Guideline (Qureshi et al 2018). The Applicant has identified Hydrea as the formulation used wholly or partially in 13 clinical studies of efficacy and 9 studies contributing to the safety and tolerability profile submitted in the Clinical Overview. Alternative branded tablet and capsule formulations, as well as compounded capsules and oral solutions have also been ascertained for a number of studies. The data generated from dissolution studies show that, regardless of the formulation, hydroxycarbamide is very rapidly dissolving. No significant differences were observed between the in vitro dissolution profiles of tablet and capsule formulations of different brands or compounded preparations. The excipients included in the composition of the formulations are established and no interaction or influence on the pharmacokinetics of the active substance is expected. In conclusion, hydroxycarbamide is not only highly soluble, but also rapidly dissolving in tablet and capsule form. This suggests that non-Hydrea oral formulations would be expected to be bioequivalent to Hydrea *in vivo*, and as such clinical efficacy and safety data based on these alternative formulations is bridgeable to Hydrea.

The applicant argued that hydroxycarbamide is a highly soluble and highly permeable compound, as *in vitro* solubility study and *in vivo* literature absorption data show. Therefore, all formulations are bioequivalent and data from non-Hydrea literature may be used. However, this argument can't be extended to all formulations without proper bridging. The applicant was able to identify for most of the submitted studies the exact formulations that were used i.e. Hydrea capsules, Droxia capsules, Siklos tablets, and gelatin capsules filled with hydroxycarbamide drug substance. For cases where capsules were prepared in hospital pharmacies, the applicant's approach was mimicking the formulation by adding hydroxycarbamide to gelatin 0 capsules. This approach is considered acceptable, since no extra excipients are expected to have been added in hospital pharmacies. Then, the applicant performed dissolution tests in pH 1.3, 4.5, and 6.8 with all solid formulations used in the submitted studies, which showed that hydroxycarbamide dissolution is rapid (>85% in 15 min) for all formulations. The excipients of each formulation were described and discussed, concluding that they are not considered to affect bioavailability. As it was shown that hydroxycarbamide is a highly soluble and highly permeable drug, the discussed quality attributes of non-Hydrea formulations (dissolution tests and excipient description) mean that bridging can be done and non-Hydrea literature can be used to justify product's efficacy/safety. As a result, the first proposed indication could be justified.

b) Justification for primary stroke prevention in children > 2 years

Adams *et al* (1992) showed that transcranial ultrasonography could be used to predict stroke in SCD, and the subsequent Stroke Prevention Trial in Sickle Cell Anemia (STOP) clearly demonstrated that chronic transfusion therapy reduces the risk of stroke in children with abnormal transcranial Doppler (TCD) velocities. This led to the inclusion of TCD screening in children with SCD as standard of care, and chronic blood transfusions for primary stroke prevention in those at highest risk. Although this approach has undoubtedly led to reduced frequency of strokes, it comes with a number of drawbacks. If a child has abnormal TCD velocities at 2 years of age, current guidelines would suggest that this child receive monthly blood transfusions indefinitely. Chronic blood transfusion therapy requires prolonged monthly hospital visits, costly iron chelation therapy, and oftentimes surgical procedures to implant central venous catheters. In addition to the cost and inconvenience, chronic transfusion therapy is associated with serious and life threatening haemosiderosis, most commonly in the liver, as well as the development of auto- or alloantibodies to erythrocyte antigens that can make it difficult to find compatible blood. For these reasons, the positive outcomes of the TWiTCH trial (Ware et al 2015), a multicentre, open-label, phase 3, non-inferiority trial, in which children with abnormal TCDs were transitioned to hydroxycarbamide therapy after 1 year of chronic transfusion therapy were considered. The patient population was carefully selected in the study, and the process of hydroxycarbamide dosing and monitoring was rigorous. Nevertheless, the report by Bernaudin *et al* (2016), a long-term cohort study, shows that transfusions can be stopped in real-life clinical practice not only in bone marrow transplanted patients, but also in a subset of patients switched to hydroxycarbamide, provided trimestrial Doppler

follow-up is carried out and transfusions are immediately re-started in case of reversion to abnormal TCD velocities. Observational cohort studies have suggested that hydroxycarbamide reduces the incidence of primary stroke. TWiTCH was the first randomised controlled study designed to evaluate if hydroxycarbamide was an effective alternative for primary stroke prevention in children with SCD. The study was stopped early after reaching its primary end-point: TWiTCH confirmed that in children with SCD and abnormal TCD velocities who had received at least 1 year of CBT and had no MRI-defined severe vasculopathy could be safely switched to hydroxycarbamide to preserve TCD velocities and prevent primary stroke. Hydroxycarbamide was non-inferior to CBT for maintaining TCD velocities, after discontinuation of initial transfusion therapy to prevent primary stroke. There were no stroke events observed in either hydroxycarbamide or the chronic blood transfusion arms of the TWITCH study. The optimal duration of transfusions prior to changing to hydroxycarbamide was not defined by the TWiTCH study. The follow-up period of the TWiTCH study was short and therefore casts some doubt on the conclusion that hydroxycarbamide can substitute for CBT indefinitely for all patients. The applicant agrees that TCD velocities in some patients will revert, and this has been reported through the observation study by Bernaudin et al (2016). On that point however, according to the applicant, the stipulation for regular TCD monitoring during hydroxycarbamide treatment should not be viewed simply as a 'safeguard' on account of the lack of long term RCT data; the importance of ongoing TCD monitoring also applies to patients managed on CBT (i.e. regardless of treatment option). The UK Forum on Haemoglobin Disorders (2016) states: "*Ongoing TCD scanning is recommended once a child has started on regular transfusions. Raised velocities may return to normal levels with transfusion, and if this fails to happen or if velocities increase, further investigations and interventions should be considered. The time interval for performing these scans should be yearly or shorter depending on the TCD velocity and the individual clinical circumstances."* Indeed it is recommended that all SCA patients are TCD screened at least annually from the age of 2 to 16 years for primary stroke prevention (UK Forum on Haemoglobin Disorders 2016) (Yawn & John-Sowah, 2015).

Regarding the second indication, it cannot be justified by the TWiTCH trial. The indicated group is children over the age of 2 years with abnormal TCD velocities (time averaged maximum mean velocity, TAMMV > 200 cm/s). However, the subjects in the trial were in the 4-16 age group and an indication for children over 2 years of age cannot be justified. Also, in the TWiTCH trial most of the participants had a TCD velocity in the normal range at both study entry and exit, as mentioned by the authors, with a baseline TCD velocity of about <210 cm/s (mainly about <190 cm/s), with only 2 out of 60 subjects having TAMMV > 200 cm/s. The mean duration of blood transfusions before switching to hydroxyurea in the alternative arm was 4.5 years and therefore it is unknown whether one year of previous transfusions is sufficient time to switch to hydroxycarbamide, even though this was an inclusion criterion in the trial. The number of children that had received only one year of transfusions and the TCD results they had achieved are not known. The follow-up of the children switched to hydroxycarbamide might not be sufficient to reveal TCD reversion as has been shown in other studies (e.g. Bernaudin *et al* 2016).

The applicant has proposed a conservative approach of documenting a normalised TAMMV (<170 cm/s) before starting hydroxycarbamide and a cautious follow-up with three monthly TCDs. However, this strategy was not part of the TWiTCH trial and cannot be supported by any prospective trial. The beneficial effect of hydroxycarbamide on TCD velocities is well-supported by several clinical observations and its use for stroke prevention can be recommended under specific circumstances within the context of national guidelines, but cannot be incorporated as an indication of the product in the lack of justification from prospective trials designed to prove this indication. Although the supportive studies show the beneficial effect of hydroxycarbamide on TCD, they cannot support the proposed indication because they were performed in a different setting, followed different methods, and replied to different questions. Lefevre *et al* 2008 and Lagunju *et al* (2015) were observational studies that compared TCD velocities in hydroxycarbamide treated and naïve patients. The retrospective study by Adegoke *et al* 2018 compared changes in the TCD velocities of children with SCD over a period of 6-48 months, in those not receiving

any specific intervention (i.e. without the use of hydroxycarbamide or transfusion) with those who were using hydroxycarbamide. Moeen *et al* 2018 compared TCD velocities between patients receiving hydroxycarbamide or transfusions, but the procedure in TWiTCH trial was not followed i.e. previous transfusion and weaning until MTD of hydroxycarbamide was achieved. Overall it has not been documented that adopting the strict follow up with 3-monthly TCDs in routine clinical practice has a beneficial effect and the frequency of investigational follow up outside clinical trials is usually either in the discretion of the clinician or addressed and recommended by national guidelines and not part of a specific product indication. In Greek Ministry guidelines, hydroxycarbamide is considered as an alternative approach to chronic transfusions only in patients in whom TCD is not available and the risk of stroke is assessed as high based on other parameters. In patients with abnormal TCD only chronic transfusions are recommended.

The CHMP agreed that the second proposed indication cannot be justified as such because of the above mentioned arguments. However, the TWiTCH trial indeed showed that hydroxycarbamide can be effective in maintaining TCD velocities after an initial period of transfusions as effectively as chronic blood transfusions. Since stroke is also of vaso-occlusive nature (like vaso-occlusive crises and acute chest syndrome), the following indication was agreed: "*Xromi is indicated for the prevention of vaso-occlusive complications of Sickle Cell Disease in patients over 2 years of age."*

2.4.6. Conclusions on clinical efficacy

The clinical data supports the following indication: "*Xromi is indicated for the prevention of vaso-occlusive complications of Sickle Cell Disease in patients over 2 years of age*."

2.4.7. Clinical safety

The safety profile for hydroxycarbamide in people with SCD is derived from 3 RCTs that enrolled 517 people and almost 50 observational studies that enrolled more than 3,000 people. In addition, in patient populations other than SCD, toxicity evidence is derived from 21 RCTs that enrolled more than 4,800 individuals and 35 observational studies that enrolled more than 7,500 individuals (NIH Expert Panel Report 2014).

Gastrointestinal disorders and skin and subcutaneous tissue disorders have been known to occur commonly in patients receiving hydroxycarbamide. Oral mucosal ulcerations and pigmentation, accompanied by nausea and vomiting, epigastric pain and diarrhoea represent the most common reported gastrointestinal side effect of hydroxycarbamide therapy. Gastrointestinal disorders and skin reactions like oral, ungual and cutaneous pigmentation, rash, melanonychia, alopecia and cutaneous dryness are generally mild to moderate in intensity, and reversible upon discontinuation. Mostly they do not impact on the patient's quality of life and have minimal clinical impact.

Hydroxycarbamide is usually well tolerated; however, long-term hydroxycarbamide therapy has been associated with cutaneous side effects, such as alopecia, diffuse hyperpigmentation, erythema, skin atrophy, and nail changes. Painful skin ulcers have been also reported and their treatment modalities mainly consisted of hydroxycarbamide discontinuation, which was generally followed by the complete or almost complete healing (Quattrone *et al* 2013). There are reports of more severe forms of skin reactions like leg ulcers, vasculitis and panniculitis with long term use of hydroxycarbamide (Mattessich *et al* 2017, Sirieix *et al* 1999, Quattrone *et al* 2013). The mechanisms underlying the pathogenesis of the hydroxycarbamide-associated cutaneous ulcer/vasculitis are not clearly defined, but probably various different elements play a role in their occurrence. Defective repair mechanisms in skin basal cells due to hydroxycarbamide cytotoxicity undoubtedly contribute to the formation of leg ulcers as well in the pathogenesis of all dermatologic hydroxycarbamide side effects. Long term hydroxycarbamide therapy leads to cumulative cytotoxicity to the basal cells of the epidermis, due to inhibition of DNA synthesis and

formation of free radicals. The injury progresses until cellular repair mechanisms are no longer able to regenerate keratinocytes and endothelial cells. Macrocytosis is now considered to be a major pathogenic factor (Quattrone *et al* 2013). The megaloblastic changes in erythrocyte geometry and deformability caused by hydroxycarbamide may prevent these cells from easily traversing the capillaries. This may impair blood flow in the microcirculation and cause relative ischaemia in the basal layer of the skin, which requires more oxygen for proliferation. The painful aspect of the ulcers suggests an anoxic mechanism (Quattrone *et al* 2013). Successful therapies are also now available for management of leg ulcers/vasculitis (Stagno *et al,* 1999; Mattessich *et al.* 2017).

Recovery from ulcers and vasculitis is usually rapid once hydroxycarbamide is stopped.

Cytotoxicity with neutropenia is the first and most common manifestation of haematological suppression, while thrombocytopenia/anaemia occurs less frequently and is rarely seen without a preceding neutropenia. Recovery from myelosuppression is usually rapid when therapy is discontinued. Treatment can then be re-initiated at a lower dose. Cytopenias were sometimes cited as reasons for discontinuation from studies, although usually hydroxycarbamide therapy was temporarily stopped. Other frequently cited reasons for discontinuation in studies were skin toxicity and pregnancy. SCD studies which used dose escalation to the MTD (MSH 1995; SCATE 2015; SWiTCH 2012; TWiTCH 2015), showed greater rates of cytopenias, which are expected (as per the pharmacology of the drug) as the dose of the drug is raised. The MSH study showed increased haematological toxicity resulting in a dose reduction in the hydroxycarbamide group as compared with placebo, but there were no infections related to neutropenia or bleeding episodes due to thrombocytopenia (MSH) 1995). Similarly, the SWiTCH study showed significantly increased rates of reticulocytopenia, neutropenia and anaemia in the hydroxycarbamide group, compared with transfusion (SWiTCH 2012). The BABY HUG study which used a standard dose of 20 mg/kg of hydroxycarbamide showed an increase in neutropenia (in terms of absolute neutrophil count) in the hydroxycarbamide group but no increase in events of thrombocytopenia and no increase in the number of infections (BABY HUG 2011). Clearly, with careful laboratory monitoring and appropriate patient education, cytopenia should rarely represent a major issue as demonstrated in the many reported studies.

Hydoxycarbamide induced symptomatic elevations in hepatic function tests appears to be a rare, idiosyncratic, and serious adverse event requiring discontinuation of hydroxycarbamide. The mechanism is likely to be direct liver toxicity, although what places an individual at risk is unknown. In the reported cases, the reaction is acute, starting within 1–4 weeks of initiating therapy and resolving in approximately 48 h after discontinuation of hydroxycarbamide. Symptoms are similar in each of the case reports, with patients experiencing rigors, fever, abdominal pain, fatigue, and confusion; as is commonly seen in acute liver injury. With the exception of HIV infected individuals receiving concurrent medications, the reaction does not appear to be dose or disease related, occurring at doses ranging from 500 mg three times a week to 1000 mg daily and in patients with psoriasis, polycythaemia vera, and essential thrombocytosis (Sulkowski et al 2000; Westerman et al 1998; Heddle et al 1980).

Potentially fatal pancreatitis and severe peripheral neuropathy have been reported in HIV patients who received hydroxycarbamide in combination with antiretroviral drugs, particularly didanosine plus stavudine. Median decline in CD4 cells of approximately 100/mm^3 is also reported with concomitant use (Bloch *et al* 2016, Halvir *et al* 2001, Rutschmann *et al* 1998, Rutschmann *et al* 2000, Swindells *et al* 2005).

Also concurrent use of hydroxycarbamide and other myelosupressive products or radiation therapy may increase bone marrow depression, gastrointestinal disturbance and mucositis. Erythema caused by radiation may be aggravated by hydroxycarbamide (Lerner *et al* 1977).

Cutaneous vasculitis toxicities including ulcerations and gangrene are more reported in patients with myeloproliferative disorder if hydroxycarbamide is used concomitantly with interferon therapy or used in patient with prior exposure to interferon therapy. (Hydrea Label).

The recent reports of long-term hydroxycarbamide exposure in adults with SCA provide further clinical evidence in support of its safety profile. The LaSHS study with 17-year follow-up demonstrated that hydroxycarbamide was well tolerated with mild, transient and reversible cytopenias as the only reported toxicities. Importantly, there were no reported cases of myelodysplasia or leukaemia in the hydroxycarbamide-treated patients (Voskaridou et al 2010). Long-term results from the MSH study with similar long-term follow-up also did not identify any long-term toxicities of hydroxycarbamide for adults with severe SCA, specifically no increase in cancer or stroke (Steinberg *et al* 2010).

Concerns about somatic and/or germ line genotoxicity underlie the potential serious late effects of hydroxycarbamide. Several retrospective studies have reported abnormal sperm parameters, including decreased numbers, forward motility, abnormal morphology, and percentage living, in men with SCD before the initiation of hydroxycarbamide with a possible increase in oligospermia during and after treatment with hydroxycarbamide (Berthaut et al 2008). These retrospective studies were performed in as few as 8 participants, without any comparative semen assessment before initiation of hydroxycarbamide in 7 participants. Recently, a larger (n=35), but still observational, prospective study to assess the potential adverse impact of hydroxycarbamide treatment of sickle cell disease on spermatogenesis was reported (Berthaut *et al* 2017). Preliminary results of this study had been provided to the EMA in March 2013. The rapporteur acknowledged that the results were sufficiently evocative of a risk of male fertility impairment after 6 months of treatment with hydroxycarbamide. In view of available data, the Pharmacovigilance Risk Assessment Committee (PRAC) ruled that the frequency of azoospermia and oligospermia in hydroxycarbamide SmPC/PL should be changed from “very rare” to “very common”. The primary purpose of the protocol was to evaluate the impact of a treatment by hydroxycarbamide (15-30 mg/kg/day), 6 months after its beginning, in 35 men with sickle cell disease (range 20-51 years of age). The main trial criterion was the average difference of the concentration of spermatozoas (millions/mL) in the ejaculate, before and after 6 months of medical treatment. After 6 months of hydroxycarbamide therapy, the mean total sperm count had reduced 5 fold from baseline. In addition, the number of cryptozoospermic (n=5) and azoospermic (n=6) men increased more than 10 fold, reaching almost 1 in 3 patients. The investigators concluded that, at current doses, hydroxycarbamide ‘causes significant, rapid, and unpredictable impairment of spermatogenesis in treated men. These results may lead to consideration of preventive sperm cryostorage for adult patients with SCA requiring this treatment.’ The investigators also added that there are few data evaluating the reversibility of the adverse effects of hydroxycarbamide on spermatogenesis, and long-term studies are required involving an adequate cohort of participants for proper counselling of our patients.

Sahoo *et al* (2017) reported on a prospective study of a 100 male SCD patients, aged 15 to 45 years. They evaluated seminal fluid indices in all patients and the effect of hydroxycarbamide on seminal fluid parameters. Group I included 50 patients without hydroxycarbamide therapy and Group II included 50 patients who needed hydroxycarbamide therapy and had normal sperm parameters prior to hydroxycarbamide therapy. Group II patients were given low dose hydroxycarbamide therapy 10 mg/kg/day. Seminal fluid analysis was done according to WHO criteria before starting hydroxycarbamide and every 3 months after initiation. In Group 1, 18% of patients developed oligospermia and 4% developed azoospermia. In group 2 (patients on hydroxycarbamide therapy) 20% developed oligospermia and 10% developed azoospermia. In 73% of patients, seminal fluid parameters reverted back to normal 3 months after stoppage of hydroxycarbamide. The authors concluded that alteration of sperm parameters is seen in a significant number of SCD patients, and is further exacerbated by hydroxycarbamide treatmenteven at low doses.

Building on the findings by Berthaut *et al* (2017), the study by Sahoo *et al* (2017) confirms that hydroxycarbamide disrupts spermatogenesis, and appears to suggest that in a majority of the patients, the effect on sperm was reversible, at least a low doses (10 mg/kg/day) - whether this is the case at higher doses remains to be shown.

The safety profile of hydroxycarbamide for children with SCD is similar to that for adults (Ferster *et al* 2001).

Patient exposure

The following publications mainly investigated the safety and tolerability of hydroxycarbamide.

Bioequivalence study

Included in the safety analysis: 28 subjects (93.3%) received Test IMP (A): Hydroxycarbamide Oral Solution (500 mg/5 mL), 30 subjects (100%) received Reference IMP (B): Hydrea 500 mg Capsule (UK), and 29 subjects (96.7%) Reference IMP (C): Hydrea 500 mg Capsule (USA).

Berthaut et al 2017

The primary purpose of the protocol in this prospective study was to evaluate the impact of a treatment by hydroxycarbamide (15-30 mg/kg/day), 6 months after its beginning, in 35 men with sickle cell disease (range 20-51 years of age).

Steinberg *et al* 2010

After 17 years of follow-up, 94 pregnancies were reported by female and male subjects enrolled in the Multicentre Study of Hydroxycarbamide in Sickle Cell Anaemia (MSH) regardless of their hydroxycarbamide exposure (Steinberg et al 2010).

Laure-Joseph *et al* 2016

In a recent report from the ESCORT-HU study, 1050 patients (496 children and 554 adults) were enrolled in ESCORT-HU from 3 European countries, from June 2008 to July 2016; Greece (11.7%), Germany (13.4%), and France (74.9%).

Castro *et al* 2014

Using health insurance claims databases, Castro et al (2014) compared the frequency/incidence of acute myeloid leukaemia (AML) and inpatient mortality in SCD subjects taking (n=1051), or not taking (n=9203) hydroxycarbamide. Patients taking hydroxycarbamide were older (median 19 vs 17 years of age), had a higher proportion of males (53% vs 38%), and their median hospitalizations per year was five times greater than in SCD patients not on hydroxycarbamide (all $P < 0.001$) implying greater disease severity.

Zimmerman *et al* 2004

Between 1995 and 2002, a total of 122 pediatric patients with SCD monitored by the Duke Pediatric Sickle Cell Program were treated with hydroxyurea for at least 6 months, including 106 patients with HbSS, 7 with sickle hemoglobin C (HbSC), 7 with sickle/β-thalassemia (HbS/β-thalassemia [6 HbS/β^0, 1 HbS/β^+], and 2 with sickle hemoglobin O_{Arab} (HbS/O_{Arab}).

Hankins *et al* 2014

The Hydroxycarbamide Safety and Organ Toxicity (HUSOFT) trial was a prospective, multicentre, open-label, single-arm, pilot study. In the first 2-year pilot study, 28 infants (median 15 months, range 6-28 months) with SCA, all unselected for disease severity, were prospectively treated.

Flanagan *et al* 2012

The Hydroxycarbamide Study of Long Term Effects (HUSTLE, ClinicalTrials.gov NCT00305175) is a prospective observational study of children (birth to 18 years) with SCA treated with hydroxycarbamide based on clinical severity, which attempts to evaluate the longterm cellular and molecular effects of hydroxycarbamide. The study consists of two patient groups. The first group (Old Cohort) includes patients previously receiving hydroxycarbamide therapy prior to enrolment on study; studies are completed at three-year treatment anniversaries. The second group (New Cohort) includes patients not on hydroxycarbamide prior to study entry, therefore allowing studies at baseline and at treatment anniversaries. Initial results on genotoxicity for both patient cohorts within the HUSTLE study have recently been analysed (Flanagan et al 2012 - n=93, age range 1.8 to 18 years).

McGann *et al* 2012

A second report from the BABY HUG trial (n=193, mean age 13.6 months) investigated the potential genotoxicity of *in vivo* hydroxycarbamide exposure by examining chromosomes directly for breakage abnormalities, including *in vitro* experiments designed to measure DNA repair capacity by directly examining chromosomes for abnormalities after damage induced by ionizing radiation. This report did not identify evidence of cumulative chromosomal damage in the hydroxycarbamide exposed group with up to 12 years of treatment exposure (McGann et al 2012).

Wang *et al* 2011

From October 2003 to September 2007, 193 infants with HbSS (187) or $S\beta^0$ thalassaemia (6), mean age 13.6 months (range 9–18), were randomised at 13 clinical centers. One hundred seventy-nine (93%) subjects who completed at least 18 months of the trial and at least one exit assessment were analysed; 167 (86%) completed the full study.

Rana *et al* 2014

This study analysed the anthropometric data collected from the children enrolled in BABY HUG (193 children aged 9-18 months.

De Montalembert *et al* 2014

ESCORT-hydroxycarbamide (clinicaltrial.gov NCT02516579) is a multicentre prospective non-interventional study implemented in Europe to collect more information about the safety profile of hydroxycarbamide and morbidity and mortality in SCD patients treated with hydroxycarbamide. ESCORT-hydroxycarbamide is in response to an EMA request and is an ongoing study involving the largest number of patients so far with SCD treated with hydroxycarbamide. Primary endpoints of ESCORT hydroxycarbamide are to determine frequency of AEs, and possible consequent changes of hydroxycarbamide treatment. Secondary endpoints are to evaluate morbidity and mortality of the disease although in the absence of control group. Preliminary results were presented at the 2014 American Society of Haematology annual conference (De Montalembert et al 2014). From June 2008 to June 2014, 483 patients (255 females; 228 males) were enrolled from 3 European countries, Greece (24%), Germany (19%), and France (56%). 67% patients were adults, median aged 37.35 years (17-83.5) and 33% were children, median aged 11.06 years (2.6-16.9). Genotypes were HbSS in 71.4% cases, and compound heterozygous HbS/β-thalassemia in 22.8%.

Adverse events

Bioequivalence study

The safety variables were AEs, laboratory safety (biochemistry, haematology, coagulation and urinalysis), vital signs (systolic/diastolic blood pressure, pulse rate and oral temperature), and 12-lead ECG (heart rate, PR interval, QRS duration, QT interval, QTcB interval).

Adverse events (AEs)

All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary, version 19.0. All AEs, including those which occurred prior to the first dose of IMP, were listed. Only treatment emergent adverse events (TEAEs), i.e., existing conditions that worsened or events that occurred during the course of the study after administration of IMP, were included within the summary tables.

An overall summary of AEs was produced including the number of TEAEs; the number and percentage of subjects reporting at least 1 TEAE, serious TEAE, TEAE leading to withdrawal from the study; the number and % of subjects reporting TEAEs by intensity and relationship to IMP. A subject with multiple occurrences of any adverse event was counted once at maximum intensity or strongest relationship to IMP.

The number of TEAEs and the number and percentage of subjects reporting at least 1 TEAE were tabulated by system organ class (SOC) and preferred term. A subject reporting multiple episodes of a particular adverse event within a treatment period, only contributed 1 count towards the corresponding SOC and preferred term.

In addition, the number and % of subjects reporting TEAEs were tabulated by maximum intensity and strongest relationship to IMP. For the summary of TEAEs by intensity, if a subject had multiple events occurring within the same SOC or preferred term the event with the highest intensity was counted. Similarly, for TEAEs by relationship to IMP, if a subject had multiple events occurring within the same SOC or preferred term, the event with the highest association to IMP was counted.

Brief Summary of AEs

There were a total of 10 AEs reported by 7 subjects during the study (Listing 16.2.7.1). One (1) event was reported by 1 subject prior to dosing (Listing 16.2.7.1) and 9 events were reported by 6 (20.0%) subjects post-dose (TEAE) (Table 29).

Table 29: Overall summary of TEAEs

	Test IMP (N=28)	Reference IMP (B) (N=30)	Reference IMP (C) (N=29)	Overall (N=30)
Number of TEAEs:	5	2	2	9
TEAE	5 (17.9)	2 (6.7)	1 (3.4)	6 (20.0)
Serious TEAE	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TEAE Leading to Withdrawal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Number (%) of subjects with TEAE by maximum intensity:				
Mild	5 (17.9)	2 (6.7)	1 (3.4)	6 (20.0)
Moderate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Severe	0 0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Number (%) of subjects with TEAE by strongest association (causality) to IMP:				
Certain	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Probable/Likely	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Possible	2 (7.1)	1 (3.3)	0 (0.0)	3 (10.0)
Unlikely	3 (10.7)	1 (3.3)	1 (3.4)	3 (10.0)
Not Related	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Conditional/Unclassified	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not Assessable/Unclassified	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

A subject with multiple AEs was counted only once at the maximum level of intensity or the strongest association to IMP.
% were calculated from the number of subjects in the safety set and treatment.
Test IMP: Hydroxycarbamide oral solution (500 mg/5 mL)
Reference IMP (B): Hydrea® 500 mg capsule UK
Reference IMP (C): Hydrea® 500 mg capsule USA
AE = adverse event, IMP = investigational medicinal product, TEAE = treatment emergent adverse event

Of the subjects reporting TEAEs, all reported mild events which were considered to be of possible or unlikely relationship to the study medication (Table 29).

When the number of subjects reporting TEAEs (related or not) were compared, there was a tendency towards a higher number reporting TEAEs following test IMP vs reference IMP (B) and reference IMP (C), although the incidence was considered low at 5 (17.9%), 2 (6.7%) and 1 (3.4%), respectively and overall did not raise any safety concerns (Table 30).

The most commonly reported TEAE was headache reported by 3 (10.0%) subjects following the test IMP (Table 30).

Table 30: TEAEs in each treatment group by System Organ Class and Preferred Term

	Number of Subjects (%)			
SYSTEM ORGAN CLASS: Preferred Term	Test IMP (N=28)	Reference IMP (B) (N=30)	Reference IMP (C) (N=29)	Overall (N=30)
NERVOUS SYSTEM DISORDERS:				
Headache	3 (10.7)	0 (0.0)	0 (0.0)	3 (10.0)
Dizziness	0 (0.0)	0 (0.0)	1 (3.4)	1 (3.3)
GASTROINTESTINAL DISORDERS:				
Abdominal Pain Upper	1 (3.6)	0 (0.0)	0 (0.0)	1 (3.3)
Diarrhoea	0 (0.0)	1 (3.3)	0 (0.0)	1 (3.3)
Nausea	0 (0.0)	0 (0.0)	1 (3.4)	1 (3.3)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS:				
Influenza Like Illness	0 (0.0)	1 (3.3)	0 (0.0)	1 (3.3)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS:				
Skin Irritation	1 (3.6)	0 (0.0)	0 (0.0)	1 (3.3)

A subject was counted only once per system organ class and preferred term in the subject counts.
% were calculated from the number of subjects in the safety set and treatment.
Test IMP: Hydroxycarbamide oral solution (500 mg/5 mL)
Reference IMP (B): Hydrea® 500 mg capsule UK
Reference IMP (C): Hydrea® 500 mg capsule USA
IMP = investigational medicinal product

There were no serious or severe TEAEs or suspected unexpected serious adverse reactions (SUSARs) reported and there were no withdrawals due to TEAEs.

All TEAEs resolved without treatment, were transient in nature and had resolved by the time of study discharge

Vital Signs

Vital signs parameters were listed with any out of normal range values flagged. There were no clinically significant changes in vital signs during the study.

Physical Examination

Physical examination (ear/nose/throat, ophthalmological, dermatological, cardiovascular, respiratory, gastrointestinal, central nervous system, lymph nodes and musculoskeletal and other) were performed and results were presented by subject. There were no clinically significant physical examination findings.

12-Lead ECG

12-Lead ECG parameters were listed with any out of normal range values flagged. There were no clinically significant changes in 12-lead ECG parameters during the study.

Concomitant Medication

Prior and concomitant medications were presented by subject. Four (4) subjects were using ongoing contraception prior to first dose of IMP. Three (3) subjects were using Dianette, Depo-provera and Microgynon (Listing 16.2.12.1) and 1 was using the Mirena coil (Listing 16.2.4.6). No other concomitant medication was taken during the course of the study. Administration of the concomitant medications detailed above was not considered to significantly affect the collection or interpretation of the safety data obtained during the study.

Palatability (test IMP only)

Palatability questionnaire data for the test IMP was presented by subject and in summary. The questionnaire was optional. Thirteen (13) subjects completed the questionnaire. Of the subjects who completed the questionnaire, the majority thought the test IMP tasted good or very good, with no aftertaste or smell and with a pleasant or very pleasant texture that they would find very easy to take regularly (Table 31).

Table 31: Summary of palatability questionnaire (Test IMP only)

	Test IMP (N=28)
Number of Subjects who Completed Questionnaire	13
Taste of Liquid Medicine:	
Bad	1 (7.7)
Okay	1 (7.7)
Good	5 (38.5)
Very Good	6 (46.2)
Aftertaste to Liquid Medicine:	
Yes	2 (15.4)
No	11 (84.6)
Review of Aftertaste:	
Okay	1 (7.7)
Good	1 (7.7)
Smell of Liquid Medicine:	
Don't Know	1 (7.7)
Neutral (No Smell)	7 (53.8)
Good	4 (30.8)
Very Good	1 (7.7)
Texture of Liquid Medicine:	
Okay	4 (30.8)
Pleasant	5 (38.5)
Very Pleasant	4 (30.8)
Easy to take Regularly:	
Easy	2 (15.4)
Very Easy	11 (84.6)

% were calculated from the number of subjects in the safety set who completed the questionnaire
Test IMP: Hydroxycarbamide oral solution (500 mg/5 mL)
IMP = investigational medicinal product

Berthaut *et al* 2017

Concerns about somatic and/or germ line genotoxicity underlie the potential serious late effects of hydroxycarbamide. Several retrospective studies have reported abnormal sperm parameters, including decreased numbers, forward motility, abnormal morphology, and percentage living, in men with SCD before the initiation of hydroxycarbamide with a possible increase in oligospermia during and after treatment with hydroxycarbamide (Berthaut et al 2008). Recently, a larger prospective study to assess the potential adverse impact of hydroxycarbamide treatment of sickle cell disease on spermatogenesis was reported (Berthaut et al 2017).

The main trial criterion was the average difference of the concentration of spermatozoas (millions/mL) in the ejaculate, before and after 6 months of medical treatment. After 6 months of hydroxycarbamide therapy, the mean total sperm count had reduced 5 fold from baseline. In addition, the number of cryptozoospermic (n=5) and azoospermic (n=6) men increased more than 10 fold, reaching almost 1 in 3 patients. Preliminary results of this study had been provided to the EMA in March 2013. The results were sufficiently evocative of a risk of male fertility impairment after 6 months of treatment with hydroxycarbamide. In view of available data, the Pharmacovigilance Risk Assessment Committee (PRAC) ruled that the frequency of azoospermia and oligospermia in the SmPC/PL should be changed from “very rare” to “very common”.

Zimmerman *et al* 2004

A large open-label study demonstrated a modest toxicity profile and overall short-term safety of hydroxycarbamide at MTD in 122 children with SCA. Mild, transient and reversible cytopenias were the only consistently observed toxicities and there were no adverse effects on growth, development, or number of acquired DNA mutations (Zimmerman et al 2004).

Hankins *et al* 2014

Hydroxycarbamide was well tolerated, produced mild toxicities (predominantly transient neutropenia), maintained elevated Hb concentration and %HbF levels, and possibly prevented loss of spleen function (Hankins et al 2005). Neutropenia occurred 57 times in 17 patients and was present in 10% of all blood counts. Neutropenia occurred a median of twice per patient (range: 1- 10 episodes) with a median duration of 1 week (range: 1-4 weeks), and was associated with an intercurrent illness (usually an upper respiratory tract infection) in 40 episodes (70%). Neutropenia was never associated with an invasive bacterial infection; in 4 episodes, patients with an ANC<500×10^9/L were hospitalized because of fever. Severe anaemia occurred 7 times in 7 patients, was associated with acute illness on 4 occasions, and required transfusion in one patient. Thrombocytopenia (platelet count 76×10^9/L) occurred once in association with a viral illness. Elevation of serum ALT (167 IU/L) occurred once without explanation and resolved within 1 week. Mean levels of serum ALT and creatinine were unchanged throughout the study. Three patients had hydroxycarbamide dose reductions for persistent or recurrent neutropenia, to 17.5 mg/kg/day in two, and to 12.5 mg/kg/day in one. These 3 infants had an average (mean ± SD) ANC of $2.5 \pm 1.1\times10^9$/L before beginning hydroxycarbamide, compared with a pre-treatment mean ANC of $4.6 \pm 2.4\times10^9$/L in patients who did not require dose modification (P=0.11). All 21 infants completing the original 2-year pilot study were offered continued oral liquid hydroxycarbamide therapy. This extension of the original HUSOFT trial investigated the effect of hydroxycarbamide dose escalation on hematologic response and provides long-term prospective data regarding the safety and efficacy of hydroxycarbamide therapy in this cohort of very young children with SCA (Hankins et al 2014). Seventeen completed the extension study with dose escalation to 30 mg/kg/day. Eight of these 17 (6 girls and 2 boys, all HbSS) continued on daily hydroxycarbamide for at least 15 years (median age at last follow-up 17.6 years)

without interruption. All hematologic indices (Hb concentration, MCV, HbF) showed sustained effect after 15 years. The median maximum tolerated dose of hydroxycarbamide has decreased from 30 to 26 mg/kg/day (range 19.5–31.2); neutropenia (ANC<1.0×10^9/L) prompting temporary drug discontinuation occurred a total of 10 times in 4 subjects and there was no severe neutropenia (ANC<0.5×10^9/L). Growth rates over 15 years continued at the 50th percentile for both height and weight, and puberty occurred without delay (age range 10–14 years). There were 5.1 vaso-occlusive events (pain and ACS)/100 patient years, 7.3 packed red blood cell transfusions/100 patient years. No malignancies, strokes, or deaths occurred. At last follow up, all subjects were at appropriate grade level (10–12 grade) with no history of repeated grades (Hankins et al 2014).

Flanagan *et al* 2012

Flanagan et al 2012 reported that children (n=93, age range 1.8 to 18 years) with SCA have a significantly higher number of circulating micronuclei-containing erythrocytes after hydroxycarbamide exposure; however, this change was observed within the first 3 months of therapy and did not accumulate over time with up to 12 years of exposure.

McGann *et al* 2012

This report did not identify evidence of cumulative chromosomal damage in the hydroxycarbamide exposed group with up to 12 years of treatment exposure (McGann et al 2012).

Wang *et al* 2011

Hydroxycarbamide had an excellent safety profile in the BABY HUG Trial. Rates of significant cytopenia including severe neutropenia, thrombocytopenia, and anaemia with reticulocytopenia were similar to the placebo group and there was no renal or liver toxicity. Children receiving hydroxycarbamide were more likely to have moderate neutropenia (ANC 500–1,250/mL) with 107 events vs 34 for placebo ($P < 0.0001$), but no increase in bacteraemia or sepsis. Also, no differences were found in genotoxicity measures including chromosome and chromatid breaks, VDJ recombination events, or micronuclei assay results.

Rana *et al* 2014

Growth impairment is a known complication of sickle cell disease. A follow up investigation from the BABY HUG trial explored the effects of hydroxycarbamide on growth in very young children. Height, weight, BMI, and head circumference were compared with WHO standards. The treatment and placebo groups were compared longitudinally by using a mixed model analysis. At study entry, the z scores of BABY HUG children were higher than WHO standard population for height, weight and BMI, while having a significantly larger head circumference. After 2 years of hydroxycarbamide or placebo treatment, there was a downward percentile trend for weight in both treatment and placebo groups; nonetheless, the mean z scores remained close to the WHO norms, suggesting that there was no significant impairment of overall growth in either group by study end. In addition, there were no significant differences for height, weight, and BMI based on study treatment. There was a significant difference for the mean head circumference z scores at study exit (hydroxycarbamide: +0.8 vs placebo: +1.0, P=0.05). Nonetheless, the head circumference remained well above the WHO 50th percentile standard. Baseline z scores were the best predictors of z scores at study exit. The ANC, ARC, and total white blood cell count had significant negative correlations with growth measures (Rana et al 2014).

Further BABY HUG and HUSTLE follow-up observational studies (clinicaltrials.gov #NCT00890396/NCT01783990 and #NCT00305175 respectively) will be of great value in describing the long-term efficacy and toxicity of hydroxycarbamide in a larger cohort of children who also have been treated since infancy, generally without interruption.

De Montalembert *et al* 2014

137 (28.4%) patients experienced 421 events (Table 32). 132 (32.2%) of these events may be attributed to hydroxycarbamide. The safety profile is roughly similar in children and adults. As expected the most frequent side effects were firstly blood disorders (n=86 events, 42.4%) such as neutropenia or thrombocytopenia. In all cases, these cytopenias were rapidly resolved with the transitory stop of hydroxycarbamide. Seventy one drug related events involving skin and subcutaneous tissue disorders were observed, mostly cutaneous dryness, skin reactions, alopecia and nails or skin pigmentation; 4 patients had a leg ulcer (34.8%). Most of these events are ongoing or stabilised despite the decrease of hydroxycarbamide. No secondary cancer has been reported until now.

Table 32: The most frequent AEs reported in the ESCORT-HU study (De Montalembert et al 2014)

	ADULTS		CHILDREN			
	No of German (%)	No of adults	No of Episodes (%)	No of children	Total (% /411)	Events Related to hydroxycarbamide treatment (Siklos®) (%**)
Blood and lymphatic system disorders (%)	**32 (17.7)**	**22**	**54 (31.03)**	**28**	**86 (20.9)**	**56 (65.1)**
Skin and subcutaneous tissue disorders (%)	**42 (23.2)**	**28**	**29 (16.7)**	**19**	**71 (17.3)**	**46 (64.8)**
Nervous system disorders: Headache (24), Dizziness/vertigo (14),	**32 (17.7)**	**23**	**12(6.9)**	**10**	**44 (10.7)**	**11 (25)**
Gastrointestinal disorders Nausea (14), diarrhoea (8), other (14)	**20 (11)**	**17**	**23 (13.2)**	**16**	**43 (10.4)**	**7 (16.3)**
Metabolic and nutrition disorders: vit D deficiency (17), weight gain (5)	**13 (7)**	**11**	**18 (18.3)**	**18**	**36 (8.75)**	**4 (11.1)**
Fever	**11 (6)**	**10**	**12(6.9)**	**7**	**23 (5.6)**	**1 (4.3)**
Cardiac disorders (hypertension, bradycardia, chest pain, cardiomegaly)	**4**	**4**	**2**	**2**	**6**	**1 (16.6)**
General disorders : fatigue	**5**	**5**	**0**	**0**	**5**	**0**
Hepatobiliary disorders	**2**	**2**	**0**	**0**	**2**	**0**
Neoplasms benign, malignant and unspecified (incl. cysts and polyps) Haematoma, benign vulvar sebaceous cyst	**2**	**2**	**0**	**0**	**2**	**0**
Renal & urinary disorders	**2**	**2**	**0**	**0**	**2**	**0**
Reproductive system and breast disorders	**3**	**3**	**0**	**0**	**3**	**0**
Other	**13**	**13**	**21**	**14**	**34**	**6 (17.1%)**
	181	80 /181 (24.8%)	174	57 / 174 (35.4%)	411	132/411 (32.2%)

**compared to the total number of "system organ class" events

Tables 33 and 34 present a summary of the toxic effects of Hydroxycarbamide observed in randomized controlled trials and observational studies in Sickle Cell Disease, respectively.

Table 33: Toxicity results in Randomized Controlled Trials of Hydroxycarbamide treatment in Sickle Cell Disease

Author, year	Intervention (N)	Mean drug duration	Formulation	Death, n (%)	Discontinuations due to AEs	Thrombo cytopenia	Neutropenia, %	Absolute neutrophil count, µL±SD	% Gastro-intestinal disturbance	% rash or nail changes	Lower extremity ulcers	Other toxicities
MSH[1]: Charache, 1995[3]	HC (152) Placebo (147)	HC: 24 mo Placebo: 28 mo	Capsules (prepared in pharmacy)	HC: (2) Placebo: (5)	**Permanently stopped:** HC: 14, medical reasons (2 myelotoxicity at a dose of 2.5 mg per kilogram per day) Placebo: 6 **Temporarily stopped** HC: 'almost all' because of marrow depression; Placebo: 4 (hyperbilirubinaemia)	HC: 4 Placebo: 5	NR	HC: 4,900± 2,000 Placebo: 6,400±2,000	NR	NR	HC: 15 Placebo: 17	HC: Hb >12.8 g/dL, 11 platelets >800,000/µL, 4 Placebo: Hb >12.8 g/dL, 1 total; bilirubin >10 mg/dL, 4
MSH: Charache, 1996[4]	HC (152) Placebo (147)	HC: 24 mo Placebo: 28±6 mo	Capsules (prepared in pharmacy)	HC: (2) Placebo: (6)	See Charache 1995	NR	66	NA	HC: 59 Placebo: 58	HC: 25 Placebo: 25	HC: 15 Placebo: 17	HC: hair loss, 18; fever,91; aplastic crisis, 1; aseptic necrosis, 9; lymphadenopathy, 45; bleeding tendency, 7 Placebo: hair loss, 28; fever, 96; aplastic crisis, 5; aseptic necrosis, 9; lymphadenopathy, 56; bleeding tendency, 3
MSH[1]: Steinberg, 1997[7]	HC (152) Placebo (147)	HC: 24 mo Placebo: 28 mo (range, 21–38)	Capsules (prepared in pharmacy)	NR	See Charache 1995	NR	NR	HC: 1,900*± 2,400 Placebo: 400± 2,200	NR	NR	NR	NR

Author, year	Intervention (N)	Mean drug duration	Formulation	Death, n (%)	Discontinuations due to AEs	Thrombo cytopenia	Neutropenia, %	Absolute neutrophil count, µL±SD	% Gastro-intestinal disturbance	% rash or nail changes	Lower extremity ulcers	Other toxicities
MSH[1]: Steinberg, 2003[8]	HC (152) Placebo (147)	HC: 7.7 yr Placebo: 7	Capsules (prepared in pharmacy)	HC: 36 (23.7) Placebo: 39 (26.5)	See Charache 1995	NR	NR	NR	NR	NR	NR	HC: malignancy, 1; sepsis/infection, 18; hepatic failure, 3; renal failure, 14 Placebo: malignancy, 1; sepsis/infection, 20; hepatic failure, 10; renal failure, 14
MSH[1]: Steinberg, 2010[9]	Never on HC (44) HC <5 yr (140) HC 5–10 yr (55) HC 10–15 yr (40) HC >15 yr (20)	Never on HC: None HC <5 yr: HC 5–10 yr: HC 10–15 yr: HC >15 yr:		Never on HC: 16 (36) HC <5 yr: 78 (56) HC 5–10 yr: 26 (47) HC 10–15 yr: 9 (23) HC >15 yr: 0 (0)	See Charache 1995	NR	NR	NR	NR	NR	NR	Never on HC: stroke: 0; renal disease: 9; hepatic disease: 5; malignancy: 0; infection: 3 HC <5 yr: stroke: 12; renal disease: 27; hepatic dis-ease: 9; malignancy: 1; infection: 27 HC 5–10 yr: stroke: 3; renal disease: 10; hepatic disease: 3; malignancy: 2; infection: 15 HC 10–15 yr: stroke: 0; renal disease: 5; hepatic disease: 1; malignancy: 0; infection: 10 HC >15 yr: stroke: 3; renal disease: 1; hepatic dis-ease: 0; malignancy: 0; Infection: 0
Belgian Study: Ferster, 1996[10]	HC or placebo (25)	6 mo 22 mo	Capsules (prepared in pharmacy)	0	0	6 mo: 2 22 mo: 0	NR	NR	NR	NR	NR	6 mo: No clinically significant toxicity

Author, year	Intervention (N)	Mean drug duration	Formulation	Death, *n* (%)	Discontinuations due to AEs	Thrombo cytopenia	Neutropenia, %	Absolute neutrophil count, µL±SD	% Gastro-intestinal disturbance	% rash or nail changes	Lower extremity ulcers	Other toxicities
BABYHUG Wang *et al* 2011	HC or Placebo	24 months	Oral solution	0	NR	HC: 12 events in 11 pts Placebo: 8 events in 7 pts	NR	ANC 500-1250/µl HC: 107 events in 45 pts (↓ dose in 9) Placebo: 34 events in 18 pts (↓ dose in 5) ANC <500/µl HC: 5 in 5, p=0.26 Placebo: 2 in 2	NR	HC; 122 in 62, p=0.08 Placebo: 165 in 69	NR	Sepsis/bactaraemia HC: 3 events in 2 pts Placebo: 6 event sin 5 pts Reticulocytopenia, chromosome and chromatid breaks, VDJ recombination vents, and micronuclei assay results were similar in both groups.

1. Follow up data from the The Multicentre Study of Hydroxycarbamide in Patients With Sickle Cell Anaemia (MSH)

* Change from baseline, mean ± SD.

NR Not reported

Table 34: Toxicities of Hydroxycarbamide in observational studies in Sickle Cell Disease

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombo cytopenia	Leuk aemia	Leg ulcers	Rash or Nail changes	Other toxicities
Berthaut, 2008	HC (44)	Yes	NR	0	NR	0	0	0	0	0	Before HC: Volume of ejaculate 3.08±1.67 mL, spermatozoa concentration 38.55±43.12 ×10⁶/mL, total sperm count 114.17±124.12 ×10⁶, initial forward motility 28.66±18.38% of motile, spermatozoa morphology 21.92±14.63% of normal, vitality 59.75±21.61% of living During HC: Volume of ejaculate 2.68±1.28 mL, spermatozoa concentration 2.66±3.75 ×10⁶/mL, total sperm count 7.02±10.18 ×106, initial forward motility 30.00±5.77% of motile, spermatozoa morphology 34.50±21.92% of normal, vitality 52.00±14.23% of living After HC: Volume of ejaculate 2.99±2.85 mL, spermatozoa concentration 18.46±26.86 ×106/mL, total sperm count 61.12±107.37 ×106, initial forward motility 29.46±20.13% of motile, spermatozoa morphology 19.16±16.3% of normal, vitality 44.40±20.12% of living

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombocytopenia	Leukaemia	Leg ulcers	Rash or Nail changes	Other toxicities
Chaine, 2001	HC (17)	Yes		NR		NR	NR	NR	5	13	Prior leg ulcer associated with ulcer on treatment (p<.005); patients with ulcer were older than those without (p<.001); 3 of 5 resolved with holding HC
Charache, 1992	HC (49)	NR	Capsules (prepared in pharmacy)	NR	2 (1 developed narcotic dependency, 1 becane HIV positive)	17	1	NR	NR	NR	No unusual infections; karotypic analysis showed no difference in % abnormal chromosomes pre- and post treatment
de Montalembert, 1997	HC (35)	NR		NR		NR	NR	NR	NR	5	NR
de Montalembert, 1999	HC (101)	Yes	NR	NR	11 (Failure 6 Pregnancy 1 Cutaneous rash 1 Leg ulcer 1 Lupus 1 ALL 1)	2 with ANC 500–1,000/µL ,3 with ANC 1,000–1,500/µL	4 with 90–100,000/µl	1	1	8	NR

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombocytopenia	Leukaemia	Leg ulcers	Rash or Nail changes	Other toxicities
de Montalembert, 2006b	HC (225)	Yes	Tablet	1	58 (Failure 30 Hypersplenism 5 TCD velocities > 200 cm/s 3 Osteonecrosis of femoral head 3 Cerebrovascular event 2 Rash 2 Dizziness 2 Headache 2 Asthenia 1 Azoospermia 1 Leg ulcer 1 Pregnancy 1 Middle cerebral artery stenosis 1 Leukaemia 1 Systemic lupus erythematosus 1 Sarcoidosis 1 Interferon for hepatitis C 1)	8	8	1 (same patient as in earlier study) 19	NR	NR	81 patients discontinued therapy, mostly for lack of efficacy
el-Hazmi, 1992	HC(21)	NR		NR		NR	NR	NR	NR	NR	6 with leukopenia (WBC <4,500/µL)
Ferguson, 2002	HC≥24 mo (30) HC<24 mo (30)	NR	NR	NR	NR	NR	NR	NR	NR	NR	HU ≥24 mo: Stated no adverse events HU <24 mo
Gulbis, 2005	HC (109)	NR	NR	1 (0.23/100)	3 (Died 1 (due to acute severe anaemia during an episode of splenic sequestration. 2 pregnancies).	NR	NR	NR	NR	NR	Transient haematological toxicity in 1.4/100 patient-yr

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombocytopenia	Leukaemia	Leg ulcers	Rash or Nail changes	Other toxicities
Hankins, 2005	HC (21)	NR	Oral solution	1	2 (death 1, pneumococcals sepsis 1)	21 episodes in 10 patients in yr 3, 21 episodes in 9 patients in yr 4	2 in year 5, 1 in year 6	NR	NR	NR	Severe anaemia 3 times in 3 patients in yr 3; 4 times in 1 patient in yr 4
Italia, 2009	HC (77)	No	Capsules (Cytodrox brand)	NR	1 (leukopoenia)	1	NR	NR	NR	NR	NR
Khayat, 2006	HC (8)	Yes	NR	NR	NR	NR	NR	NR	NR	NR	There was no significant difference in mitotic index (p>.05). There was no significant difference in chromosomal aberrations (p>.05) pre- and posttreatment
Kinney, 1999	HC (84)	Yes	Capsule	NR	5 (thrombocytopenia 1 ALT recurrent elevation 1, Severe recurrent headaches (arteriography confirmed vasculopathy with a moya-moya pattern) 1, TIA 1, Neutropenia 1	56 with ANC <2,000/µL	7	NR	NR	5	NR

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombocytopenia	Leukaemia	Leg ulcers	Rash or Nail changes	Other toxicities
Loukopoulos, 2000	HC (69)	NR	Hydrea	NR	5 (history of stroke 1, Bone marrow necrosis 1, Leukopenia or erythroid hypoplasia 3) 6 patients stopped temporarily due to '*unwilling ness to report regularly to the Clinic as agreed and gastric intolerance of hydroxycarbamide*'	NR	NR	NR	3	0	2 with severe anaemia; 0 of 40 with oncogenes; 0 of 10 with cytogenetic abnormalities
Olivieri, 1998	HC (17)	Yes		0		9	3	NR	NR	1	NR
Scott, 1996	HC (15)	NR	NR	1	5 (rash 1, Avascular necrosis of hip 1, Poor response 1, Death 1, Pregnancy 1,	NR	NR	NR	NR	1	Anaemia in 3 of 13 completing study

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombocytopenia	Leukaemia	Leg ulcers	Rash or Nail changes	Other toxicities
Schultz, 2003	Patients with SCD and cancer (49) Patients on HC with cancer (3)	Patients with SCD and cancer: Yes	NR	NR	NR	NR	NR	Patients with SCD and cancer: 7 of 16,613; not all on HU Patients on HU with cancer: 1	NR	NR	Patients with SCD and cancer: 49 cancers in patients with SCD described, in survey of providers Patients on HU with cancer: Unknown number taking HU, but among 49 patients, 3 were on HU, including 1 with leukaemia
Thornburg, 2009	HC (14)	Yes	Oral solution	NR	1 (thrombocytopaenia with splenic sequestration)	11	4	NR	NR	NR	2 patients had combined cytopenias
Thornburg, 2010	HC (75)	No	Capsule / oral liquid	NR	1 (splenomegaly and myelosuppression;)	Mean change in neutrophil count: -1,699 cells/mm3; 95% CI, -2,513–885; *P*<.0001	NR	NR	NR	NR	NR
Thornburg 2012 (BABY HUG follow up)	HC (96 Placebo (97)	Yes	Oral solution	No	See BABY HUG						HC was not associated with increased risk of bacteraemia or serious infection: 8 episodes in total (HC -3, Placebo – 5). Gastroenteritis was lower in HC group (13.8/100 pt yrs versus 37.9/100 pt yrs) (HR 0.35, p<0.0001)

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombocytopenia	Leukaemia	Leg ulcers	Rash or Nail changes	Other toxicities
Vicari, 2005	HC (22)	NR	NR	NR	3 (temporarily stopped due to neutropenia)	3	NR	NR	NR	NR	NR
Voskaridou, 1995	HC (14)	NR		NR		3NR	NR	NR	NR	NR	Leukopenia or thrombocytopenia in 6; rapidly reversed by holding therapy
Wang, 2001	HC (28)	NR	Oral solution	1	2 (death 1, splenic sequestration 1)	17 with ANC <1,500/µL 6 with ANC <500/µL	1 with <80,000/µL	NR	NR	NR	NR
Zimmerman, 2004	HC (122)	NR	Capsule / oral solution	2/455	3 (temporary interruption due to thrombocytopenia)	NR	NR	NR	NR	NR	No increase in the acquired illegitimate VDJ rearrangements
Little, 2006	A: High-risk SCD with HC intolerance (5) B: High-risk SCD with relative renal insufficiency (5) C: Misc (3)	No	Meta-analysis of studies	NR	NR	NR	NR	NR	NR	NR	Twelve of the 13 patients treated with EPO did not experience worsening of symptomatic SCD, changes in ophthalmologic symptoms, or clinical thromboses while on HU and EPO. There was no evidence for pure red cell aplasia or systemic hypertension
Lukusa, 2009	HC (4) Hematopoietic stem cell transplantation (HSCT) (6)	Yes	NR	NR	NR	NR	NR	NR	NR	NR	HU: 2 patients were azoospermic HSCT: 3 patients were azoospermic

Author	Group number	Primary Toxicity Study	Formulation	Deaths, n (per patient/yr)	Discontinuation due to AEs	Neutropenia	Thrombo cytopenia	Leuk aemia	Leg ulcers	Rash or Nail changes	Other toxicities
Voskaridou, 2010	HC (131) Non-HC (199)	Yes	NR	HC: 13 Non-HC: 49	3 due to repeated neutropenia or thrombocytopenia, but they reentered into the study after recovery). 2 due to red cell aplasia after 10 and 5 years on HU treatment, respectively, but they both reentered into the study after 6 and 7 months, respectively, when they recovered from their crises	HC:9	HC:8	NR	NR	HC:1	HC: 2 patients were azoospermic HSCT: 3 patients were azoospermic

Serious adverse event / deaths / other significant events

Malignancies

As a chemotherapeutic agent, the cytostatic effects of hydroxycarbamide are different from those of radiation, alkylating agents and other anti-cancer drugs, many of which are known to increase the risk of development of either leukaemia or cancer. There has been no evidence of malignancy in those with SCD treated with hydroxycarbamide in large observational studies (Steinberg 2010; Voskaridou 2010). Some early reports suggested that hydroxycarbamide was associated with an increased risk of leukemic transformation among patients with myeloproliferative neoplasms, which was extrapolated by both patients and healthcare providers to suggest hydroxycarbamide might also confer an increased risk of leukaemia in SCA (Weinfeld et al 1994). However, subsequent studies demonstrated that hydroxycarbamide does not lead to an increased risk of leukaemia for patients with myeloproliferative neoplasms, despite those being intrinsically preleukemic conditions (Bjorkholm et al 2011). Five malignancies were reported in 951 SCD patients taking hydroxycarbamide (0.5%) and 1 malignancy among 1736 patients not taking hydroxycarbamide (0.06%) (Gilmore *et al* 2011, Lobo *et al* 2013, Sharef *et al* 2013, Voskaridou *et al* 2010, Nzouakou *et al* 2011, Steinberg et al 2010). Three case reports were published after 2007 describing a hematologic malignancy presenting 4 to 15 years after starting hydroxycarbamide in patients with SCD (Baz et al 2012, Couronne et al 2009, Darbari et al 2011). *Ex vivo* studies of mononuclear cells taken from patients on hydroxycarbamide did not demonstrate increased genomic instability that might contribute to teratogenesis or leukemogenesis (McGann et al 2012). Wong et al (2014) in their review stated that the identification of 3 additional case reports of hematologic malignancies given the substantial increase in patient-years of exposure based on inclusion of more recent studies and *ex vivo* investigations does not provide adequate evidence to suggest an increased risk of malignancy.

Importantly, SCD is unlike myeloproliferative neoplasms such as polycythaemia vera or essential thrombocytosis, and is not considered a pre-leukaemic condition. Many patients have taken hydroxycarbamide for over 10 to 20 years, with no evidence to suggest an increased risk of malignancy. Of course, patients with SCD carry the same risk of malignancy as the general population and numerous cases in the pre-hydroxycarbamide era have been reported (Schultz et al 2003). With increased survival due to hydroxycarbamide therapy, there will inevitably be some patients with SCD who develop leukaemia or other malignancies, but this should not automatically implicate hydroxycarbamide as the causative agent (McGann *et al* 2015).

Batt *et al* (2015) in their review of data collected over 35 years revealed an increased prevalence of hematologic malignancies in the SCD population compared to the general population, predominantly of

lymphoid origin; the use of hydroxycarbamide seems to influence this finding further. In contrast, while there was also a notable mild increase in myeloid leukaemia diagnoses compared to the general population, there does not seem to be the same correlation with hydroxycarbamide. Brunson and colleagues conducted a cohort study of the incidence of cancers in Californian SCD patients compared to the general population, using population based data spanning 27 years and identifying 6423 SCD patients (Brunson *et al* 2017). SCD patients had a 72% increased risk of haematologic malignancies (leukaemia) and a 38% reduced risk of solid tumours. However, they did not observe a higher risk of leukaemia after hydroxycarbamide was approved for use in USA in 1998, suggesting that the increased risk of leukaemia is not related to hydroxycarbamide use.

Castro *et al* 2014

No new AML cases occurred in HC-treated paediatric SCD patients. For adults, the new AML incidence with HC exposure was 10.7/10,000 patient years, vs 4.0/10,000 patient years in subjects not on HC (P=0.2), a possible AML risk ratio of 3.18. Adjustment for a probable database bias for AML diagnosis/ascertainment lowered the risk ratio to 0.94 (95% CI=0.16-5.47) i.e. no increased risk for AML with HC treatment. Despite their greater disease severity, the inpatient mortality in SCD adults prescribed HC (0.29%) was lower than that of patients not taking the drug (0.42%, P=0.032).

Teratogenic effects

Some teratogenic effects of hydroxycarbamide have been observed in foetuses of treated pregnant rodents (Woo et al 2004, Katayama et al 2004; Yan and Hales 2005; Chahoud and Paumgartten 2009). Hydroxycarbamide has been demonstrated to be embryotoxic at doses over 150 mg/kg/day in rats and in monkeys (Liebelt, et al 2007). Embryotoxicity was characterized by decreased foetal viability, reduced live litter sizes, and developmental delays. On the other hand, a recent study on pregnant mice showed no evidence of teratogenic effects with intraperitoneal injection of hydroxycarbamide (25 mg/kg) on gestation day (GD) 7.5 (Guan et al 2015; Laure-Joseph et al 2016). Evidence of adverse outcomes and teratogenic effects in humans exposed to hydroxycarbamide *in utero* is limited. An expert panel report evaluating the toxicity of hydroxycarbamide has led to recommendations that hydroxycarbamide is stopped in men and women who are trying to conceive and in breastfeeding women (National Toxicology Program 2008). The risks of stopping hydroxycarbamide whilst attempting conception should be carefully discussed with the individual.

Steinberg *et al* 2010

Of female subjects with known hydroxycarbamide exposure at conception or during gestation and by male subjects with known exposure at the time of conception, 16 pregnancy outcomes were reported with 8 live births (6 term and 2 premature deliveries), 5 elective abortions, and 3 spontaneous abortions. Of the live births, no birth defects were described, consistent with findings in 3 other studies reporting pregnancy outcomes (Gilmore et al 2011; Voskaridou et al 2010; Berthaut et al 2008).

Laure-Joseph *et al* 2016

Among the 315 women with childbearing potential (aged more than 15 to 49 years), 28 pregnancies in 27 women treated with HU have been reported despite the information to stop HU before conception (Table 35). Clinical data regarding the newborn are reported for 7 pregnancies with HU exposure and no malformations were observed. In this series nested in the ESCORT HU cohort, pregnancies occurred safely despite exposure to hydroxycarbamide (Laure-Joseph et al 2016).

Table 35: Exposure of foetus *in utero* reported in ESCORT HU in women treated with Hydroxycarbamide

Foetal exposure to hydroxycarbamide	Number of pregnancies	Outcome of the pregnancy
Yes	24	7 births ≥ 35 weeks amenorrhoea
		6 premature births <35 weeks amenorrhoea
		5 abortions (4 voluntary and 1 therapeutic)
		1 miscarriage
		3 ongoing pregnancies
		2 ongoing documentation
No	2	1 premature birth
		1 normal birth
Unknown	1	Premature birth

Laure-Joseph et al Blood 2016;128:1309

Deaths

Deaths occurred rarely. The deaths observed are not alarming. It is not sure if they can be attributed to the drug in the MSH trial, since they seem not statistically significant compared to placebo. De Montalembert *et al* 2006 that reported one death is a PK study. Gulbis *et al* 2005 say that the death observed should not be interpreted as toxic death, but rather as a lack of efficacy of hydroxycarbamide. The death in Scott *et al* 1996 was unrelated to hyroxycarbamide treatment. The two deaths in Hankins *et al* 2005 and Wang *et al* 2001 are not discussed. In Rigano *et al* 2017 14 deaths were reported, but a Kaplan-Meier plot showed that hydroxycarbamide hazard ratio is 0.22. Also, in Voskaridou *et al* 2010, 13 deaths happened in the hydroxycarbamide arm vs 49 in the non-hydroxycarbamide arm.

Laboratory findings

Studies have shown that, due to the structural similarity of the drug with urea, uric acid, and lactic acid, there is an analytical interference of hydroxycarbamide with the enzymes (urease, uricase, and lactate dehydrogenase) used in the determination of urea, uric acid and lactic acid, rendering falsely elevated results of these in patients treated with hydroxycarbamide (Restituto *et al* 2006).

Safety in special populations

Impaired renal function

Impaired renal function is a relatively frequent and serious complication in patients with SCD. These patients commonly develop proteinuria, which may progress to the nephrotic syndrome and end-stage renal disease (ESRD). In an open single-dose study in adult patients with Sickle Cell disease (Yan et al, 2005) the influence of renal function on pharmacokinetics of hydroxycarbamide was assessed. Patients with normal renal function (creatinine clearance CrCl>90 ml/min, n=7), mild (CrCl 60–89 ml/min, n=2), moderate (CrCl 30 - 59 ml/min, n=3), or severe (CRCL 15-29 ml/min, n=3) renal impairment, and End Stage Renal Disease (ESRD, n = 3), with 2 of them on maintenance haemodialysis. Except for patients with ESRD, all the patients received a 15-mg/kg single oral dose of hydroxycarbamide (Droxia capsules). Patients with ESRD received a 15-mg/kg oral dose of hydroxycarbamide on 2 occasions. Blood and urine samples were collected for the assessment of hydroxycarbamide pharmacokinetics. As the degree of renal impairment worsened, the systemic exposure to hydroxycarbamide increased, and the urinary

recovery decreased. The mean AUC0-∞ was increased by 88%, 70%, 62%, 80%, and 152% for patients with mild, moderate, severe renal function impairment, and ESRD with and without hemodialysis, respectively, compared to patients with normal renal function. The changes in CLtotal/F, CLrenal, and t1/2 were also generally in keeping with this trend (i.e., higher exposure, lower clearance, and longer half-life). On the basis of the altered pharmacokinetic parameters in renally impaired patients, the authors proposed an initial dosing regimen of hydroxycarbamide (7.5 mg/kg/day) for SCD patients with CLcr <60 mL/min. Systemic exposure is likely to be unpredictable in patients with moderate to severe renal impairment (CrCL<30ml/min).

Impaired hepatic function

No studies have investigated the pharmacokinetics of hydroxycarbamide in hepatically impaired patients, but bearing in mind hepatic elimination is a significant component of overall elimination, perhaps up to 60%, it is prudent to assume that clearance of hydroxycarbamide will be reduced in patients with severe liver dysfunction and therefore careful monitoring of response is required. Systemic exposure is likely to be unpredictable in patients with severe hepatic impairment.

Immunological events

There are no literature data, however considering that the normal defence mechanisms may be suppressed by hydroxycarbamide treatment, concomitant use of hydroxycarbamide with a live virus vaccine may potentiate the replication of the vaccine virus and/or increase the adverse drug reaction of the vaccine virus. According to Lederman *et al* 2014, children with SCD receiving hydroxyurea had lower lymphocyte, CD4, and memory T-cell counts compared with those receiving placebo, but still in the range for healthy children. Because infants and young children are immunologically immature and their primary lymphoid organs must produce large numbers of naive T and B lymphocytes, the effects of hydroxyurea could be greater in this age group than in older children and adults.

Safety related to drug-drug interactions and other interactions

A justification was provided with regards to the concomitant use with antiretroviral drugs, particularly didanosine plus tavudine, other myelosupressive products or radiation therapy, interferon therapy, and live virus vaccines. Please see discussion on clinical safety.

Discontinuation due to adverse events

The majority of discontinuations reported were due to cytopenias, however often treatment has resumed after temporary cessation of therapy. Failure of therapy and pregnancy were cited in some studies as frequent reasons for discontinuation.

2.4.8. Post marketing experience

There is no current commercial experience to report with Xromi since the product has no marketing authorisation in any jurisdiction.

Safety data provided are mainly from the last decade. However, most of them originate from clinical or retrospective studies and not from spontaneous reporting. Pharmacovigilance data may provide increased and useful post-marketing experience and identify new safety issues.

A new signal with hydroxycarbamide has been confirmed by the PRAC in February 2018 concerning Lupus erythematosus. The applicant updated the SmPC and PIL to add the information proposed by PRAC.

The applicant has also investigated the available pharmacovigilance data, using Eudravigilance database (L1 level access) to discuss further the post-marketing experience and add any new identified issues in the SmPC, in order to complete the safety profile of hydroxycarbamide. It was concluded from the review that there are currently no additional data concerning possible signals. The established safety profile of hydroxycarbamide is confirmed both for the oncology indications and for the SCD indication (although to a lesser degree as there are fewer cases to examine).

The safety profile of all products containing hydroxycarbamide in the EEA has been recently thoroughly reassessed through the relevant PSUSA procedures. The assessment of the PSUR of Siklos (centrally-authorised product, SCD indication) was finalised in January 2018 without any variation. In September 2018, the assessment of the PSUR of non-centrally authorised hydroxycarbamide products, including Hydrea, was finalised with a recommendation for variation (update of sections 4.4 and 4.8 of the SmPC to add the adverse reaction interstitial lung disease with a frequency unknown and to add a warning on patients with respiratory symptoms. Update of section 4.4 of the SmPC to add a warning concerning skin cancer. The Package Leaflet to be updated accordingly). It should be noted that two of the products in the PSUSA are indicated also for SCD.

Overdose

After an acute oral overdose, nausea, vomiting, diarrhoea and constipation may occur but toxicity is mainly due to bone marrow suppression. Megaloblastic changes are detectable within 48 hours of dosing. Reversible myelosuppression affecting all haematological compartments (but particularly the white cells) may develop 1-2 weeks after exposure especially following doses greater than 800 mg/m^2. Lower doses will produce myelosuppression if renal function is impaired. Acute mucocutaneous toxicity (hand and foot erythema and scaling, generalised hyperpigmentation and acute stomatitis) has also been reported (Toxbase Hydroxycarbamide Monograph).

A 2-year-old child ingested at one time an entire 35-day supply of hydroxycarbamide (612 mg/kg body weight). Despite a serum level of 7,756 µM 4 hours post-ingestion, the only toxicity was transient mild myelosuppression (Miller *et al,* 2012).

A 3-year-old child who ingested up to 9 g of his mother's hydroxycarbamide was treated with gastric lavage and activated charcoal within 1 hour of ingestion. He remained asymptomatic, and his initial blood counts demonstrated only mild leukocytosis (usual for the patient). His WBC dropped to low normal values at 2 weeks after ingestion, and spontaneously returned to his usual values within two weeks afterwards (Cole *et al,* 2010).

A 50-year-old female receiving hydroxycarbamide treatment for thrombocythaemia took an overdose of up to 60 g of hydroxycarbamide with ethanol. She presented with agitation, myoclonic jerks and hyper-reflexia. She was drowsy at presentation, becoming unconscious with GCS 5/15 at 12 hours after overdose, when she also developed an oculogyric crisis, which was responsive to procyclidine. Blood counts started dropping at 5 days after presentation, and neutrophils reached a nadir that was below detectable limits at day 8, after which she gradually recovered. She had been treated with G-CSF starting from day 7 after presentation. She was discharged after 11 days when her full blood count stabilised, and hydroxycarbamide treatment was subsequently reintroduced (Litt *et al,* 2013).

Pregnancy and lactation

In a 17 year follow up study, 94 pregnancy outcomes were reported; 52 in 28 females exposed to hydroxycarbamide and 42 in female partners of 27 male patients exposed to hydroxycarbamide.

No relationship between hydroxycarbamide usage and neonatal abnormalities or teratogenic effects was found. Foetuses of those pregnancies that were not terminated and resulted in full-term or premature live births were normal with no evidence of any neonatal abnormalities or teratogenic effects. This seems to be true whether the parent taking hydroxycarbamide was the mother or father. Additional studies including much longer follow-up of many more hydroxycarbamide exposed sickle cell disease patients are required to establish these results conclusively.

Evidence from a patient taking hydroxycarbamide for myelogenous leukaemia shows that hydroxycarbamide is excreted into human breast milk. The patient was receiving 500 mg orally 3 times daily, with milk samples collected 2 hours following the last dose of the drug each day for 7 days. Only 3 samples were reported to clear adequately following the extraction process to ensure reliable spectrophotometric readings; these concentrations were 6.1, 3.8, and 8.4 mg/L on days 1, 3, and 4, respectively (mean, 6.1 mg/L). At these concentrations, it was calculated 3 to 4 mg/day would be received by the infant. Although amounts ingested by a breastfeeding infant would appear to be low, it is recommended that hydroxycarbamide be avoided during the breastfeeding period, with the pre-clinical evidence of teratogenic effects and the undetermined clinical effects of exposure (Sylvester et al 1987).

Other safety issues

Strawberry flavour excipient

Strawberry liquid flavour is a synthetic flavour. The strawberry flavour is a mixture of various natural and synthetic flavouring substances along with other ingredients.

The manufacturer has provided a number of compliance statements. The strawberry flavour utilises ingredients that are listed as GRAS, comply with the provisions of the EU Regulation 1334/2008 on flavourings, and all flavouring substances are listed in Annex I Part A of this Regulation, as amended (Union list of flavouring substances adopted by EU Regulation No. 872/2012). They also confirm that any additive used in the manufacture of the strawberry flavour is listed in Annex II Part B and/or Annex III Part 4 of Regulation (EC) No 1333/2008 on Food Additives and is in compliance with the specifications set out in Commission Regulation (EU) No 231/2012 laying down specifications for food additives, both regulations as amended. The flavour is confirmed to be TSE free. The strawberry flavour does not contain any EU allergens nor does it contain any ingredient for which there may be concern for paediatric use (propylene glycol, benzyl alcohol, ethanol and colours).

The levels of strawberry flavour included in the formulation is within the range recommended by the manufacturer and, in combination with the sucralose, was determined through informal, internal taste testing. The palatability (taste, aftertaste, smell, texture, ease of use and other comments) of the final formulation was assessed by questionnaire as part of the bioequivalence study (RD 729/26118) following completion of dosing. Completion of the questionnaire by the participants was optional. Thirteen (out of 28) subjects completed the questionnaire. Of the subjects who completed the palatability questionnaire, the majority thought the test IMP tasted good (38.5%) or very good (46.2%), with no aftertaste (84.6%) or smell (53.8%) and with a pleasant (38.5%) or very pleasant (30.8%) texture that 84.6% stated would find very easy to take regularly.

In addition, an evaluation of the ‘acceptability and palatability’ of Hydroxycarbamide Nova Laboratories in the paediatric SCD population will be conducted as part of a planned observational PK study in children aged 6 months – 18 years.

Syringes

The possibility of a medication error because of the inclusion of two different syringes in the same package was highlighted during evaluation. The applicant updated the product information in order to

decrease this possibility. Explanation text with guidance on which syringe to use in each case has been incorporated into the SmPC, Section 4.2 Method of Administration, and a diagram showing the package contents and the different color of the syringes has been added in the PIL. If a patient has to receive 12-15 ml, the combined use of the two syringes will provide a higher degree of accuracy.

The two syringes cover the required volumes for children 2-9 years old. However, the product's indication is not only for children, but for adults as well. An adult weighing e.g. 80 kg would need 28 ml of liquid for a 35 mg/kg dose, meaning that the 12 ml syringe should be used three times. The Applicant claims that few adults tolerate the maximum 35 mg/kg/day dose. Indeed, long term studies show that the mean maintenance dose in SCD patients is approximately 20 mg/kg/day (Voskaridou *et al* 2010, Rigano *et al* 2018), so that a typical 80 kg patient would need 16 ml of liquid, achievable with two uses of the 12 ml syringe. The syringe volume should be chosen to serve even the extreme cases (e.g. maximum 35 mg/kg/day dose). However, it is acknowledged that the choice of a larger size that would result in maximum of two uses (e.g. 18 ml) would result in an advantage for a few patients with a possible risk (inaccurate dose) for more patients. Section 4.2 of the SmPC provides a recommendation that in adults without swallowing difficulties, solid oral formulations may be more appropriate and convenient.

Regarding ease of use of the syringes, information on all centrally authorised products and authorised products in UK and Greece that are administered with syringes was provided. Among these there are no products administered with 12 ml syringe, but there are 3 products administered with 20 ml syringes. A 12 ml syringe will not be harder to use than a 20 ml syringe. Therefore, the proposed volume is acceptable. A usability and clinical performance statement by the supplier has been submitted. Regarding reproducibility, a submitted reproducibility study shows that the syringes deliver accurately the intended volume.

2.4.9. Discussion on clinical safety aspects

The applicant has provided data from a comparative safety study, RCTs, and observational studies that altogether have included a substantial number of patients. Most of these studies have been conducted after the authorisation of Siklos in EU (2007) and can therefore broaden the body of information on hydroxycarbamide safety obtained till then.

In general, hydroxycarbamide shows mild toxicity. The most frequent AEs reported in the submitted studies are cytopenias, skin disorders, gastrointestinal disorders and neurological disorders. Cytopenias, that are caused by bone marrow depression, a pharmacological effect of hydroxycarbamide, are mild, transient and reversible. The safety profile of adults and children appears to be similar. The most common side effects are bone marrow depression including neutropenia, reticulocytopenia, macrocytosis, thrombocytopenia, anaemia, headache, dizziness, nausea, constipation, skin ulcer, oral, nail and skin hyperpigmentation, dry skin and alopecia.

Preclinical toxicity studies have demonstrated the most commonly observed effects include bone marrow depression in rats, dogs and monkeys. Therefore a warning has been added in section 4.4 of the SmPC. The complete status of the blood, including bone marrow examination, if indicated, as well as kidney function and liver function should be determined prior to, and repeatedly during, treatment. If bone marrow function is depressed, treatment with hydroxycarbamide should not be initiated. Continuous follow-up of the growth of treated children and adolescents is recommended. The full blood cell count with white cell differential, reticulate count, and platelet count should be monitored regularly (see section 4.2 of the SmPC). Hydroxycarbamide may produce bone marrow suppression; leukopenia is generally its first and most common manifestation. Thrombocytopenia and anaemia occur less often and are seldom seen without a preceding leukopenia. Bone marrow depression is more likely in patients who have previously received radiotherapy or cytotoxic cancer chemotherapeutic medicinal products; hydroxycarbamide should be used cautiously in such patients. The recovery from myelosuppression is rapid when

hydroxycarbamide therapy is interrupted. Hydroxycarbamide therapy can then be re-initiated at a lower dose (see section 4.2). Xromi is contraindicated in toxic ranges of myelosuppression as described in section 4.2. of the SmPC.

Severe anaemia must be corrected with whole blood replacement before initiating therapy with hydroxycarbamide. If, during treatment, anaemia occurs, correct without interrupting hydroxycarbamide therapy. Erythrocytic abnormalities; megaloblastic erythropoiesis, which is self-limiting, is often seen early in the course of hydroxycarbamide therapy. The morphologic change resembles pernicious anaemia, but is not related to vitamin B_{12} or folic acid deficiency. The macrocytosis may mask the incidental development of folic acid deficiency; regular determinations of serum folic acid are recommended. Hydroxycarbamide may also delay plasma iron clearance and reduce the rate of iron utilisation by erythrocytes but it does not appear to alter the red blood cell survival time.

Hydroxycarbamide affects spermatogenesis, and hence oligospermia and azoospermia are very commonly reported.

Although there are concerns that hydroxycarbamide might be leukemogenic, submitted data do not support this hypothesis. Teratogenesis has not been observed in humans, however, because of the small number of studied births and of malformations observed in animals, a warning is given. Excipients present in the product do not pose safety dangers. Xromi is contraindicated in pregnant and breast feeding women (see section 4.3 and 4.6 of the SmPC).

Xromi is also contraindicated with concomitant use of anti-retroviral medicinal products for HIV disease (see sections 4.3, 4.4 and 4.5 of the SmPC).

Since renal excretion is a pathway of elimination, consideration should be given to decreasing the dosage of hydroxycarbamide in renally impaired patients. In patients with a creatinine clearance (CrCl) ≤ 60 ml/min the initial hydroxycarbamide dose should be decreased by 50%. Close monitoring of blood parameters is advised in these patients (see section 4.4 of the SmPC). Hydroxycarbamide must not be administered to patients with severe renal impairment (CrCl < 30 ml/min) (see sections 4.2, 4.3, 4.4, and 5.2).

There are no data that support specific dose adjustments in patients with hepatic impairment. Close monitoring of blood parameters is advised in these patients. Due to safety considerations, hydroxycarbamide is contraindicated in patients with severe hepatic impairment (see sections 4.2, 4.3 and 4.4).

Hydroxycarbamide must not be used in combination with anti-retroviral medicinal products for HIV disease and it may cause treatment failure and toxicities (in some cases fatal) in HIV patients (see sections 4.3, 4.4 and 4.5 of the SmPC).

Concomitant use of hydroxycarbamide with a live virus vaccine may potentiate the replication of the vaccine virus and/or may increase some of the adverse reactions of the vaccine virus because normal defence mechanisms may be suppressed by hydroxycarbamide. Vaccination with a live vaccine in a patient taking hydroxycarbamide may result in severe infection. The patient's antibody response to vaccines may be decreased. The use of live vaccines should be avoided during treatment and for at least six months after treatment has finished and individual specialist advice sought (see section 4.4 and 4.5 of the SmPC).

Hydroxycarbamide is unequivocally genotoxic in a wide range of test systems. Hydroxycarbamide is presumed to be a transspecies carcinogen (see section 4.4 and 5.3 of the SmPC).

Management of an acute hydroxycarbamide overdose should ideally be discussed with the physician responsible for prescribing cytotoxic drug or a haematologist. The benefit of gastric decontamination, e.g. activated charcoal is uncertain, but nevertheless should be considered if the patient presents within 1

hour of ingestion of any amount (because of risk of myelosuppression at all doses). Patients should be monitored for vital signs, blood and urine chemistry, renal and hepatic function and full blood counts for at least 3 weeks.

2.4.10. Conclusions on clinical safety aspects

The safety profile of the product is well described.

2.5. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	Effects on male fertility- Oligospermia and azoospermia with normal morphology and reduced semen volume Myelosuppresion
Important potential risks	Mutagenicity and Carcinogenicity – Secondary cancers (e.g. leukaemias) Off-label use in the oncology indications not approved for this formulation (with the tablet/capsule formulation) Off-label use for chronic severe anaemia – thalassemia/polycythaemia Potential medication errors – Transfer of patients from capsule and tablet to liquid formulation and two dosing syringes Skin ulceration and vasculitis Off-label use in children <2 years old Concurrent use of hydroxycarbamide with nucleoside analogue reverse transcriptase inhibitors and other myelosuppressive medicinal products or radiation therapy Interaction with live bacterial or virus vaccines The effect on embryogenesis, teratogenic potential, breastfeeding and post-natal development Safety of hydroxycarbamide in patients with underlying hepatic or renal impairment Lupus erythematosus
Missing information	Influence of hydroxycarbamide in child and adolescent growth (end of puberty)

Pharmacovigilance plan

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 3 - Required additional pharmacovigilance activities				
Phase I/II open-label observational, PK study Post current MA application	Primary objective: To determine the PK of oral hydroxycarbamide solution in children aged 6 months up to 18 years. Secondary objectives: To evaluate the efficacy/safety of oral hydroxycarbamide solution and the acceptability and palatability	Assess effects of oral solution on: • Myelosuppression • Carcinogenicity - Secondary cancers (e.g., leukaemias) • Potential medication errors – Transfer of patients from capsule and tablet to liquid formulation and two dosing syringes • Skin ulceration and vasculitis (leg ulcers) • Concurrent use of hydroxycarbamide with nucleoside analogue reverse transcriptase inhibitors and other myelosuppressive medicinal products or radiation therapy • Interaction with live bacterial or virus vaccines •Effects on male fertility- low sperm count (oligospermia) and absence of sperms (azoospermia) with normal shape and reduced semen volume • Safety of hydroxycarbamide in patients with underlying hepatic or renal impairment *Renal and hepatic impairment are*	First patient in Last patient in Last patient follow-up Final Report	January 2019 Q1 2020 Q3 2021 Q1 2022

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
		exclusion criteria for this study. If any cases are identified during the study, they will be reported and followed up appropriately. •Lupus erythematosus •Influence of hydroxycarbamide in child and adolescent growth (end of puberty) •The effect on embryogenesis, teratogenic potential, breastfeeding and postnatal development: *Pregnancy is an exclusion criterion for the study. Any pregnancies reported during the study will be followed up appropriately and relevant data will be included.*		
HCP Survey (questionnaire on additional risk minimisation measures)	Primary objective: To assess HCPs understanding of the content of the educational materials distributed as an additional risk minimization measure.	•Effects on male fertility- low sperm count (oligospermia) and absence of sperms (azoospermia) with normal shape and reduced semen volume •Myelosuppression •Mutagenicity and Carcinogenicity – Secondary cancers (e.g., leukaemias) •Potential medication errors – Transfer of patients from capsule and tablet to liquid	Protocol of the HCP Survey submitted Analysis and Final report	No later than 12 months from the date of product launch 8- 12 months from the protocol approval 20/03/2020

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
		formulation and two dosing syringes. •Skin ulceration and vasculitis •Concurrent use of hydroxycarbamide with nucleoside analogue reverse transcriptase inhibitors and other myelosuppressive medicinal products or radiation therapy •The effect on embryogenesis, teratogenic potential, breastfeeding and post-natal development •Safety of hydroxycarbamide in patients with underlying hepatic or renal impairment •Influence of hydroxycarbamide in child and adolescent growth (end of puberty)		

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Effects on male fertility-Oligospermia and azoospermia with normal morphology and reduced semen volume	Routine risk minimisation measures: • SmPC Sections 4.6, 4.8, • PIL Section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: Male patients should be informed	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: None

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	by their healthcare professionals about the possibility of sperm conservation (cryopreservation) before the start of therapy. This recommendation has been added to Section 4.6 of the Xromi 100 mg oral solution SmPC and to Section 2 of the Xromi 100 mg/mL oral solution PL. Additional risk minimisation measures: Educational materials for physicianand patient/parent	
Myelosuppresion	Routine risk minimisation measures: • SmPC Sections 4.3, 4.4, 4.5, 4.8, • PIL Section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: Regular monitoring of blood counts throughout the treatment period and discontinuation of hydroxycarbamide as felt necessary by the treating physician. Recovery from myelosuppression is usually rapid when therapy is discontinued. Treatment can then be re-initiated at a lower dose. Additional risk minimisation measures: Educational materials for physician and patient/parent	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label observational, PK study.
Mutagenicity and Carcinogenicity – Secondary cancers (e.g. leukaemias)	Routine risk minimisation measures: • SmPC Sections 4.4, 4.8, • PIL Section 4 Additional risk minimisation measures:	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	Educational materials for physician and patient/parent	activities: The Phase I/II open label observational, PK study.
Off-label use in the oncology indications not approved for this formulation (with the tablet/capsule formulation)	Routine risk minimisation measures: • SmPC Section 4.1, • PIL Section 1 Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: None
Off-label use for chronic severe anaemia – thalassemia/polycythaemia	Routine risk minimisation measures: SmPC Section 4.1, PIL Section 1 Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: None
Potential medication errors – Transfer of patients from capsule and tablet to liquid formulation and two dosing syringes	Routine risk minimisation measures: • SmPC Section 4.2, • PIL Section 3 Additional risk minimisation measures: Educational materials for physician and patient/parent	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label observational, PK study.
Skin ulceration and vasculitis	Routine risk minimisation measures: • SmPC Sections 4.4, 4.8, • PIL Sections 2, 4 Routine risk minimisation activities recommending specific clinical measures to address the risk: Hydroxycarbamide should be discontinued and/or its dose should be reduced if cutaneous vasculitic ulcerations develop. Xromi should be used with caution in patients with leg	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label observational, PK study.

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	ulcers. Avoid and/or cautious concurrent use of interferon. Additional risk minimisation measures: Educational materials for physician and patient/parent	
Off-label use in children <2 years old	Routine risk minimisation measures: • SmPC Section 4.1, • PIL Sections 1, 3 Additional risk minimisation measures: Educational materials for physician and patient/parent	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: None
Concurrent use of hydroxycarbamide with nucleoside analogue reverse transcriptase inhibitors and other myelosuppressive medicinal products or radiation therapy	Routine risk minimisation measures: • SmPC Sections 4.4, 4.5, • PIL Section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: To avoid concurrent use of Xromi with the medicinal products listed in Section, 4.4 and 4.5 of SmPC, unless the benefit of concomitant use outweighs risks and to tell you doctor, pharmacist or nurse if you are on any such medication. Additional risk minimisation measures: Educational materials for physician and patient/parent	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label observational, PK study.
Interaction with live bacterial or virus vaccines	Routine risk minimisation measures: • SmPC Sections 4.4, 4.5 • PIL Section 2 Routine risk minimisation activities recommending specific clinical measures to address the	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	risk: To avoid concurrent use of Xromi with the live vaccines as listed in Section 4.5 of SmPC, unless the benefit of concomitant use outweighs risks and to tell you doctor, pharmacist or nurse if you had recent live vaccine or planning to in near future. Additional risk minimisation measures: None	observational, PK study.
The effect on embryogenesis, teratogenic potential, breastfeeding and post-natal development	Routine risk minimisation measures: • SmPC Sections 4.3, 4.6, • PIL Section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: The patient should be instructed to immediately contact a doctor in case of suspected pregnancy. Effective method of contraception for both male and female patients in reproductive age group. Discontinuation of Xromi 3 to 6 months before, if patient on treatment want to conceive. PL Section 2 instructions - Please contact your doctor immediately if you think you may be pregnant. Additional risk minimisation measures: Educational materials for physician and patient/parent	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label observational, PK study.
Safety of hydroxycarbamide in patients with underlying hepatic or renal impairment	Routine risk minimisation measures: • SmPC Sections 4.2, 4.3, 5.2, • PIL Section 2 Routine risk minimisation	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities:

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	activities recommending specific clinical measures to address the risk: Close monitoring of blood parameters for renal and hepatic impairment and discontinuation of Xromi as deemed necessary by the treating physician. Re-starting Xromi at lower dose as deemed necessary by treating physician. Additional risk minimisation measures: Educational materials for physician and patient/parent	None
Lupus erythematosus	Routine risk minimisation measures: • SmPC Section 4.8, • PIL Section 4 Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label observational, PK study.
Influence of hydroxycarbamide in child and adolescent growth (end of puberty)	Routine risk minimisation measures: • SmPC Section 4.4 Additional risk minimisation measures: Educational materials for physician and patient/parent	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: All reports will be presented in the PSUR. Additional pharmacovigilance activities: The Phase I/II open label observational, PK study.

Conclusion

The CHMP and PRAC considered that the risk management plan version 1.0 is acceptable.

2.6. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.7. Product information

2.7.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

3. Benefit-risk balance

This application concerns a hybrid version of Hydrea 500mg. The reference product Hydrea is indicated for treatment of chronic myeloid leukaemia and cancer of the cervix in conjunction with radiotherapy. From a clinical perspective, this application does not contain new data on the pharmacokinetics and pharmacodynamics as well as the efficacy and safety of the active substance; the applicant's clinical overview on these clinical aspects based on information from published literature was considered sufficient.

One bioequivalence study forms the pivotal basis with cross-over design under fasting conditions. The study design was considered adequate to evaluate the bioequivalence of this formulation and was in line with the respective European requirements. Choice of dose, sampling points and overall sampling time as well as wash-out period were adequate. The analytical method was validated. Pharmacokinetic and statistical methods applied were adequate.

The test formulation of Xromi met the protocol-defined criteria for bioequivalence when compared with Hydrea. The point estimates and their 90% confidence intervals for the parameters AUC0-t, AUC0-72h, and Cmax were all contained within the protocol-defined acceptance range of [range, e.g. 80.00 to 125.00%]. Bioequivalence of the two formulations was demonstrated.

3.1. Therapeutic Context

3.1.1. Disease or condition

Xromi is intended for the prevention of vaso-occlusive complications of Sickle Cell Disease in patients over 2 years of age.

3.1.2. Available therapies and unmet medical need

SCD management consists of patient health maintenance and monitoring, symptom control, and disease-modifying treatment. Disease-modifying treatment includes blood transfusion and oral hydroxycarbamide administration. Because chronic blood transfusion is inevitably associated with iron overload, iron chelating drugs are administered e.g. desferrioxamine and deferasirox. Haematopoietic stem cell transplantation (HSCT) is recognised as the only cure. However, it is a rather new approach (25 years old) that has been applied only to a few hundred patients worldwide.

In 2007 Siklos 100 mg film-coated tablets was granted authorisation in European Union, with the indication of "prevention of recurrent painful vaso-occlusive crises including acute chest syndrome in

adults, adolescents and children older than 2 years suffering from symptomatic Sickle Cell Syndrome". Hydroxycarbamide Nova Laboratories 100 mg/ml oral solution covers the unmet medical need of a palatable oral liquid formulation for children and patients with dysphagia.

3.1.3. Main clinical studies

The applicant has conducted a bioequivalence study comparing Hydroxycarbamide Nova Laboratories 100 mg/mL oral solution to Hydrea capsules (UK and USA). Thirty (30) healthy subjects participated.

Four main clinical studies were submitted by the applicant: MSH (Charache *et al* 1995), Jain *et al* 2012, Ferster *et al* 1996, TWiTCH (Ware *et al* 2015).

The MSH study was a randomised, double-blind, placebo-controlled trial involving 299 adults with SCA who had experienced three or more VOCs in the previous year. The clinical end point of three or more documented VOCs was chosen because of earlier data documenting that people who experience pain at that frequency had markedly lower survival rates. The trial was conducted to test the hypothesis that Jain *et al* 2012)

Jain *et al* 2012 was a randomised, double blind, placebo controlled study conducted in a tertiary hospital in India. The study was conducted in children with sickle cell anemia (proportion with each genotype not stated) between the ages of 5 and 18 years with three or more blood transfusions or vaso-occlusive crises requiring hospitalization per year despite high HbF. The primary endpoint was the decrease in vaso-occlusive crises per patient per year. Secondary outcomes were a decrease in frequency of blood transfusions and hospitalizations and an increase in HbF levels.

Ferster et al 1996 was a placebo-controlled, randomized, crossover study. It was conducted in a single centre in Belgium and involved 25 children and young adults (age range: 2 to 22 years) with HbSS genotype and severe clinical manifestations (defined as >3 vaso-occlusive crises in the year before study entry and/or with previous history of stroke, acute chest pain, recurrent crises without a free interval, or splenic sequestration) with the primary end-point of number of hospitalizations and number of days in hospital.

TWiTCH was a NHLBI-funded Phase III multicentre RCT comparing 24-months of standard treatment (transfusions) to alternative treatment (hydroxycarbamide) in 121 children with SCA and abnormal TCD velocities. All eligible children had received at least 12 months of transfusions and did not have severe vasculopathy. TWiTCH had a non-inferiority trial design; the primary study endpoint was the 24-month TCD velocity obtained from a linear mixed model, controlling for baseline (enrolment) values, with a non-inferiority margin of 15 cm/s.

Regarding safety, the profile for hydroxycarbamide in people with SCD is derived from the bioequivalence study that was conducted by the applicant and enrolled 30 people and literature data (3 RCTs that enrolled 517 people, and almost 50 observational studies that enrolled more than 3,000 people). In addition, in patient populations other than SCD, toxicity evidence is derived from 21 published RCTs that enrolled more than 4,800 individuals and 35 observational studies that enrolled more than 7,500 individuals. Among all these studies, the bioequivalence study and the ESCORT-HU study (De Montalembert et al 2014) can be regarded as the main safety studies based on the fact that they were the only ones to tabulate the AEs according to SOC and frequency. ESCORT-HU is a multicentre prospective non-interventional study implemented in Europe involving the largest number of patients so far with SCD treated with hydroxycarbamide. Primary endpoints of ESCORT hydroxycarbamide were to determine frequency of adverse events, and possible consequent changes of hydroxycarbamide treatment. Secondary endpoints were to evaluate morbidity and mortality of the disease although in the absence of control group. From June 2008 to June 2014, 483 patients (255 females; 228 males) were enrolled from 3 European countries, Greece (24%), Germany (19%), and France (56%). 67% patients were adults,

median aged 37.35 years (17-83.5) and 33% were children, median aged 11.06 years (2.6-16.9). Genotypes were HbSS in 71.4% cases, and compound heterozygous HbS/β-thalassemia in 22.8%.

3.2. Favourable effects

The bioequivalence study conducted by the applicant showed that Xromi 100 mg/ml oral solution is bioequivalent to Hydrea capsules. This means that bridging with Hydrea-based literature is possible.

MSH trial showed beneficial effects to patients receiving hydroxycarbamide compared to those receiving placebo. A difference of 44% in the frequency of painful VOCs per year (2.5 vs 4.5, P<0.001) was observed in favor of hydroxycarbamide. When taking into account only crises severe enough to cause hospitalisation, this difference increased to 58% (1.0 vs 2.4, P<0.001). Also, a statistically significant elongation of the time until the first (3.0 vs 1.5, P=0.01) and second VOC (8.8 vs 4.6, P<0.001) was observed in patients receiving hydroxycarbamide. Finally, the number of patients in whom chest syndrome developed was significantly smaller when receiving hydroxycarbamide (25 vs 51, P<0.001).

Jain *et al* 2012 study showed that hemoglobin levels and HbF percentages increased significantly in the HU treated group compared to baseline of HU group and 18 months of placebo group. With HU therapy, event rates per patient per year for vaso-occlusive crises, blood transfusions and hospitalizations decreased by 95.0, 94.6 and 93.1%, respectively. When compared with the placebo group, patients treated with HU therapy had 94.0, 93.4 and 89.5% lesser vaso-occlusive crises, blood transfusions, and hospitalizations, respectively. Even among hospitalized patients, the duration of hospitalization was less in the HU group (3.1 ± 1.2 days) than their counterparts in the placebo group (7.1 ± 2.1 days).

Ferster *et al* 1996 showed that the number of hospitalizations was significantly reduced when patients were on HU therapy as compared with placebo. Combining the results of both periods, 16 patients of 22 (73%) did not require any hospitalisation for painful episodes when treated with HU as compared with only 3 of 22 (14%) when treated with placebo. The number of days in hospital was also significantly lower when patients were on HU therapy (range, 0 to 19 days) than when they were on placebo (range, 0 to 104 days). During the first 6-month period, the mean number of days in hospital was 5.3 days for HU, as compared with 15.2 days for placebo; during the second 6-month period, these figures were, respectively, 1.8 days for HU and 8.2 days for placebo.

TWiTCH trial showed non-inferiority ($P=8.82\times10^{-16}$) for hydroxycarbamide compared to standard transfusions when the final model-based TCD velocity difference was assessed (4.54 cm/s, 95% CI: 0.10–8.98 cm/s). TCD acts as a surrogate for stroke risk. Also, the difference of liver iron concentration from baseline was more favorable for the hydroxycarbamide treatment (final concentration difference between the two treatments was 4.3 mg Fe per g dry weight liver, P<0.001).

3.3. Uncertainties and limitations about favourable effects

Patients that participated in the MSH, Jain *et al* 2012, and Ferster *et al* 1996 trials were suffering from SCA; patients with different genotypes than HbSS and HbSβ0 thalassemia did not participate. This means that based only on this trial, an indication to all SCD patients (including rarer genotypes) is not supported. However, supportive studies that have been submitted can be used to extrapolate the indication to SCD.

In the investigational arm of TWiTCH trial 60 children were treated with hydroxycarbamide. The median duration of transfusion history in this arm was 4.5 years. The follow-up of the trial is also relatively short (24 months) for children that have been transfused so extensively before enrolment and continued to receive transfusions during an overlap period the duration of which was defined 4-9 months. Even the authors suggest that the optimal duration of transfusions prior changing to hydroxycarbamide has not been defined and that the follow-up period might be short to reveal any reversion of TCD as it was shown in the non-randomised trial of Bernaudin *et al* 2016 with a longer follow-up. Bernaudin *et al* 2016, a

supportive study giving positive results for 45 patients, has differences with TWiTCH study e.g. only patients with normalised velocities and no stenosis were switched to hydroxycarbamide, MTD was not always reached etc. Bernaudin *et al* 2016 was a longitudinal prospective observational cohort study with no control group. Effectiveness or net benefits cannot be inferred. Therefore the initially applied indication for primary stroke prevention was not accepted as such. The following indication was agreed by CHMP: "Prevention of vaso-occlusive complications of Sickle Cell Disease in patients over 2 years of age". This indication leaves the freedom to clinicians to use it for primary stroke prevention according to published studies and guidelines.

3.4. Unfavourable effects

Regarding safety, hydroxycarbamide is an active substance that has been used for many years in SCD patients, both off-label (Hydrea) and authorised (Siklos, from 2007 in European Union). This means that its safety profile is well characterised.

The majority of the unfavourable effects of the active substance concerning AEs derive from literature data. In general, hydroxycarbamide shows mild toxicity. The most frequent AEs reported in the submitted studies are cytopenias, skin disorders, gastrointestinal disorders and neurological disorders. Cytopenias, that are caused by bone marrow depression, a pharmacological effect of hydroxycarbamide, are mild, transient and reversible. The safety profile of adults and children appears to be similar. Although there are concerns that hydroxycarbamide might be leukemogenic, submitted data do not support this hypothesis. Teratogenesis has not been observed in humans, however because of the small number of studied births and of malformations observed in animals, a contraindication has been added in section 4.3 of the SmPC.

The two important identified risks are oligospermia/azoospermia in males and myelosuppression. Many important potential risks have been recognised concerning Xromi. These include mutagenicity and carcinogenicity (although according to submitted literature it seems that there is no significant danger), off-label use in the oncology indications not approved for this formulation, off-label use for chronic severe anaemia–thalassemia/polycythaemia, potential medication errors (conversion of patients from tablet to liquid formulation, two dosing syringes), skin ulceration and vasculitis, off-label use in children <2 years old, concurrent use of hydroxycarbamide and other myelosuppressive medicinal products or radiation therapy, interaction with live bacterial or virus vaccines, the effect on embryogenesis, teratogenic potential, breastfeeding and post-natal development, safety of hydroxycarbamide in patients with underlying hepatic or renal impairment and lupus erythematosus.

3.5. Uncertainties and limitations about unfavourable effects

Regarding safety, the applicant has submitted data from both Hydrea and non-Hydrea literature. Since the Applicant was able to show that non-Hydrea literature can be used based on the Quality attributes of non-Hydrea formulations (dissolution, excipients) all submitted data are acceptable.

There is missing information in literature concerning long-term safety effects in children. This will be obtained and evaluated through PSUR.

3.6. Benefit-risk assessment and discussion

3.6.1. Importance of favourable and unfavourable effects

Three studies (384 adults and children with HbSS or HbSβ⁰thal) compared hydroxycarbamide to placebo, recruiting individuals with severe disease (MSH trial; Jain *et al* 2012; Ferster *et al* 1996). There was statistically significant reduction in vaso-occlusive crises and acute chest syndrome. There were no consistent statistically significant differences in terms of quality of life and adverse events (including

serious or life-threatening events). Seven deaths occurred during the studies, but the rates by treatment group were not statistically significantly different. Supportive studies help extrapolating the positive results to SCD patients. In the TWiTCH study, hydroxycarbamide was compared with transfusion in children with abnormal TCD velocities who had received transfusion for at least one year and had no evidence of vasculopathy on magnetic resonance angiography (MRA). Hydroxycarbamide was as effective as transfusion in preventing further stroke and final TCD velocity was lower in the hydroxycarbamide group. However, there are limitations on this favourable effect, as described above. Therefore the initially applied indication for primary stroke prevention was not accepted as such. The following indication was agreed by CHMP: "Prevention of vaso-occlusive complications of Sickle Cell Disease in patients over 2 years of age". This indication leaves the freedom to clinicians to use it for primary stroke prevention according to published studies and guidelines. The bioequivalence shown between Hydroxycarbamide Nova Laboratories 100 mg/mL and Hydrea capsules is useful for bridging with Hydrea-based literature data. *In vitro* dissolution tests with non-Hydrea formulations used in the literature, the presence of excipients not affecting bioavailability, and the fact that hydroxycarbamide was shown to be highly soluble-highly permeable compound, allow bridging also with non-Hydrea literature.

Regarding safety, hydroxycarbamide profile is well characterised and shows mild toxicity.

3.6.2. Balance of benefits and risks

Overall, the benefit-risks of Xromi is considered positive in the proposed indication.

3.6.3. Additional considerations on the benefit-risk balance

Not applicable.

4. Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Xromi is favourable in the following indication:

"Hydroxycarbamide Nova Laboratories is indicated for the prevention of vaso-occlusive complications of Sickle Cell Disease in patients over 2 years of age."

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk Management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Additional risk minimisation measures

Prior to launch of Xromi in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The educational programme is aimed to ensure the safe and effective use of the product, to minimise the risks listed below and to reduce the burden of adverse reactions with Xromi.

The MAH shall ensure that in each Member State where Xromi is marketed, all healthcare professionals and patients/carers who are expected to prescribe and use Xromi have access to/are provided with the following educational package to be disseminated through professional bodies:

- Physician educational material
- Patient information pack

The **physician educational material** should contain:

- The Summary of Product Characteristics
- Guide for healthcare professionals

The Guide for healthcare professionals shall contain the following key elements:

- Indication, dosage and dose adjustment;
- Description of safe handling of Xromi, including the risk of medication error due to the use of two different dosing syringes;
- Warnings about important risks associated with using Xromi:
    - Switching patients from capsule and tablet to liquid formulation;
    - Haematological toxicity, use of other myelosuppressive agent or radiation therapy;
    - Concomitant use of nucleoside analogue reverse transcriptase inhibitors;
    - Skin ulceration and vasculitis, leg ulcers;

    - Long-term safety, in particular the development of malignancies (leukaemia, skin cancer);
    - Teratogenicity and male fertility; Need for contraception; Breast feeding;
    - Adverse drug reactions most frequently reported;
- Follow-up of patients treated with Xromi:
    - Haematological follow-up and dose adjustment;
    - Follow-up of patients with renal and/or hepatic impairment;
    - Growth follow-up in children;

The **patient information** pack should contain:

- Patient information leaflet
- A patient/carer guide

The Patient/carer guide shall contain the following key elements:

- Indication;
- Instructions for proper and safe use of the product, including clear instructions on the use of two different dosing syringes to avoid the risk of medication error;
- Follow-up of patients treated with Xromi:
    - Need for periodic blood counts; Other medicines that suppress the bone marrow and radiation therapy;
    - Concomitant use of anti-retroviral medicines;
    - Skin ulceration and vasculitis, leg ulcers;
    - Long-term safety, in particular the development of malignancies (leukaemia, skin cancer);
    - Liver and/or kidney impairment;
    - Teratogenicity and male fertility; Need for contraception; Breast feeding;
    - Growth follow-up in children;
- Key signs and symptoms of serious adverse reactions;
- Information on crisis or infections; When to seek urgent attention from the health care provider;

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

Paediatric data

Not applicable.