PASS INFORMATION

Title	ESCORT-HU Extension: European Sickle Cell Disease Cohort – Hydroxyurea – Extension study
Version identifier of the interim study report	Interim Report #4_V1.0
Date of last version of the interim study report	05-Septembre-2024
EU PAS register number (Internal reference)	EUPAS1000000066 (SIK-EU-20-1)
Active substance	L01XX05, hydroxycarbamide/hydroxyurea (HU)
Medicinal product	Siklos® (Hydroxycarbamide/hydroxyurea)
Product reference	*Siklos 100 mg film-coated tablets:* EU/1/07/397/002 *Siklos 1000 mg film-coated tablets:* EU/1/07/397/001
Procedure number	EMEA/H/C/000689
Marketing authorisation holder(s)	Theravia 16 rue Montrosier, 92200 Neuilly-sur-Seine, France Phone: +33 (0)1 72 69 01 86 https://www.theravia.com
Joint PASS	No
Research question and objectives	As part of the registration dossier for Siklos® (hydroxycarbamide/hydroxyurea [HU]), the Marketing Authorization Holder (MAH) committed to initiate a non-interventional study (ESCORT-HU) with the main goal of collecting safety information upon administration of Siklos® in patients with Sickle Cell Disease (SCD), as well as long-term follow-up data after treatment with HU. Most of the objectives of ESCORT-HU study have been fulfilled as regards the collection of information on the most common safety concerns associated with the administration of HU in patients with SCD. However, for some safety concerns, the cumulative data were not sufficient to draw meaningful conclusions. As safety information pertaining to the long-term use of HU remains incomplete in spite of the first safety study (ESCORT-HU), an extension of the latter is proposed. ESCORT-HU Extension study aims at evaluating the long-term safety of Siklos® focusing on some questions regarding its safety when used in current practice in adults and paediatric patients treated with Siklos® and followed for up to 5 years. **Primary objective** • To determine the long-term safety profile of Siklos® focusing on risks which are poorly documented or unknown (unknown meaning:

	nature and frequency of serious adverse events (SAEs) causally related to Siklos® which are not listed in the product information). **Secondary objectives** • To document changes in fertility status in males and females; in particular, to determine and document the management of male fertility through the use of semen cryopreservation in adults but also in children having started Siklos® in early childhood, • To monitor the outcome of pregnancies following exposure to Siklos® at the time of conception or at any time during the pregnancy (in participant and participant's partner), • To provide more information about the potential barriers to the compliance to the prescription, • To determine how the switch or combination with new SCD treatments is made. • To describe complications linked to SCD • To document the quality of life of SCD adult patients
Country(-ies) of study	France, Italy, Greece, Germany.
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Contract Research Organizations and Services providers

Contract Research Organization (CRO)	ICTA PM 11, rue du Bocage 21121 Fontaine les Dijon, France
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1. ABSTRACT

TITLE OF STUDY	ESCORT-HU Extension: European Sickle Cell Disease Cohort – Hydroxyurea – Extension study
KEYWORDS	Hydroxyurea, Sickle Cell Disease, malignancies, leg ulcers, fertility
RATIONALE AND BACKGROUND	As part of the Siklos® (Hydroxycarbamide/hydroxyurea [HU]) Risk Management Plan, the ESCORT-HU study addressed the most common safety concerns associated with the use of HU in sickle cell disease (SCD) patients: myelosuppression, child growth, concomitant administration of live vaccines, safety in population with renal and hepatic impairment and frequency of SCD events. The following concerns still need to be addressed: Secondary malignancies, Leg ulcers, Male fertility, Pregnancies, Potential barriers to the compliance to the prescription of Siklos®, The place of Siklos® within the environment of new SCD treatments.
RESEARCH QUESTION AND OBJECTIVES	**Aim** Evaluation of the long-term safety of Siklos® in current practice in adults and paediatric patients. **Primary objective** To determine the long-term safety profile of Siklos® focusing on risks which are poorly documented or unknown. **Secondary objectives** To document: Changes in fertility status in males and females; in particular, the management of male fertility through the use of semen cryopreservation in adults but also in children having started HU in early childhood, Outcome of pregnancies following exposure to Siklos® at the time of conception or at any time during the pregnancy, Potential barriers to the compliance to the prescription of Siklos®, Switch or combination with new SCD treatments, Complications linked to SCD, Quality of life of SCD adult patients.
STUDY DESIGN	Multicentre, prospective, non-interventional cohort study in SCD patients treated with Siklos®.
SETTING	**Countries** France, Italy, Germany and Greece. **Investigators** Physicians experienced in SCD management who are the only ones authorised to initiate Siklos® treatment. **Participants**

	Observational follow-up, for up to 5 years.
SUBJECTS AND STUDY SIZE, INCLUDING DROPOUTS	**Sample size** A sample size of 2100 patients will allow for an estimation of rates of events of interest (malignancies, leg ulcers, male fertility and serious unexpected adverse events causally related to Siklos® [SUSAR]) as low as 0.5% with a precision of 0.3%.
VARIABLES	**Primary objective assessment** Occurrence of malignancies, leg ulcers, male fertility impairment (oligospermia, azoospermia) and serious unexpected AEs (meaning SAEs which are not listed in the product information) according to the causal relationship with Siklos® or not and main features of these events. **Secondary objectives assessments** Mean age at first periods for females, mean age of puberty for males and contraception, Incidence of the use of cryopreservation for the purpose of paternity, management of semen cryopreservation and semen analysis, Management of Siklos® during pregnancy and outcome of pregnancy following exposure at the time of conception in participant and participant's partner, or at any time during the pregnancy, Frequency and reason for temporary or permanent discontinuations of Siklos®, duration of interruption, daily dose of Siklos® at the time of discontinuation, reoccurrence of vaso-occlusive crisis, Switch or combination with alternative treatment, Complications linked to SCD at enrolment and occurring during follow-up, Quality of life for SCD adult patients.
DATA SOURCES	Data from participant's medical records and data to be retrieved from ESCORT-HU are entered in an electronic case report form (eCRF). For participants already included in ESCORT-HU, data and medical history at last visit in ESCORT-HU are collected retrospectively in ESCORT-HU Extension study.
RESULTS	A total of 2093 patients were enrolled in the study at the time of this interim analysis. Among those, 2046 patients (97.8%) fulfilled the criteria for inclusion in the Safety Population. 55.8% patients of the Safety Population were females and 44.2% were males. Median exposure duration to HU was 9 years. During ESCORT-HU Extension study follow-up, 21 patients (0.8%) had a malignancy diagnosed after the start of the study. 92 patients (4.5%) developed leg ulcer(s) (125 events); 5 were related to HU 16 patients had semen analysis. Among those, 14 patients were diagnosed with male fertility impairment: 2 qualitative abnormalities, 9 quantitative abnormalities and 3 patients with both qualitative and quantitative abnormalities. 4 patients experienced a SUSAR (acute spleen sequestration, gonadal toxicity, retinal micro bleeding, type 2 diabetes). 93 pregnancies were reported in 79 female patients: 44 pregnancies were with normal birth (47.3%) and 11 with premature birth (11.8%). Twenty-nine (29)

	pregnancies (31.2%) were reported without living child, 7 ongoing and 1 missing outcome. Among those, Siklos® treatment had not been interrupted before conception in 21 cases. 27 pregnancies were reported in 24 male patients: 22 with normal birth (84.6%), 2 (7.7%) without living child,1 unknown outcome and 2 (7.7%) pregnancies were ongoing. Among those, Siklos® treatment had not been interrupted before conception in 14 cases.
DISCUSSION/ CONCLUSION	ESCORT-HU Extension study aims at collecting information on the long-term safety of Siklos® in patients with SCD. Overall, no major new safety signal has been detected so far. ESCORT-HU Extension study is expected to last 1 additional year and recruitment has ended. As the study focuses on rare events which may occur after long term exposure to HU, increased number of patients and additional follow-up duration is required and will strengthen the knowledge about safety of HU for the treatment of SCD patients.
MARKETING AUTHORISATION HOLDER(S)	Theravia 16 rue Montrosier, 92200 Neuilly-sur-Seine, France Phone: +33 1 72 69 01 86 E-mail: question@theravia.com
PRINCIPAL INVESTIGATOR(S)	The European coordinating Investigators of the study are: Pr. Frédéric GALACTEROS, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny 94000 Créteil, France. Pr. Mariane DE MONTALEMBERT, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France. Contact details of the principal and/or coordinating investigators, co-investigators and other site personnel for each country and site participating in the study are listed in a stand-alone document (see Appendix 2. List of investigators).

2. LIST OF ABBREVIATIONS

Abbreviations, specific or unusual terms and units used in this clinical study report are listed in the Table below:

Abbreviation or Term	Definition/Explanation
ACS	Acute Chest Syndrome
AE	Adverse Event
CI	Confidence Interval
CRA	Clinical Research Associate
CRO	Contract Research Organization
eCRF	Electronic Case Report Form
EMA	European Medicinal Agency
G6PD	Glucose-6-Phosphate Dehydrogenase
HAS	French National Authority for Health (Haute Autorité de Santé)
HU	Hydroxycarbamide/hydroxyurea
IQR	Interquartile Range
MAH	Marketing Authorization Holder
MedDRA	Medical Dictionary for Regulatory Activities
NA	Not Applicable
PAS	Post-Authorisation Study
PASS	Post-Authorisation Safety Study
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Sickle Cell Disease
SF-12	12-Item Short-Form Health Survey
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Event
USA	United States of America
VOC	Vaso-Occlusive Crises
WHO	World Health Organization

3. INVESTIGATORS

The European coordinating Investigators of the study are:

- Pr. Frédéric Galactéros, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny 94000 Créteil, France.
- Pr. Mariane de Montalembert, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France.

Contact details of the principal and/or coordinating investigators, co-investigators and other site personnel for each country and site participating in the study are listed in a stand-alone document (see Appendix 2. List of investigators) which is available upon request.

4. OTHER RESPONSIBLE PARTIES

4.1. MARKETING AUTHORISATION HOLDER(S)

Marketing authorisation holder(s):

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The responsibilities of the marketing authorisation holder are described in Table 21 in Appendix 2. List of investigators.

4.2. STEERING COMMITTEE

The composition of the steering committee may vary during the study and is reviewed each year depending on the recruiting sites and the most implicated. The composition of the committee as well as the roles and responsibilities are described in Table 211 in Appendix 3. Roles and responsibilities of the Marketing Authorisation Holder and other responsible parties.

The first steering committee meeting occurred on June 26th, 2021.
The second steering committee meeting was held on June 3rd, 2022.
The third steering committee meeting was held on September 22nd, 2023.
The fourth steering committee meeting is planned on September 23rd, 2024.

4.3. CONTRACT RESEARCH ORGANIZATION(S) AND OTHER SERVICE PROVIDER(S)

4.3.1. *Contract Research Organisation*

International Clinical Trial Association & Project Management (ICTA PM)

11 rue du Bocage
21121 Fontaine les Dijon.
☎: 03.80.53.40.00

Scientific Advisor: Maria GACKIERE KATSOGIANNOU

Project Manager: Sonia DAVENNE

Data Manager: Laura MOTTET

Statistician: Julie TRICHEREAU

The Contract Research Organisation (CRO) ICTA PM has been mandated by Theravia to conduct the activities described in Table 21 in Appendix 3. Roles and responsibilities of the Marketing Authorisation Holder and other responsible parties.

4.3.2. *Other service providers*

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David Pihan

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Philippe Ansar (former Arone SAS employee) and David Pihan (external consultant) have been mandated by Theravia to conduct the activities described in Table 21 Appendix 3. Roles and responsibilities of the Marketing Authorisation Holder and other responsible parties.

5. MILESTONES

Planned and actual dates for study milestones are indicated in Table 1.

Table 1. Study milestones

Milestone	Planned date	Actual date	Comments
First IEC approval	Q1 2020	18-May-2020	In France, delay between submission of the study to Ethics committee and the set-up in sites due to request from EC for modification to the patients' information letter and to refusal of simplified CV.
Last substantial modification IEC approval	NA	06-Dec-2023	Enrolment period and sample size reviewed.
Last IEC approval	NA	11-Apr-2024	At the time of this interim report, IEC approvals have been obtained for the following countries: France, Germany, Greece and Italy. Last IEC approval concerned the change of the PI in Germany. As the participation of a new investigator in the study is to be submitted to the IEC, submissions are made regularly when a new investigator participates in the study at a site already open or when a new site is opened.
Start of data collection	Q1 2020	12-Aug-2020	Slow process of contract signatures. Delay in implementing the study at sites due to Covid-19 pandemic.
End of data collection	Q3 2025	Ongoing	NA
Interim report 1	Q3 2021	05-Nov-2021	Delayed due to the delayed implementation of the study
Interim report 2	Q3 2022	21-Oct-2022	Late edition of V3.0 after correction of errors in statistical programming and in interim report V1.0.
Interim report 3	Q3 2023	01-Sep-2023	NA
Registration in the EU PAS register	NA	18-Jul-2024	EU PAS Number: EUPAS1000000066
Interim report 4	Q3 2024	05-Sept-2024	NA
Final report of study results	2026	NA	NA

6. RATIONALE AND BACKGROUND

The efficacy and long-term effectiveness of hydroxycarbamide/hydroxyurea (HU) in the prevention of painful crises and in the decrease of mortality and morbidity in sickle cell disease (SCD) patients has been established [1, 2]. As a post marketing commitment to the European Medicinal Agency (EMA) following granting of the marketing authorization for Siklos® in Europe, the non-interventional prospective ESCORT-HU study was conducted as part of the Risk Management Plan to evaluate the use of Siklos® in real-life conditions and to collect information on the long-term safety of Siklos® when used in current practice for the prevention or treatment of symptomatic complications in patients with SCD.

A total of 1906 patients were enrolled in the ESCORT-HU study in 62 centres (in Germany, Greece, Italy and France) between January 2009 and June 2017. As planned in the protocol, the follow-up was closed in Q1 2019. The main objectives of ESCORT-HU have been fulfilled as regards the collection of data on the most common concerns associated with the use of HU in SCD patients: myelosuppression, child growth, concomitant administration of live vaccines, safety in population with renal and hepatic impairment and frequency of SCD events (painful crises, acute chest syndrome, stroke, acute splenic sequestration, infections, blood transfusions and hospitalizations).

However, due to the late access to the market in France and the absence of significant sales in the United Kingdom, both accounting for the largest population of SCD patients in Europe, less than 200 participants had been enrolled in ESCORT-HU by the end of 2012. The majority of the participants have been enrolled from January 2013. Therefore, long-term follow-up data are still lacking, and the following concerns still need to be addressed:

- Drug-induced malignancies: the mean exposure in the cohort of ESCORT-HU study was 30 months, for a cumulative exposure of 7310 patient-years by 01 July 2019. Only one myelodysplastic syndrome had been reported in the cohort. It is possible that the duration of exposure to HU was too short to fully estimate the incidence of this risk; therefore, further long-term exposure data are required.
- Leg ulcers: the number of events and duration of exposure were not sufficient to provide discriminating criteria between leg ulcer caused by the disease and HU induced leg ulcer. Further data are needed to define such discriminating criteria.
- Male fertility: different publications report the deleterious effect of HU on spermatozoa count [3-5] and some cases of reversibility after HU treatment stop [6, 7]. The consequences on male fertility when HU is started before puberty, sometimes in very young children, are still not very well documented. Therefore, one of the objectives of the ESCORT-HU Extension study is to gain knowledge on how clinicians manage this concern: What is the fertility potential in patients treated with HU when HU is started before puberty? Is it possible to temporarily stop HU to allow for sperm cryopreservation and document the time to reverse HU lesions? Which alternative SCD treatment is appropriate during this HU-free period? Do the deleterious effects reoccur?
- Pregnancies: to date, there is a limited number of pregnancies with documented outcome. Despite no adverse effects on pregnancy or on the health of the foetus/new-born have been registered (Siklos® summary of product characteristics [SmPC]) to date, a careful follow-up should be considered in case of female pregnant patients or pregnant partners of male patients exposed to HU. Therefore, since an increase of the number of pregnancies with documented outcomes will allow for meaningful assessment of foetal outcome following exposure to Siklos® during pregnancy, additional data should be collected. In addition, the use of semen cryopreservation will be documented.
- Although its efficacy in the clinical management of SCD has been demonstrated, HU is often underutilised. Potential barriers to the adherence of HU have been identified at patient, provider and system levels [8, 9]. Lack of knowledge on the therapeutic potential of HU represents a barrier at the 3 above mentioned levels. Moreover, it is well known that the fear of side effects and constant laboratory monitoring are 2 factors which hinder patients to fully adhere to the treatment. Additional

data will help to better apprehend the potential barrier to patient's compliance to HU. In this regard, patients' decision to discontinue their treatment has to be distinguished from a discontinuation decided by the physician and perceived as necessary to ensure the safety and well-being of patients.

- Finally, new SCD treatments, in combination with HU or not, are or will be soon available. How does HU fit in the management of SCD within this new environment?

The data collected during this study extension supplement data from ESCORT-HU study on specific safety concerns when using Siklos® in routine clinical practice in adult and paediatric patients treated for SCD. Patients giving their consent to participate in the study or whose parents or legal guardians have been informed and given permission for their child to participate are followed for up to 5 (additional) years or until discontinuation of Siklos®.

7. RESEARCH QUESTION AND OBJECTIVES

As safety information regarding the use of HU on long-term remains incomplete despite a first safety study (ESCORT-HU), an extension of the latter has been proposed. The ESCORT-HU Extension study aims at evaluating the long-term safety of Siklos® when used in current practice in adults and paediatric patients treated with Siklos® and followed for up to 5 years.

7.1. PRIMARY OBJECTIVE(S)

To determine the long-term safety profile of Siklos® focusing on risks which are poorly documented or unknown (unknown meaning: nature and frequency of serious adverse events (SAEs) causally related to Siklos® which are not listed in the product information).

7.2. SECONDARY OBJECTIVE(S)

1. To document changes in fertility status in males and females; in particular, to determine and document the management of male fertility through the use of semen cryopreservation in adults but also in children having started HU in early childhood.
2. To monitor the outcome of pregnancies following exposure to Siklos® at the time of conception or at any time during the pregnancy (in participant or participant's partner).
3. To provide more information about the potential barriers to the compliance to the prescription of Siklos®.
4. To determine how the switch or combination with new SCD treatments is made.
5. To describe complications linked to SCD.
6. To document the quality of life of SCD adult patients.

Of note, the 6th objective is only relevant for final analysis and has not been addressed for the present interim report.

8. AMENDMENTS AND UPDATES

8.1. AMENDMENT TO THE STUDY PROTOCOL

Substantial amendments and updates to the study protocol after the start of data collection are described in Table 2.

Table 2. Amendments and updates to the study protocol

Number	Date	Section of the study protocol	Amendment or update	Reason
1	30-Oct-2020	6-Milestones	Update of the following milestones: - Start of data collection: Q3 2020 - Final report of study results: 2026	Delay in implementing the study at sites due to: - Delay between submission of the study to ethic committee and the set-up in sites due to request from EC for modification to the patients' information letter and to refusal of simplified CV in France, - Slow process of contract signatures in all countries - Covid-19 pandemic.
		9.2-Setting	Update of the description of data collection process for participants already included in ESCORT-HU study, regarding patient's visits performed between last visit in ESCORT-HU study and first visit in ESCORT-HU Extension study	Clarification of eCRF data collection process to ensure homogeneity
		9.3-Variables	Clarification in the definition of SUSARs related to Siklos	Clarification of the eCRF data collection process to ensure homogeneity
		9.7-Data analysis	Update of the definition of the analysis sets	All statistical analyses performed on the safety population
2	06-Apr-2022	8- Research question and objectives	Endpoints have been detailed in order to take into account all the data collected and to be more accurate.	Clarification of study objective and endpoints
		9.2.1.1-Inclusion criteria	The pubertal status of the patients has been clarified	Precision of an inclusion criterion
		9.3.1. Inclusion Visit and	The pubertal status of the patients has been clarified and all data collected in the database have been added	Addition of data collected in the database but not present in these sections

		9.3.2 Follow up visits		
		9.7.1. Analysis sets	Minor clarifications have been added	Minor clarifications
		11.2- Adverse event collection and reporting	Update of the definition of the analysis sets	Events reported in medical history section will not be analysed as adverse events
3	19-Sep-2023	Section 3. Responsible parties	Change of Sponsor Name and address	Name/address of Sponsor contacts and logo have been modified.
		Section 11.2 Adverse event collection and reporting		
		Section 4. Abstract	Change of end of inclusions and sample size	Sample size has been changed to 2100 patients as inclusions will end at Q1 2024.
		Section 9.2.1 Study population		
		Section 9.5 Study size		
		Section 9.9.1 Selection bias		

8.2. ADDITIONAL UPDATES

For a better understanding, endpoints listed in the protocol have been reworded with further details in the Statistical Analysis Plan (SAP).

9. RESEARCH METHODS

9.1. STUDY DESIGN

9.1.1. *Overall study design*

This is a multicentre, prospective, non-interventional cohort study in adult and paediatric patients treated with Siklos® purposed to deepen the current knowledge on low incidence and indeterminate risks including malignancies secondary to HU treatment, leg ulcers, serious unexpected adverse events related to Siklos® (SUSAR) and fertility issues. A feasibility survey for ESCORT-HU Extension was performed during summer 2018 showing that almost all centres participating in ESCORT-HU study were willing to continue the surveillance of their participants treated by Siklos® through ESCORT-HU Extension study. In addition, more than 20 additional SCD reference centres are participating in ESCORT-HU Extension study. The ESCORT-HU Extension study is thus covering almost all SCD reference centres in France and to ensure, as detailed below (section 9.2), the follow-up of more than 50% of the patient population eligible for Siklos® treatment in the participating centres.

9.1.2. *Primary endpoint*

The primary endpoint of ESCORT-HU Extension study is to determine the occurrence, the causal relationship with Siklos® or not and the main features of:

- Malignancies,
- Leg ulcers,
- Male fertility impairment (oligospermia, azoospermia)
- Serious unexpected adverse events (meaning SAEs which are not listed in the product Information).

9.1.3. *Secondary endpoints*

The secondary endpoints of ESCORT-HU Extension study are to describe:

- The mean age at first periods for females, mean age at puberty for males, and contraception,
- The incidence of the use of cryopreservation for the purpose of paternity, management of semen cryopreservation (age of performance compared to the age of occurrence of puberty/ HU treatment stop), and semen analysis results,
- The management of Siklos® during pregnancy (number of pregnancies, HU treatment interruption, mean exposure time to HU during the entire pregnancy, mean exposure time to HU per week during pregnancy) and outcome of pregnancy following exposure at the time of conception (in participant and participant's partner) or at any time during the pregnancy,
- The frequency and the reason for temporary (>15 days) or permanent discontinuations of Siklos® treatment, the duration of interruption, the daily dose of Siklos at the time of discontinuation, the reoccurrence of vaso-occlusive crisis,
- The switch(es) or combination(s) with alternative treatment (erythrocytapheresis, Hydrea® or other HU treatment, blood transfusion, bone marrow transplantation and/or other) *(not addressed in this interim analysis – this endpoint will be addressed in the 3rd and 4th interim analyses and in the final report)*,
- Complications linked to SCD at enrolment and occurring during follow-up *(not addressed in this interim analysis – this endpoint will be addressed in the 3rd and 4th interim analyses and in the final report)*
- The quality of life of adult patients through the 12-Item Short-Form Health Survey (SF-12) *(not addressed in interim analyses, only in the final report)*.

9.2. SETTING

ESCORT-HU Extension study was planned to be initiated in the same countries as ESCORT-HU (France, Italy, Germany and Greece).

9.2.1. *Site Selection*

Investigators have been recruited among physicians experienced in SCD management who are the only ones authorised to initiate Siklos® treatment hence to enrol and to follow up the participants. Investigators who have already participated in ESCORT-HU study have been asked to participate in the extension study. In addition, the study has been proposed to other physicians experienced in SCD management in France, Germany, Greece and Italy with the intent of opening additional sites motivated to participate in the ESCORT-HU Extension study.

Investigators have to fulfil the following criteria:

- Be able to enrol a minimal number of SCD patients who are potentially likely to be treated by Siklos® (minimal number of patients per investigator: 3),
- Have the adequate resources for collecting (through a web-based electronic case report form [eCRF]) and transmitting medical data and all the results of the assessments performed during the entire study,
- Have the possibility to be in contact with the participant and other medical staff to prevent loss to follow-up or to collect additional medical information.

During the study, the participating investigator is free to determine the appropriate therapy for each participant and make treatment choices as deemed clinically necessary and according to current clinical practice.

Site selection has started:

- In December 2019 in France, Germany and Italy
- In January 2020 in Greece.

At the time of this interim analysis, site selection is completed.

9.2.2. *Site Initiation*

First sites were initiated:

- On July 9th, 2020 in France,
- On November 25th, 2020 in Germany,
- On December 14th, 2020 in Greece,
- On January 14th, 2021 in Italy.

9.2.3. *Data Collection*

First inclusion in ESCORT-HU Extension study occurred on August 12th, 2020.

Participants are followed for up to 5 years. The follow-up is strictly observational and fits within routine clinical monitoring without any controlled treatment, specific examination, investigation or modification of follow-up. Participants are evaluated in hospital at inclusion. Due to the observational nature of the study, investigators are free to determine the frequency of the visits of the participant and at least one follow-up visit per year is entered in the eCRF containing all data from the previous visit.

Both retrospective and prospective data are collected in order to well document SCD history and Siklos® exposure. For participants already included in ESCORT-HU, data and medical history at last visit in ESCORT-HU study and data of any visit performed between last visit in ESCORT-HU study and first visit in ESCORT-HU Extension study are collected retrospectively. The investigator reports in each follow-up visit of the eCRF patient's relevant medical information collected since the previous visit. Temporary discontinuation of Siklos® does not lead to interruption of data collection, however, in case of permanent stop, data are no longer collected and the participant is withdrawn from the study.

9.2.4. *Site Closure*

Closures of sites that did not include any patients have been performed by letter. Sites closures concern sites n°11, 14, 26, 32, 43, 70, 73, 107 and 301. Close-out will be performed by phone after the final statistical outputs edition and a letter will be sent to each site.

9.3. SUBJECTS

9.3.1. *Subject Selection*

Sampling of study subjects

Patients are recruited over a 3.5-year period, from Q3 2020 to Q1 2024 and the follow-up is planned to end in Q3 2025. In addition to patients already included in ESCORT-HU study, new at-risk patients were recruited in centres already involved in ESCORT-HU study as well as in additional centres in France, Italy, Germany and Greece. At-risk patients are 1/ patients with a long history of HU exposure (at least 5 years, to allow a long-term follow-up), 2/ children who have not completed puberty aged more than 10 years for girls and more than 13 years for boys (in order to document puberty and cryopreservation), 3/ patients with a history of leg ulcers, 4/ pregnant women without interruption of Siklos® 3 months before the beginning of the pregnancy, 5/ males treated with Siklos® whose partner is pregnant and without discontinuation of Siklos® 3 months before the beginning of the pregnancy.

During the recruitment period, patients fulfilling the inclusion criteria were informed by their initiating physician about the ESCORT-HU Extension study and enrolled in case they agreed to participate.

Inclusion criteria

In order to enter the study, participants must fulfil the inclusion criteria reported below:

- Male or female patients with symptomatic SCD,
- ≥ 2 years old,
- Treated with Siklos® (initiation of treatment before inclusion in the study or on the day of inclusion),
- Having been informed of the study by the investigator and consenting to participate, or whose parents or legal guardians are consenting for their child to participate.

In addition, children mature enough to understand the study and what they are expected to do are asked to give their assent.

- To allow risk evaluation, participants must belong at least to one of the subpopulations defined below:
    - Participants previously enrolled in ESCORT-HU who agree to participate in ESCORT-HU Extension study,
    - New participants with any of the following criteria:
        - history of HU treatment for more than 5 years, or
        - children who have not completed puberty over 10 years of age for girls and 13 years of age for boys at enrolment (with possibility to follow them until the puberty and increase the number of patients with possible therapeutic window/stop to perform the semen cryopreservation), or
        - with history of leg ulcer, or
        - pregnant women without interruption of Siklos® 3 months before the beginning of the pregnancy or,
        - Males treated with Siklos® whose partner is pregnant and without discontinuation of Siklos® 3 months before the beginning of the pregnancy.

9.3.2. *Subject inclusion*

In centres already involved in ESCORT-HU, all participants and parents or legal guardians of children already enrolled in ESCORT-HU, still treated by Siklos® and fulfilling the eligibility criteria for

ESCORT-HU Extension were informed by the investigator about this extension study and the possibility for a longer follow-up period. Participants were included only upon given informed consent according to local regulations. For patients <18 years old, parents or legal guardians were informed and had to give permission for their child to participate. In addition, children mature enough to understand the study and what they are expected to do were asked to give their assent. Patients turning 18 years old during the follow-up period, were asked to give their consent upon their majority.

All new eligible patients in already existing centres and new centres were also asked to participate according to local regulations and are enrolled after given informed consent.

9.3.3. *Subject follow-up*

Participants' follow-up will last until Q3 2025, for a maximum duration of 5 years.

9.3.4. *Subject discontinuation*

A participant may withdraw from the study at any time without prejudice.

If the investigator decides it is in the best interest of the participant to permanently discontinue Siklos®, the participant is withdrawn from the study.

The reasons for permanent discontinuation of treatment and/or premature withdrawal from the study are documented in the e-CRF of the study.

In case the participant leaves the participating centre, every effort is made by the centre to determine whether the participant continued the treatment and if not the reason for the discontinuation. If all these attempts to contact the participant fail, the investigator then declares the participant "lost to follow-up".

9.4. VARIABLES

9.4.1. *Collected variables*

Demographic characteristics:
- Date of enrolment,
- Demographic characteristics of the participant: date of birth (month/year) and gender.

SCD history:
- Haemoglobin Genotype (SS, SC, Sβ0, Sβ+, other S genotype),
- Alpha-globin gene defect,
- Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency,

Treatments:
- HU treatment history: start date of the first administration of any specialty of HU, reason for initiation,
- Other SCD treatment: treatment, start date, stop date and reason for initiation.

Medical History
- History of malignancy: date of first diagnosis, organ, type of treatment, date of treatment, status of the malignancy at enrolment, history of malignancy in the family of the patient,
- History of leg ulcers: type of lesion, type of episode, location(s), status of leg ulcer at enrolment, causal relationship with HU,
- Type of complications linked to SCD.

Serious Unexpected AE causally related to Siklos® (SUSARs)
- Unexpected serious AEs not reported in the product SmPC effective at the time of the event and considered as causally related to Siklos®,
  For each of these events, event type, date of onset, date of end, seriousness criteria, change of Siklos® treatment, corrective treatment, severity and outcome are collected.

Malignancy, Leg ulcer, SCD complications
- Malignancy: occurrence, reoccurrence, date of first diagnosis, organ, date of treatment, type of treatment, status of the malignancy and causal relationship with Siklos®,
- Leg ulcer: occurrence, reoccurrence or worsening, causal relationship with Siklos®, date of diagnosis, type of lesion, main location, treatment, status of leg ulcer, Doppler and other exams results, predisposing factors,
- Type of complications linked to SCD.

Siklos® treatment
- Daily dose of Siklos® at the visit,
- For temporary stop longer than 15 days: reason of stop, date of interruption, date of restart, dose of Siklos® at the stop, occurrence and number of vaso-occlusive events during the treatment stop,
- Permanent stop.

Laboratory results:
- Haemoglobin,
- Foetal Haemoglobin,
- Absolute Neutrophils Count,
- Mean Corpuscular Volume

Quality of life questionnaire (for adult patients only, not mandatory, performed once or twice during the study):
- 12-Item Short-Form Health Survey (SF-12),
- Additional questionnaire about patient's quality of life.

Male fertility status
- Pubertal status: Age at puberty (Tanner V stage) for pubescent males,
- Sperm cryopreservation for pubescent males: age at sperm cryopreservation, Siklos® treatment interruption
- Semen analysis in pubescent males: results of semen analysis i.e. oligospermia / azoospermia and relationship to Siklos®,
- Partner's pregnancy and outcome of pregnancy for pubescent males: date of pregnancy, use of cryopreserved semen, outcome of pregnancy, Siklos® treatment interruption (when applicable).

Female fertility status
- Pubertal status: Age at first periods for pubescent females,
- Contraception for pubescent females,
- Pregnancy and outcome of pregnancy for pubescent females: date of pregnancy, outcome of pregnancy, number of children per pregnancy, Siklos® treatment interruption, status of the living child, birth date (month/year) and birth weight of the living child, foetal defects.

9.4.2. *Outcome variables*

Only outcome variables presented in this interim analysis report are described in this section.

Primary endpoint analysis

Events of interest

Events of interest include:

- Malignancies
- Leg ulcers
- Male fertility impairment:
  - Qualitative abnormalities
  - Quantitative abnormalities: oligospermia, azoospermia

For each event of interest, the outcome variables are:

- Number and percentage of patients with at least one occurrence of the event of interest during ESCORT-HU Extension follow-up period,
- Number and percentage of patients with at least one occurrence of the event of interest related to Siklos® during ESCORT-HU Extension follow-up period,
- Number and percentage of patients with at least one occurrence of the event of interest non-related to Siklos® during ESCORT-HU Extension follow-up period,
- Number of events
- Time between first administration of hydroxycarbamide and first occurrence of the event of interest during ESCORT-HU Extension follow-up period.
- Main features of the event of interest:
    - For malignancies: organ, reoccurrence of a previous malignancy, age at occurrence, gender, HU exposure duration, pubescent status, treatment, interruption of Siklos®, evolution, relationship with Siklos®, history of malignancy, family history of malignancy, concomitant SCD complications.
    - For leg ulcers: age at occurrence, gender, pubescent status, lesion (single or multiple), location, reoccurrence of a previous leg ulcer and number of reoccurrence(s), same location as previous ulcer, aggravation of a previous leg ulcer, treatment, recovery status, Doppler and other exams, predisposing factors, HU exposure duration, daily dose of Siklos®, relationship with Siklos®, history of leg ulcer, concomitant SCD complications.
    - For male fertility impairment: sperm cryopreservation, age at sperm cryopreservation, Siklos® stop at sperm cryopreservation; semen analysis, Siklos® stop at semen analysis, semen analysis result and relationship with Siklos®; history of fertility impairment, concomitant SCD complications.

Serious unexpected adverse events related to Siklos®

Age at the time of occurrence, date of the event, gender, medical history, description of the type of event, seriousness, severity, outcome, management of Siklos® treatment, corrective treatment if any, concomitant SCD complications.

Secondary endpoints analysis

Fertility status

For Female patients, outcome variables are:

- Number and percentage of Female patients turned pubescent since enrolment
- Age at first periods
- Number and percentage of patients at least once under contraception during treatment with Siklos®

For Male patients, outcome variables are:

- Number and percentage of Male patients turned pubescent since enrolment
- Age at onset of puberty
- Number and percentage of patients with at least one sperm cryopreservation
- Number of sperm cryopreservation
- Age at sperm cryopreservation
- Average duration between age of cryopreservation and onset of puberty (months)
- Number and percentage of patients with at least one stop of Siklos® before cryopreservation and mean duration between cryopreservation and the date of treatment stop
- Percentage of pregnancies with use of sperm cryopreservation
- Number and percentage of patients with at least one semen analysis
- Number of semen analyses
- Interruption of Siklos® for semen analysis
- Semen analysis result

- Specifications if semen analysis with quantitative abnormalities
- Relationship with Siklos® if semen analysis with quantitative abnormalities

Pregnancies

- Total number of pregnancies in female patients and in female partners of male patients
- Number and percentage of female patients and number and percentage of female partners of male patients with at least one pregnancy
- HU exposure during pregnancy
- Exposure time to HU during the entire pregnancy
- Number and percentage of pregnancies and number of patients with a pregnancy of rank 1, 2 and higher
- Number and percentage of pregnancies and number of patients with an outcome equals to: living child, without living child and with ongoing pregnancy
- Number and percentage of pregnancies and number of patients with at least one pregnancy for each outcome: normal birth, premature birth, ongoing, miscarriage, stillbirth, elective abortion, ectopic pregnancy, foetal defect, clear egg, therapeutic or medical abortion
- Birth weight of living child
- Number and percentage of pregnancies and number of patients with at least one pregnancy with HU treatment stopped before pregnancy
- Number and percentage of pregnancies (except ongoing pregnancies) and number of patients with at least one pregnancy with HU treatment stopped during the first, second and third trimester
- Exposure time to HU when treatment stopped during the first, second and third trimester
- Number and percentage of pregnancies with treatment stopped during first trimester and restarted during the first trimester
- If treatment stopped during first trimester and restarted during the first trimester, delay since treatment stopped
- Number and percentage of pregnancies with treatment stopped during first trimester and restarted during the second trimester
- If treatment stopped during first trimester and restarted during the second trimester, delay since treatment stopped
- Number and percentage of pregnancies with treatment stopped during first trimester and restarted during the third trimester
- If treatment stopped during first trimester and restarted during the third trimester, delay since treatment stopped
- Number and percentage of pregnancies with treatment stopped during second trimester and restarted during the second trimester
- If treatment stopped during second trimester and restarted during the second trimester, delay since treatment stopped
- Number and percentage of pregnancies with treatment stopped during second trimester and restarted during the third trimester
- If treatment stopped during second trimester and restarted during the third trimester, delay since treatment stopped
- Number and percentage of pregnancies with treatment stopped during third trimester and restarted during the third trimester
- If treatment stopped during third trimester and restarted during the third trimester, delay since treatment stopped
- Outcome and status of pregnancies with treatment not stopped at all

Barriers to compliance

- Number and percentage of patients with at least one temporary stop for more than 2 weeks all along the study

For patients with at least one temporary stop

- Percentage of number of stops
- Mean daily dose per kilogram at stop (mg/kg/day)
- Mean duration of stop (days)
- Number and percentage of patients with at least one vaso-occlusive episode during treatment stop period (and number of episode) and mean number of episodes
- Number and percentage of patients with at least one stop because of toxicity due to hydroxycarbamide and number of episodes
- Number and percentage of patients with at least one stop because of patient's/parent's will and number of episodes
- Number and percentage of patients with at least one stop because of compliance issue and number of episodes
- Number and percentage of patients with at least one stop because of pregnancy/sperm cryopreservation and number of episodes
- Number and percentage of patients with at least one stop because of alternative SCD treatment and number of episodes
- Number and percentage of patients with at least one stop because of medication error and number of episodes
- Number and percentage of patients with at least one stop because of another reason and number of episodes

Switch or combination with new SCD treatment

The following variables are presented for the three periods: before, after and concomitant to Siklos® discontinuation:

- Number and percentage of patients with at least one other treatment received for sickle cell disease
- Number and percentage of patients with at least one other treatment received for sickle cell disease all along the study
- If at least one other treatment received all along the study, Number and percentage of patients with at least one anti-adhesion therapy/one anti-inflammatory / one anticoagulant / at least one blood transfusion program-regular blood transfusion / one gene therapy / one L-glutamine / one modulation of intracellular levels of cGMP / one targeting foetal haemoglobin / one other hydroxycarbamide treatment
- Number and percentage of patients with at least one treatment received for insufficient effectiveness of hydroxycarbamide / one treatment received for resistance to hydroxycarbamide/ one treatment received for appearance of complications / for another reason

Complications linked to SCD

The following variables are presented by subgroups of age at the complication and by duration of the exposure to HU at the complication:

- Number and percentage of patients with at least one brain complication and if yes, number and percentage of neurocognitive dysfunction, abnormal TCD and Stroke
- Number and percentage of patients with at least one eye complication and if yes, number and percentage of retinopathy, Post-hyphema glaucoma, retinal infarction
- Number and percentage of patients with at least one lung complication and if yes, number and percentage of acute pain events, pulmonary hypertension, acute chest syndrome
- Number and percentage of patients with at least one ear complication and if yes, number and percentage of deafness

- Number and percentage of patients with at least one gallbladder complication and if yes, number and percentage of gallstone/cholelithiasis
- Number and percentage of patients with at least one heart complication and if yes, number and percentage of cardiomyopathy, cardiomegaly, diastolic heart failure, pericarditis
- Number and percentage of patients with at least one liver complication and if yes, number and percentage of cholangitis, hepatic sequestration, iron overload, intrahepatic cholestasis, sickle hepatopathy
- Number and percentage of patients with at least one spleen complication and if yes, number and percentage of functional asplenia, splenic infarction, splenic sequestration,
- Number and percentage of patients with at least one bladder complication and if yes, number and percentage of albuminuria, isosthenuria/hyposthenuria, substantial kidney injury, papillary necrosis
- Number and percentage of male patients with at least one male genitals complication and if yes, number and percentage of delayed puberty, erectile dysfunction, priapism
- Number and percentage of female patients with at least one female genitals complication and if yes, number and percentage of delayed puberty, complications of pregnancy
- Number and percentage of patients with at least one bloodstream complication and if yes, number and percentage of anaemia, thrombosis, leukocytosis
- Number and percentage of patients with at least one bones/joints complication and if yes, number and percentage of avascular necrosis, bone marrow infarction, osteomyelitis, arthritis
- Number and percentage of patients with at least one VOC

Laboratory results

- Correlation graph between each parameter of laboratory (Hb level/HbF, MCV and PNN) and the daily dose of Siklos®.

9.5. DATA SOURCES AND MEASUREMENT

Data from participant's medical records and results available from routine visits available at hospital level are entered in the eCRF by the investigator or delegated data entry specialists. Data entered in the eCRF are sent to a central database via a secure transfer. The electronic forms are used to transmit information collected during the study to Theravia and Regulatory Authorities, as applicable.

9.6. BIASES

ESCORT-HU Extension may be affected by sources of bias inherent to observational studies that result from participant/SCD specialist selection, data collection and other factors.

Selection bias

Selection bias could occur if the characteristics of the participants or the investigators (SCD specialist) are systematically different from those of the general SCD population treated with HU, thus reducing its representativeness.

Investigational sites of the ESCORT-HU Extension study are referent sites in SCD management, the only ones authorised to initiate Siklos® treatment. Taking into account that all or almost all investigators having participated to the ESCORT-HU study are expected to participate to the Extension study and that new reference sites complete the sample of investigators in participating countries, the participating sites are expected to represent about 99% of all referent centres once selection will be achieved. The characteristics of the investigators at the end of the ESCORT-HU Extension study will be then similar to the characteristics of the population of physicians initiating Siklos® and managing SCD.

In order to limit bias in the selection of patients, participants already enrolled in ESCORT-HU and treated by Siklos® are asked for their consent to participate in the ESCORT-HU Extension study and the investigators attempt to consecutively include all new patients who consent (or for whom parents or legal guardians consent) and meet the selection criteria, regardless of demography, health status, or other considerations as applicable.

Of note, at the time of final analysis, the sample of patients enrolled in ESCORT-HU Extension study should represent more than 60% of the population of patients treated by Siklos® in the participating countries. Indeed, it is estimated that about 4000 patients only are treated with Siklos® over the world. More specifically, 80% of participants to the study are expected to be recruited in France. According to the elements that appear in the opinion of the transparency Commission of the French National Authority for Health (HAS) dated on May 2018, in France, the population of patients treated with Siklos® is estimated not to exceed 2100 patients. The French population coverage is thus expected to reach 80% at a minimum.

However, due to study design, patients treated with Siklos® who have been treated with HU for 5 years or more, patients with history of leg ulcers and prepubescent children aged more than 10 years for girls and 13 years for boys may be slightly overrepresented in the study sample with respect to the population of patients treated by Siklos®.

Information bias

Information bias, also known as measurement bias, is related to the completeness or accuracy of data collected over the course of the study. Measurement bias could affect investigators and could be due to difference in sites' procedures. To limit this bias, investigators and study coordinators of each participating site are trained in the same way to the protocol and data collection tool and standard methods to manage SCD are adopted.

Confounding bias

Confounding bias may result from selective patients care or selective prescribing of a particular treatments combination to more severely affected patients. As a result of a higher background risk, reported event rate may be higher and give the appearance of an elevated risk related to the use of the studied treatment. Yet, retrospective and baseline information are collected to help to identify the presence of elevated background risk, so that event rates can be compared through subgroup analysis to determine whether the elevated risk is likely to be related to background risk or use of study drug.

This bias may be further controlled by the participation of all or almost all referent physicians in SCD management as well as the systematic consecutive invitation of patients to participate in the study.

Attrition bias

The non-interventional nature of the study may complicate the collection of follow-up data and may result in missing data and patients lost to follow-up. Even if the number of lost to follow-up in this study is limited since, by nature, the SCD management obliges the patients to be closely monitored by their referent physician, it is the study monitors' responsibility to verify investigator's efforts and means through which study participants are contacted in order to avoid lost to follow-up.

Because the follow-up duration is up to 5 years, the proportion of patient withdrawals/discontinuations might be significant. Safety data collection is stopped in case of permanent discontinuation of Siklos® and switch to another therapeutic regimen excluding Siklos® or bone marrow transplantation or gene therapy because it is not possible to define if any occurrence of any event of interest after Siklos® discontinuation is linked to the previous use of Siklos® or to the new therapy or procedure. However, in patients secondarily treated with Hydrea® or other HU presentation, long term risks using HU are reported to the MAH of Hydrea®.

Missing data

Missing data may bias the statistical analyses of a study if there are not missing completely at random; and even in this case, missing data may impact the precision of the estimates. The use of an e-CRF by

investigators (including drop down list, single entry option and online checks for missing information) reduces the risk of missing data and ensures that missing data are limited to non-existing information and exclude non-reported information.

The handling of missing data is of special importance in longitudinal studies and thereby statistical analyses; its management and the methodology have been anticipated in the statistical analysis plan (SAP) of this study.

9.7. STUDY SIZE

The total number of patients expected to be enrolled in the ESCORT-HU Extension study over the 5-year period, from Q3 2020 to Q1 2024, was estimated to be of 2100:

- According to the results of a feasibility study performed during Summer 2018, it was estimated that more than 60% of the participants enrolled in ESCORT-HU (that is about 1200 patients) would be still treated by Siklos® (and would accept a follow-up in ESCORT HU Extension)
- Based on the patients' recruitment rate in the ESCORT-HU study, approximately 1200 new patients were expected to be recruited by the ESCORT-HU centres over the 3.5-year duration of the study
- In addition, at least 10 participants in each of the 10 additional SCD reference centres were expected to participate in ESCORT-HU Extension study

This sample size should allow the estimation with a sufficient precision of the rate of patients treated with Siklos® experiencing any of the events of interest (malignancies, leg ulcers, male fertility impairment (oligospermia and azoospermia) or serious unexpected adverse events) causally related to Siklos® (primary objective of ESCORT-HU Extension study). The rates of these events were expected to be low (≤ 5 %). They were estimated using two-sided 95% Confidence Interval (95%CI). The precision e in the estimation of a rate p for a given sample size n was calculated using the following sample size:

$$e = \sqrt{\frac{1.96^2 p(1-p)}{n}}$$

The Table 3 provides the precision of the estimation with n=2100 patients to estimate rates from 0.5% to 5%.

Table 3: Accuracy of the proportion estimations according to the sample size

Expected rate of the events of interest	**Precision in the estimation with n=2100 patients**
p=5%	e=0.93%
p=2,5%	0.67%
p=1%	0.42%
p=0,5%	0.30%

This allows for an estimation of frequencies of events as low as 0.5% with a precision <0.3%.

9.8. DATA MANAGEMENT

The Contract Research Organization (CRO) ICTA PM has been appointed by the Sponsor for management of collected data (setting up of a database and validation).

Access to the electronic data capture system has been provided to the CRO personnel responsible for data management for the duration of the trial through a password-protected method of internet access.

For this interim analysis, SAS® software version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for data management process and data were stored in the clinical database stored in a secure folder. No coding of AE nor coding of treatments was performed.

9.9. STATISTICAL METHODS

These statistical interim analyses were performed using SAS® software version 9.4.

Statistical methods used for this interim analysis are fully described in the SAP version 7.0 dated on 26th June 2024.

9.9.1. *Main summary measures*

The following descriptive statistics were used:

- Quantitative variables: number of available answers and number of missing values, mean, standard deviation, median, first and third quartiles and range (minimum and maximum observed values), Wald 95% confidence interval (CI) (if relevant).
- Qualitative variables: number of available answers and number of missing values, frequency, and percentage of each modality based on non-missing data, Agresti-Coull 95% CI of the proportion (if relevant). When available, the modalities Not done, Unknown and Not applicable were not considered as missing values and were taken into account in percentage calculation.

9.9.2. *Main statistical methods*

Population Sets

All statistical analyses (i.e. patients' disposition and characteristics, primary and secondary endpoints) were performed on the Safety Population, defined as all enrolled patients meeting:

- all of the following inclusion criteria:
  - Male or female patients with symptomatic SCD
  - ≥ 2 years old
  - Treated with Siklos® (initiation of treatment before inclusion in the study or on the day of inclusion)
  - Having been informed of the study by the investigator and consenting to participate, or whose parents or legal guardians are consenting for their child to participate
- At least one of the following criteria
  - Participants previously enrolled in ESCORT-HU
  - For new participants:
    - History of HU treatment for more than 5 years, or
    - Prepubescent over 10 years of age for girls and 13 years of age for boys at enrolment (possibility to follow them until the puberty and increase the number of patients with possible therapeutic window/stop to perform the semen cryopreservation), or
    - With history of leg ulcer, or
    - Pregnant women without interruption of Siklos® 3 months before the beginning of the pregnancy, or

  - Males treated with Siklos® whose partner is pregnant and without discontinuation of Siklos® 3 months before the beginning of the pregnancy.

- And who received at least one dose of Siklos®.

Sub-Groups Definitions

No subgroup analyses were produced for this interim report.

Protocol deviations

The list of protocol deviations including non-respected inclusion criteria was written by ICTA PM in a dedicated document and approved by the Sponsor. This list was reviewed with the Sponsor during the pre-analysis data review meeting. Number and percentage of patients in each deviation were presented on all enrolled patients.

Softwares:

Statistical analyses were performed with SAS® version 9.4 (SAS institute, North Carolina, USA).

General statistical tests:

The analyses were mainly descriptive. No formal statistical testing was carried out.

9.9.3. *Missing values*

In accordance with General Data Protection Regulation, the day of birth of participants was not collected (only month and year collected). Missing day of date of birth was replaced by 01 for calculations only.

Replacement rules applied for missing day and/or missing month of date of first administration of HU, date of permanent stop of Siklos®, date of interruption of Siklos®, date of restart of Siklos®, date of first diagnosis of malignancy and of diagnosis of leg ulcer, date of pregnancy and of complication linked to SCD, date of Siklos® treatment stopped before the pregnancy are those described in the SAP.

Neither missing data for quantitative variables nor missing data on categorical variables were replaced. Missing data items were indicated in each table.

9.9.4. *Handling of dropouts*

No general assessment of the dropouts in the study was made. Only the number of patients who drop out and the reason for dropout was presented in the disposition table.

9.9.5. *Sensitivity analyses*

No sensitivity analysis was performed for this interim report.

9.9.6. *Amendments to the statistical analysis plan*

Amendment to the SAP was produced for this analysis. The applicable version of the SAP for this fourth interim analysis was the SAP version 7.0 dated on 26th June 2024. This amendment mainly consisted in:

- Update of the sample size (according to protocol amendment, version 4.0 09SEP2023).
- Fertility status: description of semen analysis results for patient with status of treatment initiation unknow and with status of treatment interruption unknown (Section 7.2.7 of the SAP).
- Minor modifications of imputation rules for missing dates (sections 7.1.4, 7.4.1.4 of the SAP).
- Minor modifications in description of exposure time during pregnancy for patients who stopped treatment during the pregnancy only for patient who stopped definitively treatment during pregnancy (section 7.5.2 of the SAP).
- Addition of details of SCD complication in table (section 7.4.1.1 of the SAP)

- Addition of a "VOC" complication in the list of complications linked to SCD (Section 7.5.5 of the SAP).

9.10. QUALITY CONTROL

9.10.1. *Remote Monitoring*

A monitor from the CRO mandated by the Sponsor performed regular remote monitoring of the investigation centres during the setting up and conduct of the study. Frequency of the remote monitoring depended on the recruitment rate, agreement between the investigator and the monitor and the Sponsor's specifications.

9.10.2. *On-site Data Quality Control*

Prior to the initiation of the study, each investigator and study coordinator at participating sites were trained on the protocol and eCRF completion. This training was either provided via an investigator's meeting (face to face, virtual, or hybrid), or an onsite initiation visit performed by a Clinical Research Associate (CRA). Sites were provided with a workbook (CRF completion guide) describing completion of eCRF.

Data QC was performed on-site by a monitor from the CRO mandated by the Sponsor in all sites in each country involved in the study. At the time of the fourth interim report, 46 QC had been performed in 27 sites in France and 1 site in Greece. In each site, following an iterative approach, 5% of the participants underwent QC. Each CRF page was reviewed completely (100%) by the CRA for accuracy and completion. If the control was not satisfying (% of error >5%), other 5% of participants underwent QC.

It was the responsibility of the investigators to ensure that all data collected were recorded with minimum delay, accurately and legibly.

During the QC visits, the investigator allowed the monitor to:

- Inspect the site and the facilities where the study takes place as well as the material used for the study in order to ensure compliance with the protocol requirements,
- Meet all members of their team involved in the study,
- Consult all the documents relevant to the study,
- Have access to the eCRF,
- Directly access source documents for comparison of data therein with the data in the eCRF,

The monitor:

- Checked that the eCRF have been filled out correctly,
- Verified that the study is carried out in compliance with the protocol and local regulatory requirements,
- Paid attention to patients becoming adult during the course of the study. For children, consent was under the decision of parents/legally acceptable representatives until the 18th birthday. At this time the investigator had to discuss with the participant the possibility to continue with the study (during the first visit after 18th birthday) and consent had to be obtained and documented in the participant's medical file.

After the QC visit, CRA discussed with the investigator or delegated data entry specialist the reported findings and their resolution. If not possible on site, the list of findings was communicated after the visit in a follow-up email.

If corrections could not be obtained during the visit, written instructions were given for data entry correction according to the findings.

If computerised medical files were used, the investigator printed the medical files of the participants and securely archived them.

The investigator personally signed and dated the first page of each print-out, indicating the total number of pages. During a monitoring visit, the investigator provided print-outs of the participant's medical files to the monitor. Those print-outs have to remain on site after monitoring visit.

If the computer system used allowed the tracking of the changes made to the medical files, the investigator supplied the monitor, at each visit, with a print-out of the medical files of the participants and the records of the changes made. Each print-out was personally dated and signed, by the investigator on the first page. The number of pages was also indicated by the investigator on the first page.

All information dealt with during these visits were treated as strictly confidential.

The investigator guaranteed the safety of the study data in the medical files by implementing security measures to prevent unauthorised access to the data and to the computer system.

9.10.3. *Statistical validation*

A visual review of all statistical outputs via review of SAS code, SAS log and outputs, was performed by the ICTA PM Lead Statistician.

Once the statistical outputs were produced, an external review of the TFLs was performed by the ICTA PM Project Manager and by the ICTA PM Medical and Scientific Advisor.

10. RESULTS

The statistical analyses were performed according to the SAP version 7.0 dated on 26th June 2024. Tables, figures and graphs produced for this fourth interim analysis are provided in Appendix 4. Tables, Figures and Listings.

All statistical analyses (i.e. patients disposition and characteristics, primary and secondary endpoints) are presented on the Safety Population.

10.1. PARTICIPANTS

10.1.1. *Disposition of patients*

The disposition of patients is described in Figure 1 below, and in Table 1.1.1 in Appendix 4. Tables, Figures and Listings Inclusion criteria fulfilled by patients previously enrolled in ESCORT-HU study are displayed in Table 1.1.6 in Appendix 4. Tables, Figures and Listings, and inclusion criteria fulfilled by new participants are displayed in Table 1.1.7 in Appendix 4. Tables, Figures and Listings.

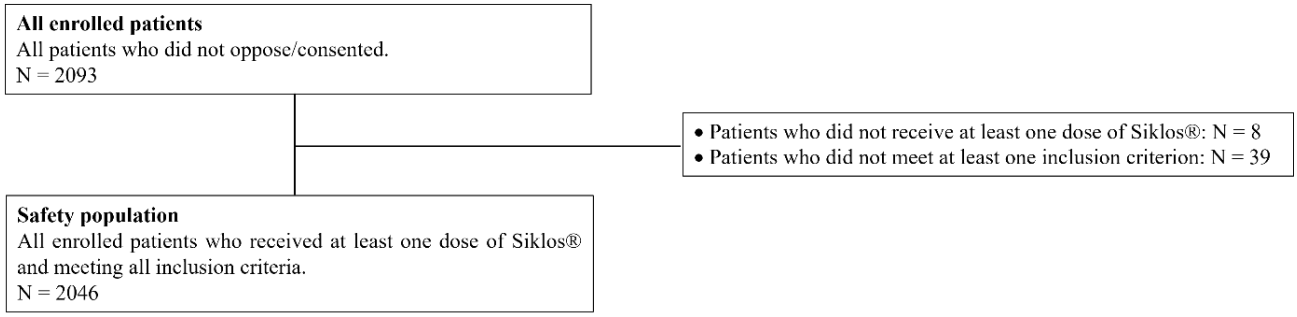
A total of 2093 patients were enrolled in the study at the time of interim analysis:

- 874 patients were previously enrolled in ESCORT-HU
- 1219 patients were new participants and among those,
    - 971 patients were treated with HU for more than 5 years,
    - 303 patients were prepubescent girls over 10 years of age or prepubescent boys over 13 years of age,
    - 126 patients had a history of leg ulcer,
    - 18 patients were pregnant women who did not stop Siklos® at least 3 months before the beginning of the pregnancy,
    - 3 patients were Males treated with Siklos® whose partner was pregnant and who did not discontinue Siklos® 3 months before the beginning of the partner's pregnancy.

  Of note, each patient could fulfil one or more of those criteria.

Among all enrolled patients, 2046 patients (97.8%) fulfilled the criteria for inclusion in the Safety Population. Patients not included in the Safety Population (47 patients) were either patients who did not meet one of the inclusion criteria at least, as defined in section Population Sets (39 patients) or patients who had not received at least one dose of Siklos® (8 patients). It is noteworthy that these 8 patients were included just before the database lock, so their data were not available yet at the time of this report.

Figure 1: Flowchart of the disposition of patients (All enrolled patients: N = 2093).



Source: ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Flowchart 1.1.1

10.1.2. *Protocol deviations*

Protocol deviations are detailed in Table 1.1.5 in Appendix 4. Tables, Figures and Listings.

Overall, 89 deviations were recorded. Recorded protocol deviations were mainly related to eligibility criteria not fulfilled (77 deviations) which concerned 88 patients (one patient presented more than one deviation):

- Twenty-two (22) patients were not treated with Siklos® at the time of inclusion
- Twenty-one (21) patients were not informed of the study or did not consent to participate
- Twenty-four (24) patients were females who did not meet at least one of the inclusion criteria required for inclusion of new female participants
- Ten (10) patients were males who did not meet at least one of the inclusion criteria required for inclusion of new male participants

The other reported deviations (12) were mainly due to consent/non-opposition not completed in the CRF (10 patients), sequence number of SUSAR not completed (1 patient), wrong version of consent signed (1 patient).

10.1.3. *Disposition of visits and study duration*

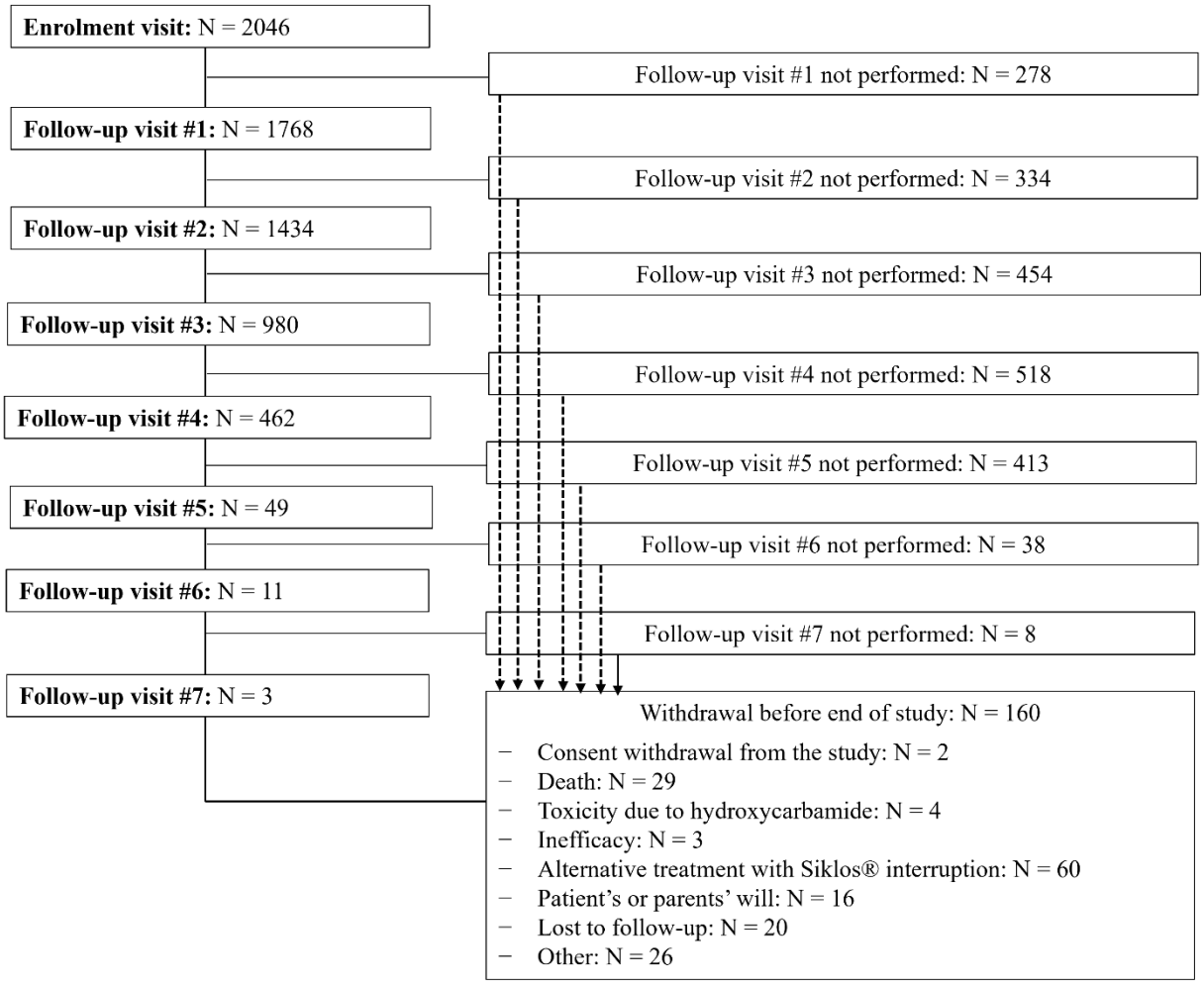
The disposition of visits completed for patients of the Safety Population is depicted in Figure 2. Reasons for premature discontinuation are displayed in Table 1.1.2 and detailed in Listing 1.1.1 in Appendix 4. Tables, Figures and Listings. Study duration is displayed in Table 1.1.3 in Appendix 4. Tables, Figures and Listings.

Enrolment visit was completed for the 2046 patients of the Safety Population. The first, second, third, fourth, fifth, sixth and seventh follow-up visits were completed for 1768 patients, 1434 patients, 980 patients, 462 patients, 49 patients, 11 patients and 3 patients respectively. A hundred and sixty (160) patients had withdrawn prematurely. Reasons for premature discontinuation were: alternative treatment with Siklos® interruption (60 patients), death (29 patients), lost to follow-up (20 patients), consent withdrawal (2 patients), toxicity due to HU (4 patient), inefficacy of HU (3 patients), patient's or parent's

will (16 patients) and other reasons (26 patients). Other reasons of withdrawal reported included "transplant" for 1 patient, "haplo-identical allograft" for 1 patient, "decline in kidney function" for 1 patient, "inclusion criteria not fulfilled" for 1 patient, "move to Canada" for 1 patient, and "switch to adult center" for 2 patients (all other reasons are listed in Listing 1.1.1).

Median study duration for the enrolled population, corresponding to duration from enrolment to withdrawal (or last follow-up visit for patients who had not withdrawn), was approximately 1.96 years (IQR: 0.97-2.71).

Figure 2: Flowchart of attended visits (Safety Population: N = 2046).



Source: Based on ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Flowchart 1.1.2.
Note: One patient withdrawn before the end of the study for "other" reason reported as "patient's will", was reclassified in this report under reason "patient's or parent's will".

10.2. DESCRIPTIVE DATA

10.2.1. *Demographics and other baseline characteristics*

Tables displaying the demographic data and other baseline characteristics are presented in Appendix 4. Tables, Figures and Listings, in Tables 1.2.1.1, 1.2.1.2, 1.2.1.3, 1.2.1.4, 1.2.1.5, 1.2.1.6 and 1.2.1.7.

For new participants, baseline characteristics correspond to characteristics at the time of enrolment in ESCORT-HU Extension study. For participants previously included in ESCORT-HU study, baseline characteristics correspond to the characteristics of patients at the time of their last visit in ESCORT-HU

study. As such, all events of interest which occurred between end of ESCORT-HU study and start of ESCORT-HU Extension study were taken into account in primary and secondary endpoints analyses.

- Demographic characteristics

Demographic characteristics of patients included in the Safety Population are presented in Table 4 and in Appendix 4. Tables, Figures and Listings, Table 1.2.1.1.

Female patients represented 55.8% of the Safety Population and male patients represented 44.2% of the Safety Population. Age at inclusion ranged from 5 to 80 years old with a median age of 23 years.

The vast majority of patients (86.8%) were homozygous for HbS allele. Sß+, Sß0 and SC genotypes represented 5.2%, 5.3% and 1.3% of patients respectively. Other encountered S genotypes (1.5% patients) are detailed in Listing 1.2.1.1, available in Appendix 4. Tables, Figures and Listings.

Among the patients with available information on alpha-globin gene defects (2039 patients), 14.8% presented with a deletion of alpha-globin gene and 32.9% had no deletion. For the remaining 52.2% patients, alpha-deletion status was either not assessed or unknown.

Among the patients with available information on associated G6PD defect (2045 patients), deficiency was reported for 8.9% of patients and absence of deficiency was reported for 70.2% patients. For the remaining 20.9% patients, G6PD deficiency status was either not assessed or unknown.

Table 4: Demographic characteristics - Safety population (N=2046 patients)

		Safety population (N = 2046)
Gender	N	2046
Female	n (%)	1142 (55.8%)
Male	n (%)	904 (44.2%)
Age at the time of inclusion (years)	N	2046
	Mean ± SD	27.39 ± 16.31
	Median	23.00
	Q1 ; Q3	13.00 ; 40.00
	Min ; Max	5.0 ; 80.0
Genotype	N (missing values)	2046 (0)
SS	n (%)	1776 (86.8%)
Sß0	n (%)	106 (5.2%)
Sß+	n (%)	108 (5.3%)
SC	n (%)	26 (1.3%)
Other S genotype	n (%)	30 (1.5%)
Deletion of alpha-globin gene	N (missing values)	2039 (7)
Presence of deletion	n (%)	301 (14.8%)
G6PD deficiency	N (missing values)	2045 (1)
Presence of deficiency	n (%)	182 (8.9%)

Source: ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Table 1.2.1.1

- Hydroxycarbamide history

ESCORT-HU Extension study enrolled both patients already treated with HU at the time of enrolment and patients initiating HU at the time of inclusion. Data related to HU treatment history are provided in Table 5 and in Appendix 4. Tables, Figures and Listings, Table 1.2.1.2.

Time since first administration of HU at enrolment in ESCORT-HU Extension study could be calculated for 2044 patients of the Safety Population. Median time between first administration and enrolment was almost 6 years (range: 0 to 32.7 years). Median daily dose of Siklos® was 20 mg/kg/day (IQR: 15-24 mg/kg/day).

Reasons of initiation for HU are detailed in Appendix 4. Tables, Figures and Listings, Table 1.2.1.2. The majority of patients (76.5%) initiated HU because of vaso-occlusive crisis/acute chest syndrome.

Table 5: Hydroxycarbamide history - Safety population (N=2046 patients)

		Safety population (N = 2046)
Time since first administration of any specialty of hydroxycarbamide (years)	N (missing values)	2044 (2)
	Mean ± SD	7.06 ± 4.77
	Median	5.84
	Q1 ; Q3	4.15 ; 8.83
	Min ; Max	-0.0 ; 32.7

Patients ESCORTHU2-031-1997, ESCORTHU2-031-2060 had their date of first administration of hydroxycarbamide (respectively 17NOV2023 and 01FEB2024) after their date of enrolment (respectively 03NOV2023 and 31JAN2024) but it was decided to keep them in the safety set.

Source: ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Table 1.2.1.2

- Other SCD treatments

Several patients also received other treatments for SCD, of which the nature is provided in Table 6 (and detailed Appendix 4. Tables, Figures and Listings, Table 1.2.1.3and Listing 1.2.1.2).

Among patients in the Safety population, 16.4% received at least one other treatment for SCD, at enrolment and during the study, of which blood transfusion program / regular blood transfusion for 47.6% of them.

Table 6. Other SCD treatments

		Safety population (N = 2046)
Another treatment received for SCD	N (missing values)	2046 (0)
At least one other treatment received for SCD	n (%)	336 (16.4%)
	N (missing values)	336 (0)
	anti-adhesion therapy	4 (1.2%)
	anti-inflammatory	2 (0.6%)
	anti-sickling agent	9 (2.7%)
	anticoagulant	25 (7.4%)
	blood transfusion program / regular blood transfusion	160 (47.6%)

		Safety population (N = 2046)
	L-glutamine	10 (3.0%)
	other hydroxycarbamide treatment	1 (0.3%)
Reason of initiation	treatment received for insufficient effectiveness of hydroxycarbamide	43 (12.8%)
	treatment received for resistance to hydroxycarbamide	1 (0.3%)
	treatment received for appearance of complications	40 (11.9%)
	treatment received for another reason (main reasons: VOC/ACS, vasculopathy, anemia, transplantation and pregnancy, ulcer…)	151 (44.9%)

Source: ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Table 1.2.1.2

- History of malignancies

Table 7 provides information on patients' history of malignancies (detailed in Appendix 4. Tables, Figures and Listings, Table 1.2.1.4, Listings 1.2.1.5 and 1.2.1.6).

Twenty-two (22) patients (1.1%) of the Safety Population had history of malignancies at the time of enrolment in ESCORT-HU Extension study. Reported organs were breast (7 patients; 1 with family history of breast malignancy), cervix (2 patients – 1 reported as "neck of the uterus"), thyroid (3 patients), kidney (2 patients), bladder, skin, tongue, bone, bone marrow, liver, ovary and parathyroid (1 patient each). Mean age at diagnosis was 41.03 ± 11.96 years.

Fifteen (15) patients (68.2%) had received a treatment for their malignancy at the time of enrolment in ESCORT-HU Extension. Fourteen (14) of them had received a surgical treatment, 2 patients had received a chemotherapy treatment and 2 patients had received a treatment with radiotherapy at the time of enrolment in ESCORT-HU Extension. Fourteen (14) patients had completely recovered at the time of enrolment in ESCORT-HU Extension or at the time of last visit performed for ESCORT-HU study, 1 patient was on path to recovery and 2 patients had remission. The malignancy of 2 patients was ongoing at this time and status was unknown for 3 patients.

Table 7: Malignancy history - Safety population (N = 2046)

		Safety population (N = 2046)
Medical history of malignancy	N (missing values)	2045 (1)
History of malignancy	n (%)	22 (1.1%)
Treatment at enrolment or at last visit in ESCORT-HU	N (missing values)	22 (0)
Malignancy treated	n (%)	15 (68.2%)

		Safety population (N = 2046)
Status of the malignancy at enrolment or at last visit in ESCORT-HU	N (missing values)	19 (3)
Complete recovery	n (%)	14 (73.7%)
Recovering	n (%)	1 (5.3%)
Remission	n (%)	2 (10.5%)
Ongoing	n (%)	2 (10.5%)

Source: ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Table 1.2.1.3

- History of leg ulcers

Table 8 provides information on patients' history of leg ulcers (detailed in Appendix 4. Tables, Figures and Listings, Table 1.2.1.5). A listing specifying leg ulcer locations is provided in Appendix 4. Tables, Figures and Listings, Listing 1.2.1.7.

History of leg ulcers was encountered in 209 patients (10.2%) of the Safety population. Among those, 46.9% had a single lesion while 40.2% patients had multiple lesions. Approximately 51.7% patients had experienced multiple episodes of leg ulcers. For 8.1% of patients, leg ulcers were assessed as related to HU treatment. At the time of enrolment in ESCORT-HU Extension study, 71.3% of patients had completely recovered of their last episode of leg ulcer.

Table 8: History of leg ulcers - Safety population (N = 2046)

		Safety population (N = 2046)
Medical history of leg ulcer	N (missing values)	2042 (2)
History of leg ulcer [a]	n (%)	209 (10.2%)
Type of lesion [b]	N (missing values)	209 (0)
	Multiple lesions	84 (40.2%)
	Single lesion	98 (46.9%)
Episode(s) [c]	N (missing values)	209 (0)
	Multiple episodes	108 (51.7%)
	Single episode	67 (32.1%)
Status of the last leg ulcer at enrolment [d]	N (missing values)	209 (0)
	Complete recovery	149 (71.3%)
	Ongoing	36 (17.2%)
	Recovering	14 (5.6%)
Relationship with HU [e]	N (missing values)	209 (0)

		Safety population (N = 2046)
Related to HU Yes	n (%)	17 (8.1%)

[a] For 2 patients history of leg ulcer was reported as unknown.
[b] For 23 patients (11.0%) the type of lesion was reported as unknown and for 4 patients (1.9%), as not applicable.
[c] For 29 patients (13.9%) the type of episode was reported as unknown and for 5 patients (2.4%), as not applicable.
[d] For 5 patients (2.4%) the status of the last leg ulcer was reported as unknown and for 5 patients (2.4%), as not applicable.
[e] For 44 patients (21.1%) the relationship with HU was reported as unknown, for 1 patient (0.5%) it was not done and for 5 patients (2.4%), as not applicable.
Source: ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Table 1.2.1.4

- Fertility status

Female fertility status

A summary of female fertility status information at enrolment in ESCORT-HU Extension study or at last visit in ESCORT-HU study is provided in Table 9 and detailed in Table 1.2.1.7 in Appendix 4. Tables, Figures and Listings.

Overall, 1142 Females were included in the Safety Population and pubescent status was available for 1141 patients. At the time of enrolment in ESCORT-HU Extension study or at last visit in ESCORT-HU study, 824 Females (72.2%) were pubescent. Summary statistics on age at onset of puberty could be calculated for 380 of those: median age was 14 years (range: 8 to 23 years).

Among pubescent females, 47.5% had completed at least one pregnancy before enrolment in ESCORT-HU Extension study or at last visit in ESCORT-HU study. Approximately one-third (30.5%) had completed one pregnancy while the others had completed two pregnancies or more. Among females with at least one pregnancy, 46.3% had experienced at least one pregnancy without living child and among those, 52.0% had experienced two or more pregnancies without living child. Most of them (90.0%) had also completed at least one pregnancy with a living child.

Table 9: Female fertility status at enrolment - Safety population (N = 2046)

		Safety population (N = 2046)
FEMALE	N (missing values)	1142 (0)
Pubescence status	N (missing values)	1141 (1)
Pubescent [a]	n (%)	824 (72.2%)
Pregnancy before enrolment for pubescent Females	N	824
Pregnancy [b]	n (%)	391 (47.5%)

[a] For 19 patients (1.8%) the status was reported as unknown, not applicable or not done.
[b] For 36 patients (4.3%) the status was reported as unknown or not applicable or not done.
Source: ESCORT-HU2_IA4_ TFLs_V1.0_03SEP2024– Table 1.2.1.7

Male fertility status

A summary of male fertility status information at enrolment in ESCORT-HU Extension study or at last visit in ESCORT-HU study is provided in Table 10. Additional information is provided in Table 1.2.1.7 available in Appendix 4. Tables, Figures and Listings.

Overall, 904 males were included in the Safety Population and pubescent status was available for 899 patients. At the time of enrolment in ESCORT-HU Extension study or at last visit in ESCORT-HU

study, 511 males (56.8%) were pubescent. Summary statistics on age at onset of puberty could be calculated for only 102 of those: median age was 15 years (range: 11 to 20 years).

Sperm cryopreservation had been performed for 142 pubescent males (27.8%) before enrolment, at a median age of 26 years (range: 11 to 50 years). Of note, whether cryopreservation had been performed or not was unknown for almost 10.4% of pubescent males.

For 77.5% of males who had cryopreservation, the cryopreservation had been performed before HU treatment initiation. For 6 patients (4.2%), the timing of cryopreservation was reported as not known or not done and 26 patients (18.3%) performed a cryopreservation while HU had already been initiated. Among patients already initiated with HU, treatment status at the time of cryopreservation was not known for 5 patients, HU treatment of 17 patients was interrupted before cryopreservation and 4 patients performed cryopreservation while on treatment.

For 103 pubescent males (20.2%), semen analysis had been performed at the time of enrolment in ESCORT-HU Extension study. For 74.8% of them (77 patients), analysis had been performed before HU initiation (unknown, not applicable and not done was reported for 7.8% patients): 34 patients had normal results, 7 patients had qualitative abnormalities, 6 patients had quantitative abnormalities and 8 patient had both qualitative and quantitative abnormalities (unknown result for other 22 patients).

Among patients with semen analysis performed after HU treatment initiation (18 patients), HU was stopped before analysis for 11 patients with 4 normal results, 2 quantitative abnormalities, 1 qualitative and quantitative abnormality and 4 results not known. Five (5) patients had semen analysis performed without HU interruption beforehand. Among those, 3 patients had both qualitative and quantitative abnormalities and 1 patient had quantitative abnormalities (unknown result for the last patient). Two (2) patients had semen analysis performed with HU status unknown; results were normal for both patients.

Table 10: Male fertility status at enrolment - Safety population (N = 2046)

		Safety population (N = 2046)
MALE	N (missing values)	904 (0)
Pubescence status	N (missing values)	899 (5)
Pubescent [a]	n (%)	511 (56.8%)
Sperm cryopreservation for pubescent Males	N	511
Cryopreservation performed [b]	n (%)	142 (27.8%)
Semen analysis for pubescent Males	N (missing values)	510 (1)
Semen analysis performed [c]	n (%)	103 (20.2%)

[a] For 30 patients (3.4%) the status was reported as unknown, not applicable or not done.
[b] For 70 patients (13.8%) the status was reported as unknown, not applicable or not done.
[c] For 95 patients (18.6%) the status was reported as unknown, not applicable or not done.
Source: ESCORT-HU2_IA4_ TFLs_V1.0_03SEP2024– Table 1.2.1.7

10.2.2. *Treatment exposure*

Treatment exposure data is presented in Table 11 and in Appendix 4. Tables, Figures and Listings, Table 1.3.1.

ESCORT-HU Extension study enrolled both patients already treated with HU at the time of enrolment and patients initiating HU at the time of inclusion. Treatment exposure reflects duration of exposure to any specialty of HU.

Median exposure duration to HU for patients of the Safety Population was 9 years (range: 0 to 38.3 years) and 75% patients were exposed to HU for more than 6 years at least (Q1: 6.9 years) at the time of this interim analysis.

Table 11: Treatment exposure - Safety population (N = 2046)

		Safety population (N = 2046)
Exposure (years)	N (missing values)	2044 (2)
	Mean ± SD	10.11 ± 5.15
	Median	9.00
	Q1 ; Q3	6.90 ; 12.27
	Min ; Max	-0.0 ; 38.3

Note: Patients ESCORTHU2-031-1997, ESCORTHU2-031-2060 had their date of first administration of hydroxycarbamide (respectively 17NOV2023 and 01FEB2024) after their date of enrolment (respectively 03NOV2023 and 31JAN2024) but it was decided to keep them in the safety set.
Source: ESCORT-HU2_ IA4_TFLs_V1.0_03SEP2024– Table 1.3.1

10.3. OUTCOME DATA

Main outcome results were calculated on the Safety Population (2046 patients) and, for results related to male fertility impairment, also on the subpopulation of pubescent males of the Safety Population with at least one semen analysis performed (16 patients).

10.4. MAIN RESULTS

10.4.1. *Analysis of the Primary Endpoint*

The primary endpoint of ESCORT-HU Extension study was to determine the occurrence, the causal relationship with Siklos® and the main features of malignancies, leg ulcers, male fertility impairment (oligospermia, azoospermia) and SUSARs.

These sections focus on new events of interest that have occurred after the start of ESCORT-HU Extension study, i.e. after the date of enrolment in ESCORT-HU Extension for new participants or after the date of last visit in ESCORT-HU for patients previously enrolled in ESCORT-HU.

The tables displaying the occurrence of these events of interest are presented in Appendix 4. Tables, Figures and Listings, in Table 2.1.1.1 and Table 2.1.1.2, and events are detailed in Listing 2.1.1.1, Listing 2.1.1.2 and Listing 2.1.1.3.

- Malignancies

Twenty-one (21) patients (1.0%) of the Safety Population (16 Females and 5 Males, all pubescent) had at least one malignancy diagnosed after the start of ESCORT-HU Extension study (Table 12):

Table 12. Malignancies diagnosed during ESCORT-HU Extension study - Safety population (N = 2046)

	Gender, Age at the inclusion in ESCORT-HU Extension study (years)	Age at diagnosis of malignancy (years)	Time between first exposure to HU and diagnosis (years)	Relationship with Siklos® treatment	Status at the time of the 4[c] interim analysis
Hematological malignancies					
Acute lymphoblastic leukemia	Female, 15	15	6	Unknown	Ongoing
Bone marrow malignancy	Female, 26	27	7	Unknown	Ongoing
Blood malignancy	Female, 54	57	22.5	Unknown	Ongoing
Chronic lymphatic leukemia	Female, 65	68	19	Unknown	Ongoing
Myeloma	Female, 38	41	14	Unknown	Ongoing
Lymphoid glands malignancy	Female, 65	65	12	No	Completely recovered
Lymphoma large cells stage IV	Female, 58	59	8	No	Ongoing
Lymphoma T	Female, 38	40	14	No	Ongoing
Solid tumors					
Left axillary adenocarcinoma	Female, 38	40	6.5	No	Ongoing
Breast malignancy	Female, 54	55	8.5	Unknown	remission
Cervix	Female, 38	40	6.5	No	Ongoing
Uterus malignancy	Female, 37	35	9	No	Completely recovered
Thyroid gland	Female, 53	56	7	No	Completely recovered
Lungs	Male, 52	55	13	No	Ongoing
Muscle malignancy	Female, 13	16	5.3	No	Ongoing
Colon malignancy	Female, 29	30	5.5	No	Ongoing
	Female, 43	44	10	No	Ongoing
Kidney malignancy	Male, 67	67	23	Unknown	Ongoing
Skin malignancy	Male, 70	71	21	Unknown	Completely recovered
Liver malignancy	Male, 61	60	19.5	No	Remission
Malignancy of the retina	Male, 58	59	13	No	Completely recovered

Note: patients with age of malignancy diagnosis younger than age at inclusion to ESCORT-HU Extension study, are those who were included in ESCORT-HU study.
Source: ESCORT-HU2_IA4_ TFLs_V1.0_03SEP2024– Listing 2.1.1.1.

- Leg ulcers

Overall, 92 patients (4.5%) of the Safety Population developed at least one leg ulcer (125 events) since the start of ESCORT-HU Extension study. All patients were pubescent at the time of occurrence; mean age at occurrence was 47.9 ± 11.0 years.

Eighty-two (82) reported leg ulcers were with a single lesion while 34 were with multiple lesions (unknown for 9 events). Leg ulcer locations are detailed in Table 2.1.1.2 in Appendix 4. Tables, Figures and Listings. For 71 events (56.8%), those were reoccurrence of previous leg ulcers with same localisation as the previous one in 56 cases (78.9%). For 20 events (16.0%), the leg ulcers reported were aggravation of a leg ulcer already known. Most of the diagnosed leg ulcers (84.0%) during the follow-up period occurred in patients who had a history of leg ulcer.

At the time of this interim analysis, recovery status was complete recovery for 63 leg ulcers (50.4%), recovering for 16 leg ulcers (12.8%) and 46 leg ulcers (36.8%) were ongoing.

Time between first administration of HU and first leg ulcer incident during the study could be calculated for the 91 patients who experienced one leg ulcer and is presented in Table 13. Median time was approximately 9.2 years (IQR: 5.1-13.4 years). For leg ulcers with a relationship with HU exposure, median time between first administration of HU and first leg ulcer incident was 5.7 years (IQR: 2.4-9.9 years).

Five (5) leg ulcers (4.0%) were reported as related to Siklos®, 35 (28.0%) had unknown relationship to Siklos® and 85 (68.0%) were not related to Siklos®.

Table 13: Time between treatment initiation and first incident leg ulcer - Safety population (N = 2046)

		Safety population (N = 2046)
Time between first administration of HU and first incident leg ulcer (meaning occurring during the follow-up) (years) [a]	N (missing values)	91 (1)
	Mean ± SD	10.22 ± 6.33
	Median	9.17
	Q1 ; Q3	5.11 ; 13.42
	Min ; Max	0.0 ; 28.2
For Leg ulcer with a relationship with HU exposure		
Time between first administration of hydroxycarbamide and first incident leg ulcer (meaning occurring during the follow-up period) (years)	N (missing values)	4 (0)
	Mean ± SD	6.14 ± 5.34
	Median	5.69
	Q1 ; Q3	2.40 ; 9.88
	Min ; Max	0.2 ; 13.0

[a] For 23 patients in the safety population relationship of leg ulcer with HU exposure is unknown.
Source: ESCORT-HU2_IA4_TFLs_V1.0_03SEP2024– Table 2.1.1.5

- Male fertility impairment

Male fertility impairment related events were analysed on the population of pubescent males of the Safety Population, i.e. males of the Safety Population who were already pubescent at the time of inclusion and males who turned pubescent during the study, who had at least one semen analysis performed during study follow-up (17 semen analyses in 16 patients).

Thirteen (13) patients were diagnosed with at least one male fertility impairment after the start of ESCORT-HU Extension study (14 fertility impairments overall). One fertility impairment (1) concerned a patient with history of fertility impairment (data was missing for 10 patients).

- Siklos® was not stopped before performing 6 semen analysis:
  - One (1) semen analysis result was qualitative abnormality.
  - Five (5) results were quantitative abnormalities (1 unknown detail, 1 oligospermia and 3 azoospermia).
- Siklos® was stopped before performing 8 semen analysis:
  - One (1) semen analysis result was qualitative abnormality.
  - Four (4) results were quantitative abnormalities (3 oligospermia and 1 azoospermia).
  - Three (3) results were both qualitative and quantitative abnormalities. Oligospermia was reported as quantitative abnormality.

▪ Serious unexpected adverse events related to Siklos®

Four (4) patients experienced a SUSAR during the period of interest for this fourth interim analysis (Appendix 4. Tables, Figures and Listings, Listing 2.1.2.1):

- One SUSAR was an acute spleen sequestration of mild severity which occurred in a 10-year-old male (age at enrolment; age at occurrence was 11 years old). Red blood cells transfusion was performed as corrective treatment. This event was resolved at the time of this interim analysis.
- The second SUSAR was a retinal micro bleeding of mild severity which occurred in a 13-year-old male (age at enrolment; age at occurrence was unknown). No corrective treatment was performed. This event was ongoing at the time of this interim analysis.
- The third SUSAR was a severe type 2 diabetes which occurred in a 49-year-old female (age at enrolment; age at occurrence was 47 years old. Diamicron and metformine treatments were administered as a corrective treatment. This event was ongoing at the time of this interim analysis.
- The fourth SUSAR was a gonadal toxicity (“insuffisance gonadotrope avec une testostérone et une Inhibine B effondrée”) which occurred in a 16-year-old male (age at enrolment; age at occurrence was 18 years old). No corrective treatment was performed. This event was recovering at the time of this interim analysis.

10.4.2. *Analysis of the Secondary Endpoints*

The secondary endpoints for this interim analysis related to Female and Male fertility, management and use of cryopreservation and pregnancies.

▪ Fertility status

The other secondary endpoints of the study related to fertility status, were to document the mean age at puberty for females (age at first period) and males (age at Tanner V stage), the use of contraception, the use of cryopreservation, the management of Siklos® during pregnancy and the outcome of pregnancies following exposure to Siklos® (detailed in section 9.1.3).

Female fertility status

A summary of female fertility status information during follow-up is provided in Table 14 and available with more details in Table 3.1.1 in Appendix 4. Tables, Figures and Listings.

Table 14: Female fertility status during study follow-up – Safety population (N=2046)

		Safety population (N = 2046)
Females turned pubescent since enrolment	N [a]	318
	n (%)	121 (38.1%)

		Safety population (N = 2046)
Age at first period (years)	N (missing values)	105 (16)
	Mean ± SD	13.19 ± 1.44
	Median	13.00
	Q1 ; Q3	12.00 ; 14.00
	Min ; Max	11.0 ; 17.0

[a] Number of prepubescent Females of the Safety population at enrolment in Escort-HU Extension study
Source: ESCORT-HU2_ IA4_ TFLs_V1.0_03SEP2024– Table 3.1.1

Among the 318 prepubescent female patients at enrolment, 121 (38.1%) turned pubescent during the study follow-up period. Age at onset of puberty could be calculated for 105 of them: median age at first periods was 13 years (range 11-17 years).

Overall, among the 945 female patients who were pubescent at enrolment or turned pubescent during follow-up, information on use of contraception during follow-up was available for 558 of them. Use of contraception during treatment period was reported at least once for 179 (32.1%) of these pubescent female patients.

Male fertility status

A summary of male fertility status information during follow-up is provided in Table 15 and available with more details in Table 3.1.1 in Appendix 4. Tables, Figures and Listings.

Table 15: Male fertility status during study follow-up – Safety population (N=2046)

		Safety population (N = 2046)
Males turned pubescent since enrolment	N [a]	393
	n (%)	96 (24.4%)
Age at puberty (years)	N (missing values)	70 (26)
	Mean ± SD	15.21 ± 1.53
	Median	15.00
	Q1 ; Q3	14.00 ; 16.00
	Min ; Max	12.0 ; 19.0

[a] Number of prepubescent Males of the Safety population at enrolment in Escort-HU Extension study
Source: ESCORT-HU2_ IA4_TFLs_V1.0_03SEP2024– Table 3.1.1

Among the 393 prepubescent male patients at enrolment, 96 (24.4%) turned pubescent during the study follow-up period. Age at onset of puberty could be calculated for 70 of them: median age at puberty was 15 years (range 12-19 years).

Overall, among the 607 male patients of the Safety population who were pubescent at enrolment or turned pubescent during follow-up, information on sperm cryopreservation during follow-up was available for 566 patients (data was missing for 41 patients): 14 pubescent Males underwent sperm cryopreservation (≥4 sperm cryopreservations for each patient). Age at sperm cryopreservation could be calculated for 2 patients only: cryopreservations were performed at 22 and 31 years. Median average

duration between age of onset of puberty and age of cryopreservation could be calculated only for 3 patients and was 60 months. Nine (9) patients had at least one cryopreservation with Siklos® interrupted beforehand. Three (3) cryopreservations were used for pregnancy purpose.

As reported in section 10.4.1, 16 pubescent male patients of the Safety population had a semen analysis during the study follow-up period. Although features of semen abnormalities are already described in this section, detail on management and results of all semen analyses is reported in Table 3.1.1 in Appendix 4. Tables, Figures and Listings.

Among the 17 semen analyses performed during Escort-HU Extension follow-up, 8 were performed after Siklos® stop. Three semen analyses had normal results, the 14 other analyses presented semen qualitative and/or quantitative abnormalities (already described in section 10.4.1).

- Pregnancies

Information on pregnancies of patients or patient partners who turned pregnant during follow-up is displayed in Table 3.1.2 and listings 3.1.1 and 3.1.2 in Appendix 4. Tables, Figures and Listings.

Pregnancies in female patients

Main features of pregnancies experienced by female patients during Escort-HU Extension follow-up period are summarized in Table 16.

Among pubescent female patients of the Safety population, 79 Females (8.4%) had at least 1 pregnancy during the study follow-up period and 93 pregnancies were reported, of which 77 exposed to Siklos®.

Among the 93 reported pregnancies, 57 (61.3%) were with living children as outcome: 44 with normal birth (47.3%) and 11 with premature birth (11.8%) (1 with an outcome unknown and 1 "not applicable"). Twenty-nine (29) pregnancies (31.2%) were reported without living child as outcome: 19 (20.4%) due to miscarriage, 1 (1.1%) stillbirth, 6 (6.5%) due to elective abortion, 1 (1.1%) due to therapeutic or medical abortion, 1 (1.1%) due to ectopic pregnancy (the status of 1 pregnancy (without a living child) was completed as "not applicable"). At the time of this interim analysis, 7 pregnancies (7.5%) were ongoing. No foetal defect of living child was reported for 54 pregnancies, and it was reported as "not applicable" (1 pregnancy), or "not done" (1 pregnancy) or "unknown" (1 pregnancy).

Table 16: Pregnancies outcomes in female patients

		Safety population (N=2046)
Total number of pregnancies in female patients	N	93
Pregnancy outcomes	N (missing)	93 (0)
Pregnancy with a living child	n (%)	57 (61.3%)
Pregnancy without a living child	n (%)	29 (31.2%)
Ongoing	n (%)	7 (7.5%)
Pregnancy outcome status [a]	N (missing)	93 (0)
Normal birth	n (%)	44 (47.3%)
Premature birth	n (%)	11 (11.8%)
Miscarriage	n (%)	19 (20.4%)
Stillbirth	n (%)	1 (1.1%)

		Safety population (N=2046)
Elective abortion	n (%)	6 (6.5%)
Ectopic pregnancy	n (%)	1 (1.1%)
Therapeutic or medical abortion	n (%)	1 (1.1%)
Unknown	n (%)	1 (1.1%)
Not applicable	n (%)	9 (9.7%)

[a] The ongoing pregnancies (n=7) were classified under 'not applicable' category. The status of 1 pregnancy (without a living child) was completed as “not applicable”.
Source: ESCORT-HU2_ IA4_TFLs_V1.0_03SEP2024– Table 3.1.2

Exposure to HU during pregnancy are resumed in Table 17 and detailed in Table 3.1.2 in Appendix 4. Tables, Figures and Listings. Overall, 77 pregnancies were exposed to HU. Exposure time to HU during the entire pregnancy could be calculated for 35 pregnancies with an outcome completed. Median exposure time was approximately 6.3 weeks (IQR: 3.9-13.0 weeks).

Among the 93 reported pregnancies, Siklos® treatment was stopped before conception for 16 pregnancies (25.3%); exposure status was unknown for 2 pregnancies and “not done” for 2 pregnancies.

In pregnancies other than ongoing (86 pregnancies), where Siklos® was not stopped before conception (71 pregnancies), treatment was stopped during the first trimester for 42 of them (60.9%) (median exposure duration of 38 days, IQR: 23-58 days, calculated for 21 pregnancies). Among them, treatment restarted during the first trimester for 7 of them (median delay since treatment stopped of 32 days, IQR: 16-47 days), during the second trimester for 2 pregnancies (delay since treatment stopped of 27 and106 days) and during the third trimester for 7 pregnancies (median delay since treatment stopped of 228 days, IQR: 177-239 days).

Treatment was stopped during the second trimester for 6 pregnancies (8.7%) (median exposure duration of 4 pregnancies was 118 days, IQR: 110- 131.50 days). Among them, treatment restarted during the second trimester for 1 pregnancy (delay since treatment stopped of 33 days) and during the third trimester for 1 pregnancy (delay since treatment stopped of 172 days).

No pregnancies had treatment stopped during the third trimester at the time of the interim analysis.

Treatment was not stopped at all for the 21 remaining pregnancies (2 pregnancies with an unknown status of treatment during pregnancy and without date of treatment restart were considered as missing). Among these pregnancies, 4 normal births, 3 premature births, 8 miscarriages, 1 stillbirth, 4 elective abortions, 1 therapeutic or medical abortion were reported.

Table 17. HU exposure during pregnancies of female patients

		Safety population (N=2046)
HU exposure during pregnancy [a]	N	93
	Yes	77 (82.8%)
Exposure time to HU during pregnancy for patients with an outcome completed (weeks)	N (missing values)	35 (42)
	Mean ± SD	9.07 ± 7.44
	Median	6.30
	Q1 ; Q3	3.90 ; 13.00
	Min ; Max	0.0 ; 32.9
Treatment stopped before pregnancy	Yes	16 (17.2%)
Treatment not stopped before pregnancy [b]	N	86

		Safety population (N=2046)
Pregnancies with treatment stopped during the **first trimester** [c]	Yes	71 (82.6%)
	N (missing values)	69 (2)
Pregnancy with treatment stopped during first trimester restarted during the first trimester	Yes	42 (60.9%)
	N	42
Pregnancy with treatment stopped during first trimester restarted during the second trimester	Yes	7 (16.7%)
	N	42
Pregnancy with treatment stopped during first trimester restarted during the third trimester	Yes	2 (4.8%)
	N	42
Pregnancies with treatment stopped during the **second trimester** [c]	Yes	7 (16.7%)
	N (missing values)	69 (2)
Pregnancy with treatment stopped during second trimester restarted during the second trimester	Yes	6 (8.7%)
	N	6
Pregnancy with treatment stopped during second trimester restarted during the third trimester	Yes	1 (16.7%)
	N	6
	Yes	1 (16.7%)

[a] Pregnancies with treatment stopped before pregnancy equal to « Not done » or « Unknown » were considered as exposed. Exposure time = baby birth date#1 - date of pregnancy - duration of temporary Siklos stops or baby birth date#1 # Duration of stops = sum(Date of restart - Date of stop).
[b] For pregnancies other than "ongoing".
[c] Pregnancies with an unknown status of treatment during pregnancy and without date of treatment restart were considered as missing.
Source: ESCORT-HU2_ IA4_TFLs_V1.0_03SEP2024– Table 3.1.2

Pregnancies in female partners of male patients

Main features of pregnancies experienced by female partners of male patients during Escort-HU Extension follow-up period are summarized in Table 18.

Among pubescent male patients of the Safety population, 24 (4.0%) had at least 1 pregnancy reported for their female partners during the study follow-up period and 27 pregnancies were reported.

Among the 27 reported pregnancies, 22 (84.6%) were with living children as outcome, 2 pregnancies (7.7%) were reported without living child as outcome, 2 pregnancies (7.7%) were ongoing and 1 had unknown outcome (1 missing value). Regarding pregnancy status details, 21 pregnancies were with normal birth (77.8%) and the outcome was a miscarriage for 2 pregnancies (7.4%). No foetal defect was reported.

Siklos® treatment was stopped before conception for 13 pregnancies (48.1%).

Table 18: Pregnancies outcome in female partners of male patients

		Safety population (N=2046)
Total number of pregnancies in female partners	N	27
Pregnancy outcomes	N (missing value) [a]	26 (1)
Pregnancies with a living child	n (%)	22 (84.6%)
Pregnancies without a living child	n (%)	2 (7.7%)

		Safety population (N=2046)
Ongoing	n (%)	2 (7.7%)
Pregnancy outcome status [a][b]	N	25
Normal birth	n (%)	21 (77.8%)
Miscarriage	n (%)	2 (7.4%)
Treatment stopped before pregnancy	N	13 (48.1%)

[a] Outcome status was reported as "unknown" for 1 pregnancy.
[b] The ongoing pregnancies are classified under 'not applicable' category. The status of 1 pregnancy (without a living child) was completed as "not applicable".
Source: ESCORT-HU2_ IA4_TFLs_V1.0_03SEP2024– Table 3.1.2

- Barriers of compliance

Barriers of compliance are presented in Table 19 and detailed in Table 3.1.3 and Listing 3.1.3 in Appendix 4. Tables, Figures and Listings. Among patients of the Safety population, 296 (14.5%) had at least one temporary stop for more than 2 weeks all along the study, and the majority (75.0%) had 1 stop; median daily dose of HU at stop was 17 mg/kg/day (ranged from 2 to 35 mg/kg/day). Median duration of stop could be calculated for 233 patients and was approximately 94 days (ranged from 0.3 to 1731 days). Among the 296 patients who stopped HU for more than 2 weeks all along the study, 117 patients (84.8%) had at least one vaso-occlusive episode during treatment stop period (158 missing value). Among reasons for treatment stop, there was toxicity (12.8%), patient's/parents will (23.0%), compliance issue (12.8%), pregnancy/sperm cryopreservation (28.7%), alternative SCD treatment (11.1%), other reason (16.9%). Among other reasons frequently reported by investigators, reasons were related to pregnancy, chemotherapy, surgery, inefficacy, infections (viral or respiratory), thrombopenia and anaemia, ulcer, other disease (complication or treatment of other disease), compliance issue.

Table 19. Barriers of compliance

		Safety population (N=2046)
At least one temporary stop for more than 2 weeks all along the study	N (missing values)	2036 (10)
	Yes	296 (14.5%)
Number of stops	N	296
	1	222 (75.0%)
	2	60 (20.3%)
	More than 2	14 (4.7%)
Mean daily dose per kilogram at stop (mg/kg/day)	N (missing values)	257 (39)
	Mean ± SD	17.65 ± 6.66
	Median	17.00
	Q1 ; Q3	13.00 ; 22.50
	Min ; Max	2.0 ; 35.0
Mean duration of stop (day)	N (missing values)	233 (63)

		Safety population (N=2046)
	Mean ± SD	162.83 ± 206.56
	Median	94.00
	Q1 ; Q3	27.00 ; 233.00
	Min ; Max	0.3 ; 1 731.0
At least one vaso-occlusive episode during treatment stop period	N (missing values)	138 (158)
	Yes	117 (84.8%)
At least one stop because of toxicity due to hydroxycarbamide	N (missing values)	296 (0)
	Yes	38 (12.8%)
At least one stop because of Patient's/parents will	N (missing values)	296 (0)
	Yes	68 (23.0%)
At least one stop because of Compliance issue	N (missing values)	296 (0)
	Yes	38 (12.8%)
At least one stop because of Pregnancy / sperm cryopreservation	N (missing values)	296 (0)
	Yes	85 (28.7%)
At least one stop because of Alternative SCD treatment	N (missing values)	296 (0)
	Yes	33 (11.1%)
At least one stop because of other	N (missing values)	296 (0)
	Yes	50 (16.9%)

Source: ESCORT-HU2_ IA4_TFLs_V1.0_03SEP2024– Table 3.1.3

- Switch or combination with new SCD treatment

Switch or combination with new SCD treatments are presented in Table 20 and detailed in Table 3.1.4 in Appendix 4. Tables, Figures and Listings:

- Twenty (20) patients had a new SCD treatment before Siklos® discontinuation (i.e. new treatment in combination with Siklos®), of whom 13 had a new treatment all along the study[1]:
  - At least one anti-adhesion therapy (n=3),
  - At least one blood transfusion program / regular blood transfusion (n=10),
  - At least one other hydroxycarbamide treatment (n=1),
  - At least one treatment received for insufficient effectiveness of hydroxycarbamide (n=7),
  - At least one treatment received for appearance of complications (n=4)
  - At least one treatment received for another reason (n=13).
- A hundred and thirteen (113) patients had a new SCD treatment after Siklos® discontinuation, of whom 83 had a new treatment all along the study:
  - At least one anti-adhesion therapy (n=5),
  - At least one anti-inflammatory (n=1),
  - At least one anti-sickling agent (n=6),
  - At least one anticoagulant (n=7),
  - At least one blood transfusion program / regular blood transfusion (n=27),

[1] defined as a treatment with a date of initiation posterior or equal to the date of enrolment or date of last visit in ESCORT-HU for participants previously enrolled in ESCORT-HU

  - At least one L-glutamine (n=8),
  - At least one other hydroxycarbamide treatment (n=6),
  - At least one treatment received for insufficient effectiveness of hydroxycarbamide (n=23),
  - At least one treatment received for appearance of complications (n=14),
  - At least one treatment received for another reason (n=58).
- Ten (10) patients had a new SCD treatment concomitant to Siklos® discontinuation, of whom 10 had a new treatment all along the study:
  - At least one other hydroxycarbamide treatment (n=10),
  - At least one treatment received for appearance of complications (n=2),
  - At least one treatment received for another reason (n=10).

Table 20. Switch or combination with new SCD treatment

		Safety population (N=2046)
Patient with other new SCD treatments **before Siklos® discontinuation** [a]	N	2046
	Yes	20 (1.0%)
At least one other treatment received for sickle cell disease all along the study	N	20
	N (%)	13 (65.0%)
Patients with new SCD treatment **after Siklos® discontinuation** [b]	N	2046
	Yes	113 (5.5%)
At least one other treatment received for sickle cell disease all along the study	N	113
	N (%)	83 (73.5%)
Patients with new SCD treatment **concomitant Siklos® discontinuation** [c]	N	2046
	Yes	10 (0.5%)
At least one other treatment received for sickle cell disease all along the study	N	10
	N (%)	10 (100.0%)

[a] Before Siklos discontinuation defined as: date of initiation of other new SDC treatments < date of Siklos discontinuation.
[b] After Siklos discontinuation defined as: date of initiation of other new SDC treatments > date of Siklos discontinuation.
[c] Concomitant to Siklos discontinuation defined as: date of initiation of other new SDC treatments = date of Siklos discontinuation.
Source: ESCORT-HU2_ IA4_TFLs_V1.0_03SEP2024– Table 3.1.4

- Complication linked to SCD

Complication linked to SCD by age at complication and by duration of the exposure to HU are detailed in Tables 3.1.5 and 3.1.6 and Figures 3.1.1 and 3.1.2 in Appendix 4. Tables, Figures and Listings. Among the 2046 patients in the Safety population, complications linked to SCD were reported for 1551 (87.7%) patients (values were missing for 277 patients) who presented a VOC (77.4%) or at least one organ complication during follow-up: lung (30.6%), bloodstream (28.6%), eye (27.7%), bones/joints

(18.4%), liver (17.0%), male genitals (16.9%), kidney (16.7%), spleen (15.7%), heart (13.7%), gallbladder (11.7%), brain (7.4%), ear (3.4%), female genitals (2.9%), bladder (1.9%).

- Laboratory results

Correlation of laboratory results (Hemoglobin and foetal Hemoglobin level (Hb/HbF), mean corpuscular volume (MCV) and polynuclear neutrophils (PNN)) and patients' daily dose of Siklos® is presented in Figures 3.2.1 – 3.2.4, in Appendix 4. Tables, Figures and Listings.

10.5. ADVERSE EVENTS/ADVERSE REACTIONS

This study focused on specific safety concerns whose occurrence and incidence was analysed as primary endpoint (namely leg ulcers, malignancies, male fertility impairments and SUSARs). Other events were not actively solicited as these events are not likely to contribute to the further understanding of the safety profile of the product. Any other adverse reactions are reported by healthcare professionals and consumers to the MAH (Theravia) or to the concerned Competent Authority via the national spontaneous reporting system.

11. DISCUSSION

Study population and follow-up

ESCORT-HU Extension study was set up to collect information on the long-term safety of Siklos® when used in current practice for the prevention or treatment of symptomatic complications in patients with SCD and especially to deepen the current knowledge on low incidence and indeterminate risks including secondary cancers, leg ulcers, serious unexpected adverse events related to Siklos®, fertility and pregnancy issues. This study thus focussed on inclusion of patients at-risk for developing these events of interest, i.e. patients with a long history of HU exposure, prepubescent patients expected to turn pubescent within the study follow-up period, patients with a history of leg ulcers, pregnant female patients exposed to HU at the time of conception and/or during pregnancy and male patients who did not interrupt HU before conception and whose partner is pregnant. Patients eligible to participate in the study were thus either patients already enrolled in ESCORT-HU study or new patients fulfilling one or several of these characteristics.

A total of 2093 patients from 4 countries (France, Germany, Greece and Italy) were enrolled in the study at the time of this fourth interim analysis.

Among the populations of interest for ESCORT-HU Extension study, 874 patients were previously enrolled in ESCORT-HU and 1219 were new participants (as described in section 10.1.1). Yet, pregnant women who did not stop Siklos® at least 3 months before the beginning of the pregnancy and Males treated with Siklos® whose partner was pregnant and who did not discontinue Siklos® 3 months before the beginning of the partner's pregnancy were poorly represented (18 and 3 enrolled patients, respectively).

Among enrolled patients, 2046 patients were included in the Safety Population. Male and female patients' representation was balanced (44.2% males and 55.8% females) and most patients had an SS genotype (86.8%).

Median study duration at the time of this interim analysis was 1.96 years and median exposure duration to HU was approximately 9 years and the vast majority of the patients (75%) were exposed to HU for 6 years or more (up to 38 years). Compared to the ESCORT-HU study where the median follow-up duration was 45 months (i.e. less than 4 years), one of the objectives to increase the duration of exposure to HU is reached. Exposure duration was increased as compared to duration reported at the time of the third interim analysis for ESCORT-HU Extension study (median duration was 8.5 years), and the number of patients of the Safety population increased for this fourth interim analysis (+12% patients and +250% since the first interim analysis). This interim analysis thus provides a reliable insight on safety issues that could be related to long-term exposure to HU in SCD patients.

Malignancies

Twenty-two patients (1.1%) of the Safety population had experienced malignancies before enrolment in ESCORT-HU Extension study. During study follow-up, at the time of the fourth interim analysis, 21 patients (1.0% of the Safety population) were diagnosed with malignancies: 8 patients had hematological malignancies and 13 had solid tumours. Among them, relationship with Siklos® was reported unknown by investigators in 8 cases. In the third interim analysis, malignancies were reported for 15 patients (0.8%) of the Safety population, among which relationship with Siklos® could not be excluded in 6 cases. The present results thus consolidate previous findings that there is no increased occurrence of malignancies upon HU treatment.

Based on other surveys conducted on SCD patients treated with HU, no increased risk for developing malignancies upon HU treatment has been evidenced so far. Schultz *et al.* attempted to assess baseline

cancer incidence in SCD patients [10]. Fifty-two (52) cases of malignancy were reported among 49 patients in a cohort of 16,613 patients with SCD. This survey did not permit an accurate calculation of the malignancy incidence in SCD patients treated with HU. No evidence for increased risk for Acute Myeloid Leukemia in SCD patients treated with HU was identified in the survey conducted by Castro *et al*. in 2014 and in the Multicenter Study of Hydroxyurea conducted by Steinberg *et al*. in 2003, no adults developed an HU-induced leukemia after up to 9 years of drug exposure [11, 12]. The exact incidence of malignancy has not been accurately determined due to a lack of long-term follow-up. The first description of SCD coexisting with acute leukemia has been reported by Goldin et al. in 1953 in a 38-year-old black man with SCD and acute myeloid leukemia (AML) [13]. Since then, the occurrence of acute leukemia has been reported in several cases of patients with SCD.

In a study summarising HU-associated data from both preclinical and clinical studies, no mutagenic activity of HU could be identified. This was consistent with the observed lack of any compelling carcinogenic potential [14].

Several recent reports describing the widespread usage of HU have brought to light adverse effects linked to tissues characterized by high cellular turnover, such as the skin [15]. The pathogenesis behind the augmented risk of skin malignancies is associated with UV exposure[16].

However, despite its generally favourable tolerability, as HU is unequivocally genotoxic in a wide range of test systems, this risk should be followed. Case-report studies describing malignancies or myelodysplasia in patients with SCD on hydroxyurea therapy are still emerging and the debate on an increased risk of malignancy in SCD patients treated with HU is ongoing [17-19]. ESCORT-HU Extension study is expected to last 1 additional year and recruitment has ended. Increased number of patients and additional follow-up duration will strengthen the knowledge about a potential contribution of HU in the emergence of neoplasia in SCD patients.

Leg ulcers

Causal implication of HU in the onset of leg ulcers in SCD is controversial. Overall, 10.2% patients of the Safety population had developed leg ulcers before the start of ESCORT-HU Extension study, of whom 8.1% were related to Siklos®. A small increase in the occurrence of leg ulcers was observed during the study follow-up: 4.5% patients of the Safety Population experienced leg ulcers (125 events in 92 patients), compared to the time of the third interim analysis (3.8%; 88 events in 71 patients). Of note, patients who experienced leg ulcers during ESCORT-HU Extension study follow-up period were mainly patients with a history of leg ulcers (81.8%). Results are quite similar to those of the third interim analysis.

Median time between first administration of HU and first incidence of a leg ulcer in ESCORT-HU Extension study was approximately 9 years. Among the 125 reported leg ulcers, only 5 were considered as related to HU and for 35 leg ulcers relationship to HU was unknown and can therefore not be excluded. However, due to the low prevalence of leg ulcers in the study, no conclusion could be drawn regarding relationship between HU treatment and incidence of leg ulcers.

In an observational study conducted on 131 SCD patients treated with HU and 199 patients not receiving this treatment with a median follow-up of 8 years, there was no difference in incidence of leg ulcers [20]. However, the total prevalence of leg ulcers in this study was low (3%). In a systematic literature review, HU treatment was not significantly associated with an increased risk of leg ulcers in SCD patients as well [21]. A study from the Consortium for the Advancement of Sickle Cell Research group conducted on 659 SCD patients from 3 countries (USA, Ghana, Italy) did not show any increased prevalence of leg ulcers history among patients treated with HU (overall prevalence of 10.3% vs 4.3% in patients treated with HU) [22]. In a recent study, Tolu *et al*. investigated the impact of HU therapy on leg ulcer prevalence in SCD patients. Leg ulcer incidence appeared to be independent of HU use [23].

Fertility

One of the main concerns about HU utilisation in SCD patients is whether HU treatment alters fertility potential in SCD patients when started before puberty. Mean age at puberty of the 511 Males who were pubescent at enrolment in ESCORT-HU Extension study and of the 96 Males turned pubescent during the follow-up period was approximately 15 years old. Only 16 patients performed semen analyses during this period. Semen abnormalities were detected in 14 patients during the study follow-up period. Among them, Siklos® was not stopped before performing the semen analysis for 6 patients: (1 patient had a qualitative abnormality and 5 patients had a quantitative abnormality: 1 unknown, 1 oligospermia and 3 azoospermia). Siklos® was stopped before performing the semen analysis for 8 patients (1 patient had a qualitative abnormality, 4 patients had a quantitative abnormality (3 oligospermia and 1 azoospermia) and 3 patient had both qualitative and quantitative abnormalities. Oligospermia was reported as quantitative abnormality). Due to the low number of semen analyses and incomplete information or inconsistencies at the time of this interim analysis regarding timing of Siklos® initiation in relation to onset of puberty, and treatment interruption before semen sampling, impact of HU on semen abnormalities cannot be discussed at this stage of the study.

Yet, a recent study addressed the specific effect of HU on the spermatogonial pool in boys with SCD [24]. The spermatogonial quantity in testicular tissue collected for fertility preservation in prepubertal SCD boys was compared between 17 HU-exposed and 13 non-exposed patients. Median HU-exposure duration in HU-exposed patients was 36 months (range 8-67 months) and a washout period with a median duration of 5 months was observed in 11 patients. Histologic analysis revealed that the spermatogonial pool was not statistically different between HU-exposed and non-exposed patients nor between HU-exposed patients with or without washout period. As sample size was very small, these results which suggest that depletion of the spermatogonial pool in prepubertal patients with severe SCD genotypes is related to the disease itself and not to HU toxicity still need to be confirmed. In another recent exploratory study, limited by its small sample size, results showed that an irreversible toxic effect on spermatogenesis may not be a major concern in boys with severe disease requiring HU treatment before puberty [25]; the drug's many beneficial effects largely outweigh its main negative effect in males [26]. The effect of HU exposure on infants is still a relevant open question, given that a “mini-puberty” characterized by the multiplication of spermatogonia cells occurs between the ages of 12 and 18 months. So, the true risk-benefit ratio of HU initiation during this period in asymptomatic infants is still in question. It also remains to be determined whether the recovery of spermatogenesis in boys treated with HU in childhood necessarily requires transfusion therapy [25].

Moreover, 14 pubescent Males underwent sperm cryopreservation up (≥4 sperm/patient) during the study follow-up. Age at sperm (22 and 31 years) cryopreservation could be calculated for 2 of the 14 patients only. Nine (9) patients had at least one cryopreservation with Siklos® interrupted beforehand. Three (3) cryopreservations were used for pregnancy purpose.

Finally, mean age at puberty of the 824 females, pubescent at enrolment in ESCORT-HU Extension study was 14 years old and mean age of first period of the 121 females who turned pubescent during the follow-up period was 13 years old.

Pregnancies

Despite the warning in Siklos® SmPC, pregnancies without interruption of HU treatment remain common among female patients treated with HU or partners of patients treated with HU: among the 93 pregnancies that occurred in female patients and the 27 pregnancies that were reported for female partners of male patients during ESCORT-HU study follow-up, Siklos® treatment had not been interrupted before conception in 21 and 14 cases, respectively. These results are in line with the high rate of females without contraception during treatment with Siklos® (67.9%) and suggest that most of

these pregnancies were unplanned; nevertheless, data on contraception uptake were missing for 387 out of the 945 female pubescent patients.

Among pregnancies reported in female patients without interruption of Siklos® before conception (excluding ongoing pregnancies at the time of the interim analysis), Siklos® was interrupted within the first trimester of pregnancy for 60.9% of them, of which treatment restarted during the first trimester for 7 of them, during the second trimester for 2 pregnancies and during the third trimester for 7 pregnancies. Siklos® was interrupted within the second trimester for 8.7% of pregnancies, of which treatment restarted during the second trimester for 1 pregnancy and during the third trimester for 1 pregnancy. No interruption of Siklos® was reported during the third trimester, meaning that for the other 21 pregnancies, Siklos® treatment was maintained for the entire duration of the pregnancy irrespectively of the outcome (with or without live birth). Of note, a high rate (31.2% of pregnancies with unfavourable outcome (mainly miscarriage, ectopic pregnancies, therapeutic or medical abortion) were reported among female patients who experienced pregnancies during ESCORT-HU Extension study. These results were similar to results obtained during the third interim analysis.

However, SCD is associated with an increased risk of miscarriage and complications during pregnancy. An American survey comparing perinatal outcomes among women of African ancestry with and without SCD reported adjusted odds of foetal death among deliveries to women with SCD 2.2 fold higher than those among women without SCD [27] and a recent review of articles published on maternal outcomes in sickle cell disease reported abortion/stillbirth rates ranging from 10 to 33% depending on the studies [28]. In a recent self-report survey including 1285 women from the Sickle Cell Disease Implementation Consortium, 737 (57.4%) reported 1788 pregnancies (1079 live births, 394 miscarriages, 40 stillbirths, 207 abortions, 48 current pregnancies, and 20 missing outcomes) of which 241 (15.9%) live births, miscarriages or stillbirths were conceived while on hydroxyurea [29]. Authors suggest that a history of prior poor foetal outcome and severe sickle cell disease are significant risk factors for miscarriage or stillbirth in women with sickle cell disease; and use of hydroxyurea up to the time of conception does not seem to have an adverse effect on the fetus and may be considered safe. Meanwhile, women that continue taking hydroxyurea during pregnancy may have an increased risk of low foetal viability or low infant birth weight. For live births, there does not appear to be an increase in birth defects when hydroxyurea is used during pregnancy.

Very recently, the Society for Maternal-Fetal Medicine has published management recommendations for SCD pregnant women based on observational data. It is recommended that discontinuation of HU in the event of unexpected pregnancy should be individualized, and risk stratified by trimester in collaboration with SCD experts along with principles of informed consent and shared decision making [30]. In agreement with previous reports [31, 32], a recently published systemic review showed that the prevalence of congenital abnormalities in babies born to women exposed to HU for the treatment of SCD during pregnancy is low [33].

So far, based on results of ESCORT-HU Extension study, there is no clear evidence of increased rate of complications during pregnancy in SCD women treated with HU.

Among pregnancies experienced by female partners of male patients during ESCORT-HU Extension study, most pregnancies were with a favourable outcome (22 pregnancies with a living child, 2 without living child and 2 ongoing). These results are comparable to those obtained during the third interim analysis. However, the number of pregnancies reported at this time is not sufficient to draw any sounded conclusion on the impact of male exposure to HU treatment at the time of conception on partner's pregnancy outcome.

Complications linked to SCD

At least one organ complication linked to SCD was reported in 87.7% of patients. These results were not surprising since it is well known that patients with SCD present progressive deterioration of organ function and end-organ damage is inevitable and appears to be irreversible [34, 35].

12. CONCLUSION

ESCORT-HU Extension study aims at collecting information on the long-term safety of Siklos® in patients with SCD. The present interim analysis was based on data collected on a total of 2046 patients included in the safety population with a median duration of exposure to HU of approximately 9 years. Overall, no major new safety signal has been detected so far. ESCORT-HU Extension study is expected to last 1 additional year and recruitment has ended. Yet, as the study focuses on rare events which may occur after long term exposure to HU, increased number of patients and additional follow-up duration is required and will strengthen the knowledge about safety of HU for the treatment of SCD patients.

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14. APPENDICES

APPENDIX 1. LIST OF STAND-ALONE DOCUMENTS

APPENDIX 2. LIST OF INVESTIGATORS

Site n°	Country	Site name	Investigators
1	France	Hôpital Saint-Antoine	Dr Latifatou Boukari
1	France	Hôpital Saint-Antoine	Dr Maria Chauchard
2	France	Paris HEGP	Pr Jean Benoît Arlet
3	France	Paris Necker	Dr Laure Joseph
4	France	Paris Necker	Dr Mariane de Montalembert
4	France	Paris Necker	Dr Melissa Taylor
4	France	Paris Necker	Dr Slimane Allali
5	France	Paris-Trousseau	Dr Marie Hélène Odièvre
5	France	Paris-Trousseau	Dr Jenny Youn
5	France	Paris-Trousseau	Dr Assa Niakate
5	France	Paris-Trousseau	Dr Anne-Sophie Romain
6	France	Robert Debré	Dr Malika Benkerrou
6	France	Robert Debré	Dr Laurent Holvoet
6	France	Robert Debré	Dr Florence Missud
6	France	Robert Debré	Dr Berengere Koehl
6	France	Robert Debré	Dr Ghislaine Ithier
6	France	Robert Debré	Dr Valentine Brousse
7	France	Ambroise Paré	Dr Valérie Soussan-Banini
9	France	Aulnay	Dr Marie Belloy
9	France	Aulnay	Donald Celicourt
10	France	Bobigny Avicenne	Dr Sylvain Le Jeune
10	France	Bobigny Avicenne	Dr Marilucy Lopez-Sublet
10	France	Bobigny Avicenne	Dr Antonio Morabito
12	France	Bondy	Dr Marine Houlier
12	France	Bondy	Dr Lahoueri Amor Chelihi
13	France	Corbeil Essonnes	Dr Célia Salanoubat
13	France	Corbeil Essonnes	Dr Elsa Ngwem
16	France	Colombes	Dr Louis Affo
17	France	Colombes	Dr Nevena Danekova
17	France	Colombes	Dr Hélène SEE
18	France	Créteil CHIC	Dr Isabelle Genty
18	France	Créteil CHIC	Dr Annie Kamdem
18	France	Créteil CHIC	Dr Cécile Arnaud
19	France	Créteil Henri Mondor	Pr Frédéric Galactéros
19	France	Créteil Henri Mondor	Dr Gonzalo De Luna Cardenal
19	France	Créteil Henri Mondor	Dr Anoosha Habibi
19	France	Créteil Henri Mondor	Pr Pablo Bartolucci

Site n°	Country	Site name	Investigators
19	France	Créteil Henri Mondor	Dr Suella Martino
20	France	Gonesse	Dr Michel Benemou
20	France	Gonesse	Dr Lazarevic
20	France	Gonesse	Dr Sarakbi
21	France	Jossigny	Dr Nathalie Garrec
21	France	Jossigny	Dr Kokou Agbo Kpati
22	France	Le Chesnay	Dr Maud Guillon
24	France	Saint Denis	Dr Stéphanie Ngo
25	France	Saint Denis	Dr Aurore Malric
25	France	Saint Denis	Dr Basil Coulon
25	France	Saint Denis	Dr Laure Nizery
27	France	Amiens	Pr Loïc Garcon
27	France	Amiens	Dr Lavinia Merlusca
28	France	Amiens	Dr Valérie Li Thiao Te
29	France	Angers	Dr Corentin Orvain
29	France	Angers	Dr Martine Gardembas
30	France	Bordeaux	Dr Arnaud Desclaux
30	France	Bordeaux	Dr Thierry Pistone
30	France	Bordeaux	Dr Alexandre Duvignaud
30	France	Bordeaux	Dr Denis Malvy
30	France	Bordeaux	Dr Duc Nguyen
31	France	Bordeaux	Dr Camille Runel-Belliard
31	France	Bordeaux	Dr Marie Lagarde
33	France	Grenoble	Dr Caroline Makowski
33	France	Grenoble	Dr Alexis Bocquet
33	France	Grenoble	Dr Laurence Bouillet
34	France	Grenoble	Dr Corinne Armari-Alla
35	France	Lille	Dr Anne Lambilliotte
35	France	Lille	Dr Melissa Barbati
36	France	Limoges	Dr Christophe Piguet
37	France	Lyon	Dr Giovanna Cannas
38	France	Lyon	Dr Nathalie Garnier
38	France	Lyon	Dr Cécile Renard
38	France	Lyon	Dr Carine Halfon Domenech
38	France	Lyon	Pr Yves Bertrand
38	France	Lyon	Dr Kamila Kebaili
39	France	Marseille	Dr Estelle Jean Mignard
39	France	Marseille	Dr Benoît Faucher
40	France	Marseille	Dr Isabelle Thuret
40	France	Marseille	Dr Sarah Szepetowski
41	France	Montpellier	Dr Ivan Bertchansky
42	France	Montpellier	Dr Perrine Mahe

Site n°	Country	Site name	Investigators
45	France	Nancy	Dr Aurelie Phulpin
46	France	Nantes	Dr Agathe Masseau
46	France	Nantes	Dr Julie Graveleau
46	France	Nantes	Dr Alexandra Espitia
46	France	Nantes	Dr Marine Eustache
47	France	Nantes	Dr Marie-Laure Couec
47	France	Nantes	Dr Caroline Thomas
47	France	Nantes	Dr Alice Huault
48	France	Nice	Pr Pierre Simon Rohrlich
48	France	Nice	Dr Lionel Mannone
48	France	Nice	Dr Joy Benadiba
49	France	Orleans	Dr Hélène Bens
51	France	Poitiers	Dr Gellen Dautremer
51	France	Poitiers	Dr Alain Ramassamy
52	France	Reims	Dr Violaine Laurant-Noel
52	France	Reims	Dr Ailsa Robbins
53	France	Reims	Dr Claire Pluchart
54	France	Rennes	Dr Stanislas Nimubona
55	France	Rouen	Dr Maximilien Grall
55	France	Rouen	Dr Gaetan Sauvetre
56	France	Rouen	Dr Bruno Filhon
56	France	Rouen	Dr Nimrod Buchbinder
59	France	Strasbourg	Dr Catherine Paillard
60	France	Toulouse	Dr Marie-Pierre Castex
60	France	Toulouse	Dr Marie Lamant
61	France	Toulouse	Dr Pierre Cougoul
62	France	Tours	Dr Jean-Baptiste Valentin
62	France	Tours	Dr Laurent Ardillon
63	France	Tours	Dr Jill Serre
64	France	Meaux	Dr Caroline Masserot-Lureau
64	France	Meaux	Dr François Gouraud
64	France	Meaux	Marie Monfort-Gouraud
65	France	Angers	Dr Jean-François Brasme
65	France	Angers	Dr Isabelle Pellier
66	France	Saint-Brieuc	Dr Amélie Vareliette
67	France	Meaux	Dr Jamile Frayfer
68	France	Dreux	Dr Désiré Ndayarinze
69	France	Kremlin Bicêtre	Dr Corinne Guitton
69	France	Kremlin Bicêtre	Dr Ferrielle Ferielle Zenkhri
71	France	Le Mans	Dr Vannina Giacobbi-Milet
72	France	Lille	Dr Benjamin Carpentier
74	France	Corbeil Essonnes	Dr Cécile Guillaumat

Site n°	Country	Site name	Investigators
74	France	Corbeil Essonnes	Dr Bérénice Chailloux Mirat
74	France	Corbeil Essonnes	Dr Guillaume Costa
74	France	Corbeil Essonnes	Dr Melek Djaziri
100	France	Guadeloupe - Pointe à pitre	Dr Maryse Etienne-Julan
100	France	Guadeloupe - Pointe à pitre	Dr Emmanuelle Bernit
100	France	Guadeloupe - Pointe à pitre	Dr Scylia Alexis-Fardini
101	France	Guadeloupe - Pointe à pitre	Dr Lydia Divialle-Doumdo
101	France	Guadeloupe - Pointe à pitre	Dr Marie Petras
103	France	Martinique - Le Lamentin	Dr Gylna Loko
103	France	Martinique - Le Lamentin	Dr Romana Ifrim
103	France	Martinique - Le Lamentin	Dr Anaïs Bourguignon d'Herbigny
104	France	Martinique - Fort de France	Dr Claire Dichamp
104	France	Martinique - Fort de France	Dr Hanine Mansour
104	France	Martinique - Fort de France	Dr Anne-Caroline Isidore
105	France	Guyane - Cayenne	Dr Narcisse Elenga
105	France	Guyane - Cayenne	Dr Joddy Mafema Missindu
105	France	Guyane - Cayenne	Dr Noelis Boizan-Perez
108	France	Guyane - St Laurent du Maroni	Pr Narcisse Elenga
109	France	Réunion	Dr Cécile Stoven
109	France	Réunion	Dr Quentin Cabrera
109	France	Réunion	Dr Catherine Mohr
109	France	Réunion	Dr Patricia Zunic
109	France	Réunion	Dr Hugo Legendre
200	Greece	Athens	Dr Maria Dimopoulou
200	Greece	Athens	Dr Pagona Flevari
200	Greece	Athens	Dr Sevastianos Chatzidavid
300	Germany	Berlin	Dr Lena Oevermann
300	Germany	Berlin	Dr Dani Hakimeh
301	Germany	Koblenz	Dr Stephan Lobitz
302	Germany	Dusseldorf	Dr Sonya El Amouri
303	Germany	Freiburg	Dr Sarah Salou
303	Germany	Freiburg	Dr Alexander Puzik
303	Germany	Freiburg	Dr Charlotte Niemeyer
303	Germany	Freiburg	Dr Eva Maria Kornemann
304	Germany	Hamburg	Dr Uwe Kordes
304	Germany	Hamburg	Dr Matthias Bleeke
400	Italy	Modena	Dr Giovanni Palazzi
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401	Italy	Napoli	Dr Silverio Perrotta
401	Italy	Napoli	Dr Maddalena Casale
401	Italy	Napoli	Dr Maraia Paola Boaro
402	Italy	Padova	Dr Raffaella Colombatti

Site n°	Country	Site name	Investigators
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402	Italy	Padova	Dr Maria Paola Boaro
403	Italy	Verona	Dr Simone Cesaro
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APPENDIX 3. ROLES AND RESPONSIBILITIES OF THE MARKETING AUTHORISATION HOLDER AND OTHER RESPONSIBLE PARTIES

Table 21: Roles and responsibilities of the MAH and other responsible parties

Responsible parties	Composition and/or Contact information	Roles and responsibilities
Market Authorization Holder	**MAH contact person:** **Laura Fabre Thomas-Bourgneuf**, PharmD - Clinical Operation Manager Theravia 16 rue Montrosier, 92200 Neuilly-sur-Seine, France Phone : +33 (0)6 50 63 93 63 E-mail: laura.thomas-bourgneuf@theravia.com	• Supervision of the study and all CROs implicated. • Sites follow-up • Organisation of investigators meetings • Newsletters • Pharmacovigilance • Management of the Steering Committees • Publications
Steering committee	**Members of the 4th steering committee (September 23rd, 2024):** • **Pr Frédéric GALACTÉROS** Genetics and internal medicine Hôpital Henri Mondor, Créteil, France +33 149812440 frederic.galacteros@aphp.fr • **Pr Mariane DE MONTALEMBERT** Pediatrician Hôpital Necker Enfants Malades, Paris, France +33 144494896 mariane.demontal@nck.aphp.fr • **Dr Giovanna CANNAS** Hematologist Hôpital Edouard Herriot, Lyon, France +30 2132060940 giovanna.cannas@chu-lyon.fr • **Dr Malika BENKERROU** Pediatrician	The main mission of the Steering committee is to oversee the conduct and reporting of the study, to ensure expert clinical guidance and a high standard of scientific quality. The Committee is not directly responsible for executing project activities, but it provides vision, support and guidance for those who do. The primary responsibilities of the Steering Committee are to: • Review, endorse and implement the charter, • Take steps to reduce deviations from the protocol to a minimum • Periodic review of the progress of the study • Review safety data • Make recommendations to the Sponsor concerning the study.

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Contract Research Organisations	**International Clinical Trial Association & Project Management (ICTA PM)** 11, rue du Bocage 21121 Fontaine les Dijon. Phone: 03.80.53.40.00	ICTA PM has been mandated by Theravia to conduct the following activities: • Project management • Study set-up and preparation • Regulatory submissions

		• Sites follow-up (data collection visits, quality control visits, remote monitoring and close-out) • Pharmacovigilance: Reconciliation of pharmacovigilance data • Data management and Statistical analysis • Study Report writing • Archiving
	Innopharma c/o Polo Tecnologico Brianza Via Lavoratori Autobianchi, 1 Edificio 9 - Ufficio 14 - Piano Terra 20832 Desio (MB) – Italy **Monipol** Heilsbachstr.22-24, 53123 Bonn - Germany **Qualitis Ltd** 13 Aetideon & Voutsina str Holargos 155 61 Attiki, Greece	ICTA PM has mandated the following companies for: • Regulatory submissions • Sites management in countries other than France.
Other service providers	Philippe Ansar (former Arone SAS employee) ph_ansar@hotmail.com David Pihan david.pihan@gmail.com	Philippe Ansar (former Arone SAS employee) and David Pihan (supporting external consultant) have been mandated by Theravia to conduct the following activities: • eCRF installation, updating and configuration • Main support for eCRF problems • eCRF design and development • Management of the Data management module • Hosting monitoring (checks on system updates, creation of tickets, technical interface between THERAVIA and Hosteur) • Addition of documents to be uploaded to the eCRF

APPENDIX 4. TABLES, FIGURES AND LISTINGS



ESCORT-HU2_Interim
_Analysis_Main_TFLs_V

APPENDIX 5. APPROVAL

Title	**ESCORT-HU Extension: European Sickle Cell Disease Cohort – Hydroxyurea – Extension study**
Report version and date	Version 1.0 – 05-Sep-2024
Study number (Internal reference)	SIK-EU-20-1
Study type / Study phase	PASS
Medicinal product	L01XX05, hydroxycarbamide/hydroxyurea (HU)
Sponsor	Theravia S.A.S

The undersigned confirms that she/he has read this report and confirms that to the best of her/his knowledge it accurately describes the conduct and results of the study.

For the Sponsor

Clinical Operation Manager: [THOMAS-BOURGNEUF Laura] Signature: Signé par : Laura Thomas-Bourgneuf Nom du signataire : Laura Thomas-Bourgneuf Motif de la signature : J'approuve ce document Heure de signature : 06/09/2024 | 11:09:29 AM CEST C8E641ACD4E64000B390FCF3F7C96E14 Date: 06/09/2024

EU QPPV: [DUGUET Corinne] Signature: Signé par : Corinne Duguet Nom du signataire : Corinne Duguet Motif de la signature : J'approuve ce document Heure de signature : 10/09/2024 | 5:01:51 PM CEST 43C966C2FE64479F8D7B86CC67D2F8CA Date: 10/09/2024

For the CRO

Scientific advisor / Medical writer: [GACKIERE KATSOGIANNOU Maria] Signature: Signed by: Maria GACKIERE Signer Name: Maria GACKIERE Signing Reason: I approve this document Signing Time: 9/6/2024 | 11:12:08 AM CEST BAF014D1336A486B830ECABDF5BD90BC Date: 9/6/2024

Project Manager: [DAVENNE Sonia] Signature: Signé par : Sonia DAVENNE Nom du signataire : Sonia DAVENNE Motif de la signature : J'ai examiné ce document Heure de signature : 06/09/2024 | 11:45:16 AM CEST 1FFDB9F82F9A44B8ADD70A4C081C25DF Date: 06/09/2024