



# Sevuparin for the treatment of acute pain crisis in patients with sickle cell disease: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial

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## Summary

**Background** There are no approved treatments for vaso-occlusive crises in sickle cell disease. Sevuparin is a novel non-anticoagulant low molecular weight heparinoid, with anti-adhesive properties. In this study, we tested whether sevuparin could shorten vaso-occlusive crisis duration in hospitalised patients with sickle cell disease.

**Methods** We did a multicentre, double-blinded, placebo-controlled, phase 2 study in 16 public access clinical hospitals in the Netherlands, Lebanon, Turkey, Bahrain, Oman, Saudi Arabia, and Jamaica. Patients aged 12–50 years with a diagnosis of sickle cell disease (types HbSS, HbSC, HbSβ<sup>0</sup>-thalassaemia, or HbSβ<sup>+</sup>-thalassaemia) on a stable dose of hydroxyurea, hospitalised with vaso-occlusive crisis for parenteral opioid analgesia with a projected stay of more than 48 h were included in the study. Patients were randomly assigned (1:1) using a computer-generated randomisation scheme to receive sevuparin (18 mg/kg per day) or placebo (NaCl, 0.9% solution) intravenously for 2–7 days until vaso-occlusive crisis resolution. All individuals involved in the trial were masked to treatment allocation. The analysis was done in the intention-to-treat population. The primary endpoint was time to vaso-occlusive crisis resolution defined as freedom from parenteral opioid use (in preceding 6–10 h); and readiness for discharge as judged by the patient or physician. The trial is registered with ClinicalTrials.gov, NCT02515838.

**Findings** Between Oct 7, 2015, and Feb 10, 2019, 144 patients were randomly assigned and administered sevuparin (n=69) or placebo (n=75). The median age was 22.2 years (range 12.2–33.6), 104 (72%) 144 were adults (18 years or older), and 90 (63%) were male and 54 (37%) were female. The intention-to-treat analysis for the primary endpoint showed no significant difference in median time to vaso-occlusive crisis resolution between the sevuparin and placebo groups (100.4 h [95% CI 85.5–116.8]) vs 86.4 h [70.6–95.1]; hazard ratio 0.89 [0.6–1.3]; p=0.55). Serious adverse events occurred in 16 (22%) of 68 patients in the sevuparin group and in 21 (22%) of patients in the placebo group. The most frequent treatment-emergent adverse events were pyrexia (17 [25%] in the sevuparin group vs 17 [22%] in the placebo group), constipation (12 [18%] vs 17 [22%]), and decreased haemoglobin (18 [26%] vs 9 [12%]). There were no deaths in the sevuparin group and there was one (1%) death in the placebo group after a hyper-haemolytic episode due to alloimmunisation.

**Interpretation** This result, as well as the results seen in other clinical studies of inhibitors of adhesion in sickle cell disease, suggest that selectin-mediated adhesion might be important in the initiation, but not maintenance of vaso-occlusion, indicating that strategies to treat vaso-occlusive crises differ from strategies to prevent this complication.

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## Introduction

Sickle cell disease is characterised by chronic haemolytic anaemia, painful vaso-occlusive episodes, progressive organ damage, and early mortality.<sup>1–4</sup> Vaso-occlusive crises are the main reason for the hospitalisation of these patients. On average, patients have 2.5 vaso-occlusive crises per year, of which 1.5 led to hospitalisation.<sup>5</sup> In particular, young adults aged 18–30 years appear to have the highest health-care utilisation due to vaso-occlusive crises and are at risk for complications like acute chest syndrome, one of the most important causes of early mortality in adult

patients with sickle cell disease.<sup>6</sup> There is currently no approved therapy for acute vaso-occlusive crisis besides pain management. Pain treatment is often already initiated at home by use of pain relief (eg, non-steroidal anti-inflammatory drugs and oral opioids), rest, and rehydration; however, hospitalisation for intravenous opioids, intravenous fluids, oxygen, and sometimes antibiotics and transfusion, are frequently needed.<sup>7</sup> The scarcity of specific treatments for sickle cell disease,<sup>8–10</sup> frequent pain, risk of opioid dependency, disease confinement to socioeconomically challenged groups, and the difficulty to maintain school and work activities

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## Research in context

### Evidence before this study

We searched for clinical trials published from inception to Dec 1, 2020, using the search terms “sickle cell disease” combined with either “adhesion” or “heparin”. We identified 46 clinical trials. Abstracts were reviewed to identify all phase 2 and 3 trials studying anti-adhesive therapy directed at selectins or using heparins in sickle cell disease. This review resulted in two relevant clinical studies before the initiation of our trial and one relevant clinical study after initiation of our trial. In one randomised controlled clinical trial done in 2007, treatment with low molecular weight heparin (tinzaparin) significantly reduced the duration of vaso-occlusive crisis in comparison with placebo in patients with sickle cell disease admitted to hospital with vaso-occlusive crisis. In 2015, a small randomised phase 2 study showed that the pan-selectin inhibitor (GMI-1070 [rivipansel]) had no effect on the duration of a vaso-occlusive crisis, but significantly reduced opioid use in patients with sickle cell disease admitted with a vaso-occlusive crisis as compared with patients in the placebo group. In another randomised clinical study done in 2017, preventive P-selectin inhibition with a neutralising monoclonal antibody reduced the incidence of vaso-occlusive crisis by 45% but has not been

studied in patients with sickle cell disease admitted with a vaso-occlusive crisis.

### Added value of this study

This study showed that anti-adhesive therapy aimed primarily at P-selectin is not able to change the duration or the severity of pain in patients with sickle cell disease. This finding suggests that different from preventive strategies, P-selectin inhibition is not able to change the clinical course of patients with sickle cell disease and with an already manifest vaso-occlusive crisis.

### Implications of all the available evidence

An implication of this important finding is that the role of anti-adhesive strategies as a single drug in general might need to be limited to the prevention and not to the treatment of vaso-occlusive crises. The findings also show that a non-anticoagulant heparinoid might not be beneficial in the vaso-occlusive crisis setting. Whether anti-adhesive strategies could be useful in addition to other drugs that modify other aspects of sickle cell pathophysiology, should be subject of future studies. Another goal in future studies could be to administer anti-selectins or non-anticoagulant heparinoid drugs very early in the course of a vaso-occlusive crisis to prevent the crisis developing further.

due to sickle cell disease all contribute to the overall disease burden and reduced quality of life for patients.<sup>11–13</sup>

Vaso-occlusion in sickle cell disease is a complex phenomenon, involving increased adhesion of erythrocytes and leukocytes to activated endothelial cells, which results in vascular obstruction.<sup>14,15</sup> Anti-adhesive P-selectin or pan-selectin inhibition has prevented vaso-occlusion in several animal models<sup>16–19</sup> and published data from a randomised controlled clinical trial<sup>8</sup> showed that P-selectin inhibition is effective in preventing vaso-occlusive crisis. In a clinical trial published in 2007,<sup>20</sup> full anticoagulant therapy using tinzaparin (175 IU/kg) reduced vaso-occlusive crisis duration and time of severe pain in patients with sickle cell disease hospitalised for vaso-occlusive crisis.<sup>20</sup> In addition to their anticoagulant properties, heparinoids are also potent multimodal effectors of blood rheology with activities on adhesive, inflammatory, and immune-related components.<sup>16,21</sup> The anticoagulant properties, however, limit the therapeutic use of heparinoids due to the risk of bleeding. We hypothesised that a heparinoid with markedly attenuated anticoagulant activity, but retaining its broad anti-adhesive properties, could be effective in treating vaso-occlusive crises in patients with sickle cell disease who are hospitalised. Sevuparin is a novel short heparinoid modified to be non-anticoagulant by the elimination of the anti-thrombin binding domain resulting in abrogated anti-factor II and anti-factor X activities.<sup>22</sup> Sevuparin retains some degree of isolated residual activated partial thromboplastin time (aPTT) activity, which is likely

attributable to heparin cofactor II activity.<sup>22</sup> Although its anticoagulant properties are markedly attenuated, sevuparin maintains the anti-adhesive, anti-aggregate, and anti-inflammatory properties of heparin as shown by its activities on P-selectin, L-selectin, thrombospondin, von Willebrand factor, and fibronectin.<sup>23,24</sup>

The primary objective of the present study was to assess whether sevuparin could shorten the time to vaso-occlusive crisis resolution in patients with sickle cell disease who were hospitalised compared with placebo, and to evaluate the effect of sevuparin on the mean change in pain intensity, duration of severest pain, and cumulative dose of parental opioids. This study investigated the assumed benefit of non-adhesive treatment to shorten the duration of vaso-occlusive crisis strategies in general and specifically if a non-anticoagulant heparinoid would be beneficial in that setting.

## Methods

### Study design and participants

We did a multicentre, randomised, double-blind, placebo-controlled, phase 2 study in 16 public access clinical hospitals in the Netherlands, Lebanon, Turkey, Bahrain, Oman, Saudi Arabia, and Jamaica (appendix p 2). Patients aged 12–50 years with sickle cell disease (types HbSS, HbSC, HbS $\beta^0$ -thalassaemia, or HbS $\beta^+$ -thalassaemia), on a stable dose of hydroxyurea, and hospitalised with vaso-occlusive crisis for parenteral opioid analgesia with a projected stay of more than 48 h were included. Patients with more than five hospitalisations for vaso-occlusive

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crisis during the last 6 months (to exclude patients with exacerbations of chronic pain rather than true vaso-occlusion); a history of clinically significant bleeding in vital organs or current clinically significant bleeding; currently being treated with aspirin, anti-platelet therapy, anticoagulation therapy, or prophylactic or therapeutic low molecular weight heparin or unfractionated heparin; aPTT above normal range and international normalised ratio (INR) greater than 1.4; platelet count less than 75 000 cells per  $\mu\text{L}$ ; evidence of acute sickle cell disease complications other than vaso-occlusive crisis at screening; use of strong opioids for more than 3 consecutive days during the 15 days before presenting to hospital; and known infection with HIV and active infection with hepatitis B or C virus were excluded. Full details of selection criteria are in the appendix (pp 45–46).

All participants or their parents provided written informed consent. The clinical study protocol was approved by the appropriate Independent Ethics Committees, Institutional Review Boards, and regulatory agencies in all countries. The final clinical study protocol (version 4.2; appendix pp 21–75) had several approved amendments, which are detailed in the appendix (pp 76–88). Some of the amendments had the potential to affect study recruitment and conduct because of the modification of selection criteria, sample size, and number of sites.

#### Randomisation and masking

Patients were randomly assigned to sevuparin for up to 7 days or placebo (saline). Both groups received a standard of care therapy for vaso-occlusive crisis. Patients were randomly assigned by a computer-generated randomisation scheme by an independent statistician and programmer of PCG Clinical Services, Uppsala, Sweden, who had no further involvement in the trial, using unstratified block randomisation in fixed block sizes of six, to receive either sevuparin or placebo in a 1:1 ratio. Randomisation took place centrally by Interactive Web Response System. Vials for preparing intravenous infusions were identical with contents not differing in appearance or smell. Participants were enrolled and assigned to treatment by trained site staff who had continuous involvement in the trial while being masked. Throughout the study, participants, site staff, investigators, data managers and statisticians were masked to the treatment allocation until database lock was established. There was no formalised evaluation of masking.

#### Procedures

Sevuparin 150 mg/mL solution for intravenous infusion (formulated in a 0.015 M phosphate buffer at a pH of 7.0, manufactured by Opocrin SpA, Formigine, Italy) was administered as a 3 mg/kg loading dose followed by 18 mg/kg per day continuous infusion. Placebo (9% sodium chloride solution) was administered intravenously in parity to sevuparin.

Investigational medicinal product was administered as an intravenous continuous infusion for 2–7 days until resolution of vaso-occlusive crisis (for a minimum of 2 days). The following dosing modification was applied in response to effects on aPTT according to the protocol: investigational product infusion rate was reduced by 25% when aPTT was more than  $1.5 \times$  upper limit of normal (ULN) and stopped when aPTT was more than  $2.5 \times$  ULN until aPTT was less than  $1.5 \times$  ULN, at which point the infusion rate could be recommenced at 50% of the initial rate. No other reason for dosing modification or interruption was allowed. To secure blinding, the clinicians taking care of the patients were blinded to the aPTT results and an independent person in the laboratory instructed the clinicians whether the study drug administration had to be changed. Study medication was given as an add-on to the standard care according to local hospital. This study refrained from the use of a centralised standard of care given the known variations between countries and sites and the fact that randomisation within the study would correct for these differences.

A follow-up visit was done at days 4–11 and again at days 23–33 after the last dose of the study drug to record the reoccurrence of vaso-occlusive crisis and potential adverse events. Laboratory monitoring during the study was done at screening (baseline), day 3, day 5, and last day of study treatment; and days 4–11 and days 23–33 after the last dose of the study drug.

Reasons to withdraw an individual from treatment were medical necessity judged by an investigator or at the request of the patient, major protocol violation that would confound interpretation of the results, patient's condition no longer requiring treatment, need to administer non-permitted concomitant medication, or pregnancy. Patients who had a serious adverse event judged to be related to study medication discontinued treatment. After inclusion patient reported adverse events were recorded on all study days 1 up to 8 and then on all follow-up visits at 7 and 28 days, as well as 3 and 6 months after the last dose. Pain intensity assessment was done using the visual analogue scale (0–100 mm [0 mm meaning no pain and 100 mm the worst possible pain]). The pain was assessed 30 min before the start of infusion and then every 4 h (while awake) until vaso-occlusive crisis resolution.

Safety was assessed by recording the type, duration, seriousness, and intensity (mild, moderate, or severe) of adverse events along with relatedness to study drug and outcomes. Serious adverse events were defined as any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of hospitalisation, results in persistent or substantial disability or incapacity, or is a congenital anomaly or birth defect.

To record any adverse events, we monitored vital signs (done every 8 h during study); did electrocardiogram

(ECG) screening on day 2 and at the end of study drug administration; did laboratory examination, including liver and renal function examination (at screening, day 3 and 5, and last day of study treatment; and days 4–10 and 23–33 after the last dose of the study drug); and coagulation tests consisting of aPTT and INR (every 6 h during the first 24 h of study drug administration and then every 12 h until the end of study treatment). A decrease in haemoglobin and C-reactive proteins was not defined by a central laboratory but was judged by individual investigators taking into account local circumstances. Full safety assessments are summarised in the appendix (pp 59–62).

Pharmacokinetic assessments were done on day 3–8, depending on treatment length, in the first 25 patients recruited and in 20 adolescents at pre-dose, 1 h, 2 h, 24 h, and at end of infusion. In all remaining patients, pharmacokinetic data were measured on day 2.

### Outcomes

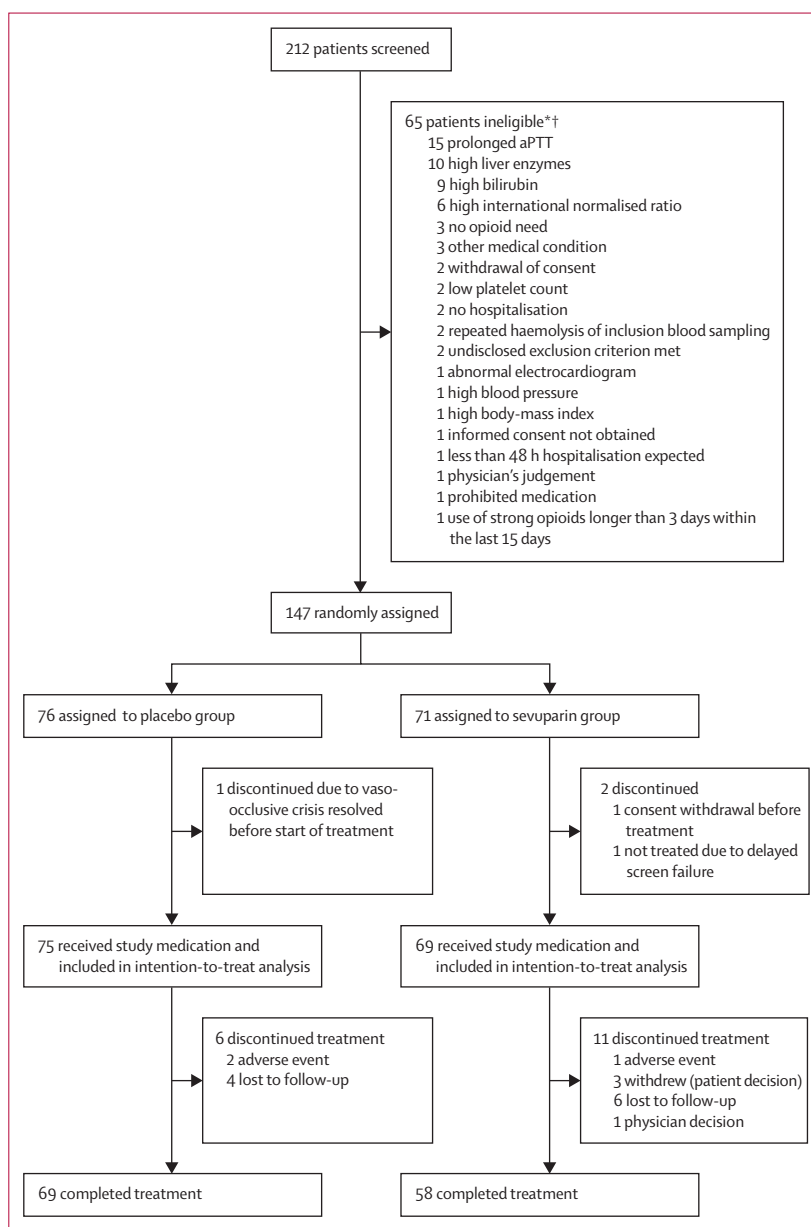
The primary outcome was time to vaso-occlusive crisis resolution defined as the time from the start of study treatment infusion until the fulfilment of the two following criteria: freedom from parenteral opioid use (in preceding 6–10 h) and readiness for discharge as judged by the patient or physician.

The protocol-specified key secondary outcomes were change in pain intensity from baseline recorded every 4 h during the awake time until vaso-occlusive resolution; duration of the most severe pain (defined as time to a 30% reduction in pain intensity compared with baseline and maintained during at least 8 h); and accumulated dose of parenteral opioids from baseline until vaso-occlusive crisis resolution or readiness for discharge. The other secondary outcomes were mean daily dose of parenteral opioids per day until vaso-occlusive crisis resolution or readiness for discharge; time to discontinuation of intravenous or parental opioids; clinician and patient global impression of change (measured on day 3 of treatment until the end of treatment on a 6 point scale ranging from 1 (very much improved) to 6 (very much worse)); time to discharge; time to readiness for discharge (as judged by the patient or investigator); proportion of patients with vaso-occlusive crisis resolution at 24, 48, 72, 96, and 120 h based on the Kaplan-Meier curve estimates; estimated 25th, 50th, and 75th percentiles for time to vaso-occlusive crisis resolution using the Kaplan-Meier curve; cumulative pain intensity difference (derived over time from the pain intensity differences as the area under the pain intensity difference vs time curve); proportion of patients with re-hospitalisation for vaso-occlusive crisis within 3 and 28 days after discharge; pharmacokinetic characteristics of sevuparin; and safety and tolerability of the treatments by recording vital signs, physical examination, ECG, laboratory safety analyses, and occurrence of adverse events. Exploratory endpoints were frequency of re-hospitalisation due to acute complications; proportion

of patients that received a blood transfusion during the study; proportion of patients who had acute chest syndrome; and biomarkers associated with the disease mechanism (the results of the biomarker exploratory endpoint are not reported in this Article as the data are still being analysed and may be reported separately).

### Statistical analysis

The full statistical analysis plan is shown in the appendix (pp 89–129). The study sample size was



**Figure 1: Trial profile**

\*Only one reason is counted here. An individual patient could have met several ineligibility criteria within the same screening occasion. †In this study, ineligibility reasons were not recorded in the clinical data base (Study Data Tabulation Model), these data are therefore extracted from the electronic data capture without formal data cleaning and verbatim reasons have been redacted to allow descriptive tabulation.

calculated on a target of 120 events of vaso-occlusive resolution (primary endpoint). Overall, approximately 133 patients were projected for enrolment assuming a non-event in 10%. The power calculation assumed that the hazard ratio (HR) between the treatment groups was 0.6, corresponding to a median time to crisis

resolution of 48 h in the seviparin group and 72 h in the placebo group. There was an aim to include at least 30% adolescents (aged 12–17 years). The intention-to-treat population constituted the primary analysis population for all efficacy and exploratory analyses in the study consisting of all randomly assigned patients. Further analysis populations are the per-protocol population defined as all randomly assigned patients without major protocol deviations deemed to have major impact on primary endpoint evaluation, the safety population defined as all subjects treated, grouped by treatment actually received, and the pharmacokinetic population defined as all patients treated with seviparin who had valid pharmacokinetics sampling data available (appendix p 66).

The intention-to-treat analysis of the primary endpoint used survival analysis by the Cox proportional hazards regression model with treatment as a fixed factor in testing the following statistical hypothesis: there is no difference between the treatment groups with regard to time to vaso-occlusive crisis resolution (null hypothesis; HR 1.0); and there is a difference between the treatment groups with regard to time to vaso-occlusive crisis resolution (alternative hypothesis; HR not equal to 1.0)

Censoring was at vaso-occlusive crisis resolution or 7 days in case of non-resolution or discontinuation, replacing missing data by means of placebo multiple imputation. HR comparisons were at 95% CI should this be: HR comparisons were accompanied by 95% CIs and all statistical tests were two-sided with a type I error of 0.05. The primary analysis was complemented by non-responder imputation and observed cases analyses (appendix p 106). Analysis was also done on predefined subgroups: age, gender, mutation type, vaso-occlusive crisis occurrence, number of vaso-occlusive crisis hospitalisations in past 12 months, use of hydroxy-carbamide, history of acute chronic syndrome, and concomitant oral opioid treatment (appendix pp 101–102). A full account of the analysis methods of the primary endpoint is in the appendix (p 109).

The key secondary endpoints (change in pain intensity, duration of most severe pain, and accumulated dose of parenteral opioids) were tested with serial multiplicity correction provided that the P-value for the null hypothesis was less than 0.05 for the primary efficacy objective (appendix p 108). The other secondary endpoints were not adjusted for multiplicity in any situation and their associated p-values were considered as descriptive (appendix pp 109–112).

All time-to-event secondary efficacy endpoints were analysed with the same survival analysis method as for the primary endpoint. Secondary efficacy endpoints were patient global impression, clinical global impression, and cumulative pain intensity; which were done using ANCOVA, in which treatment was a fixed factor (appendix p 111). Two post-hoc analyses were done on the primary endpoint: use of an alternative censoring method

	Treatment		Overall (N=144)
	Seviparin (N=69)	Placebo (N=75)	
<b>Age, years</b>			
Mean (SD)	23.6 (8.79)	23.4 (7.95)	23.5 (8.33)
Median (range)	21 (12–49)	22 (12–45)	22 (12–49)
<b>Body-mass index (kg/m<sup>2</sup>)*</b>			
Mean (SD)	22.8 (4.07)	23.0 (4.17)	22.9 (4.11)
Median (range)	22.2 (12.2–33.6)	22.6 (15.2–35.0)	22.3 (12.2–35.0)
<b>Race</b>			
White	55 (80%)	57 (76%)	112 (78%)
Black or African American	14 (20%)	14 (19%)	28 (19%)
Did not specify	0	4 (5%)	4 (3%)
<b>Sex</b>			
Female	27 (39%)	27 (36%)	54 (38%)
Male	42 (61%)	48 (64%)	90 (63%)
<b>Age category</b>			
Adolescent, ages 12–17 years	20 (29%)	20 (27%)	40 (28%)
Adult	49 (71%)	55 (73%)	104 (72%)
<b>Mutation</b>			
Sickle cell anaemia	47 (68%)	54 (72%)	101 (70%)
HbSβ-thalassaemia	11 (16%)	7 (9%)	18 (13%)
HbSβ-thalassaemia	6 (9%)	10 (13%)	16 (11%)
Sickle cell haemoglobin C	5 (7%)	4 (5%)	9 (6%)
<b>History of acute chest syndrome</b>			
Yes	11 (16%)	16 (21%)	27 (19%)
No	58 (84%)	59 (79%)	117 (81%)
<b>Hydroxyurea use</b>			
Used in the past 6 months	40 (58%)	41 (55%)	81 (56%)
Not used in the past 6 months	29 (42%)	34 (45%)	63 (44%)
<b>Hospitalisations due to vaso-occlusive crisis in the past 12 months</b>			
Mean (SD; median [range])	1.7 (2.77; 1[0–17])	1.4 (1.66; 1[0–7])	1.5 (2.26; 1[0–17])
One or no episodes	46 (67%)	46 (61%)	92 (64%)
Two episodes	9 (13%)	16 (21%)	25 (17%)
Two or more episodes	14 (20%)	13 (17%)	27 (19%)
<b>Pain intensity at baseline†</b>			
Mean (SD)	69.4 (22.3)	64.7 (24.1)	66.9 (23.3)
Median (range)	73 (0–100)	71 (0–100)	72 (0–100)
<b>Haemoglobin (g/dL)†</b>			
Mean (SD)	9.58 (1.77)	9.18 (1.53)	9.37 (1.65)
Median (range)	9.65 (5.10–14.70)	8.85 (5.64–13.50)	9.30 (5.1–14.70)
<b>Leucocytes (10<sup>9</sup> cells per L)†</b>			
Mean (SD)	12.70 (6.19)	14.0 (5.88)	13.40 (6.04)
Median (range)	13.2 (3.04–26.8)	14.7 (3.82–26.3)	14.0 (3.04–26.8)
<b>Neutrophils (10<sup>9</sup> cells per L)†</b>			
Mean (SD)	8.30 (5.49)	9.10 (4.98)	8.69 (5.23)
Median (range)	7.00 (1.80–22.63)	8.89 (1.30–20.52)	7.57 (1.30–22.63)

(Table 1 continues on next page)

in which censoring for the derivation of the primary endpoint was set to the actual timepoint of discontinuation instead of at 168 h (ie, 7 days); and the effect of aPTT-triggered dose reductions. The data safety monitoring board interim analyses are described in further detail in the appendix (p 72). Sevuparin plasma concentration analyses are presented as arithmetic mean (SD) according to sex (male or female) and age (adult [aged 8–50 years] or adolescent [aged 12–17 years]).

We did all statistical analyses using SAS (version 9.3 or higher). We used SPSS (version 27) to generate figures 2 and 3. This trial is registered with ClinicalTrials.gov, NCT02515838 and EudraCT, 2014–004416–11.

### Role of the funding source

The funder was involved in study design, data collection, data analysis, data interpretation, and writing of the report.

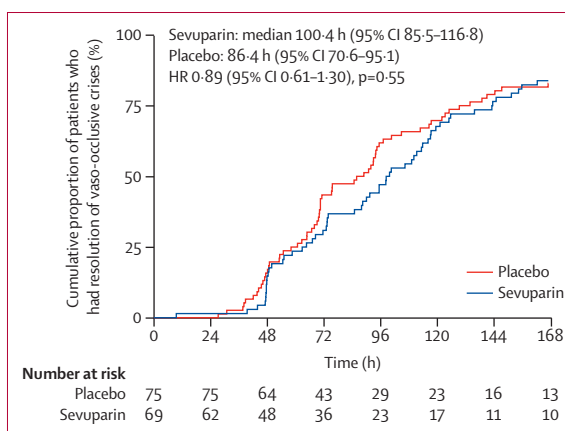
### Results

Between Oct 7, 2015, and Feb 10, 2019, 212 patients were screened and 147 patients were randomly assigned to the sevuparin group (n=71) or the placebo group (n=76; figure 1). Of those, two patients from the sevuparin group were excluded because not receiving study medication and one patient from the placebo group because not receiving study medication. Consequently, 144 received treatment (69 patients in the sevuparin group and 75 patients in the placebo group) and were included in the per modified intention-to-treat population. Treatment groups were balanced with respect to demographic and baseline characteristics and treatment duration (table 1, appendix pp 3–4). Major protocol deviations occurred in 49 (71%) of 69 patients in the sevuparin group and in 44 (59%) of 75 in the placebo group. Not all major protocol deviations led to exclusion from the per-protocol population because they were not relevant to the efficacy analysis. The major protocol deviations are listed in the appendix (pp 130–149).

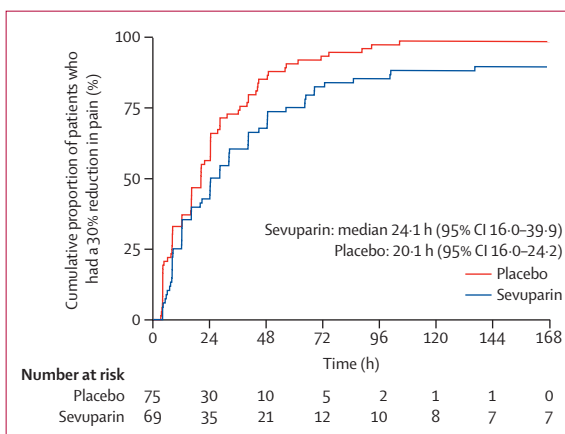
120 patients fulfilled the criteria for the primary endpoint: 58 (84%) of 69 patients (11 patients censored) in the sevuparin group and 62 (83%) of 75 patients (13 patients censored) in the placebo group. Reasons for censoring were missing time or date for primary endpoint and absence of 6 h opioid freedom and readiness occurring after 168 h (figure 1).

The primary analysis of the intention-to-treat population showed no significant difference in median time to resolution of vaso-occlusive crisis between the sevuparin and placebo groups: 100.4 h (95% CI 85.5–116.8); 25th percentile, 65.1 h (48.3–74.3); and 75th percentile, 144.3 h (118.1–not available [NA]) versus 86.4 h (70.6–95.1); 25th percentile, 58.5 h (47.7–69.6); 75th percentile, 130.1 h (101.3–NA; figure 2). The HR for the primary intention-to-treat analysis was 0.89 (0.6–1.3; p=0.55) and for the per protocol population was 0.81 (0.5–1.2; p=0.32; figure 2;

	Treatment		Overall (N=144)
	Sevuparin (N=69)	Placebo (N=75)	
(Continued from previous page)			
<b>Lactate dehydrogenase (U/L)†</b>			
Mean (SD)	429.3 (191.9)	448.0 (154.7)	439.3 (172.7)
Median (range)	401 (149–986)	428 (170–914)	411 (149–986)
<b>Creatinine (mg/dL)</b>			
Mean (SD)	0.53 (0.19)	0.56 (0.19)	0.55 (0.19)
Median (range)	0.52 (0.20–1.15)	0.53 (0.22–1.12)	0.53 (0.20–1.15)
Data are n (%), unless otherwise specified. *Data missing for one patient in the sevuparin group and two patients in the placebo group. †Safety population had 68 patients assigned to sevuparin and 76 patients assigned to placebo (one patient randomly assigned to sevuparin but received placebo).			
<b>Table 1: Baseline clinical characteristics of the modified intention-to-treat population</b>			



**Figure 2: Vaso-occlusive crisis resolution in the intention-to-treat population**  
All censoring was done at 168 h due to missing time or date for the readiness for discharge assessment, absence of 6 h opioid freedom, and readiness for discharge occurring after 168 h. HR=hazard ratio.



**Figure 3: Duration of severest pain in the intention-to-treat population**  
All censoring was at 168 h due to missing visual analogue scale assessments.

appendix p 5). The median duration of drug administration in the sevuparin group was 74.8 (range 7.92–167.1) h versus 72.9 (range 30.1–176.2) h in the placebo group. There were no significant differences

between treatment groups in the time to vaso-occlusive crisis resolution for the predefined subgroup analyses (appendix pp 6–7).

The results of the key secondary efficacy endpoints in both the intention-to-treat and per-protocol populations showed no significant differences between patients given sevuparin and patients given placebo. Specifically, mean change in pain severity from baseline until resolution of the vaso-occlusive crisis on the visual analogue scale was  $-35.3$  (SD  $19.7$ ) mm for patients in the sevuparin group and  $-34.1$  (SD  $18.8$ ) mm for patients in the placebo group (treatment effect assessed with ANCOVA for fixed effects  $p=0.726$ ; appendix p 8). The median time to a 30% pain reduction was not significantly different between the two treatment groups (24.1 h [95% CI 16.0–39.9] in the sevuparin group vs 20.1 h [95% CI 16.0–24.2] in the placebo group; figure 3).

The intention-to-treat analysis of the accumulated dose of parenteral opioids from baseline until readiness for discharge showed no significant difference between the sevuparin group and placebo group (median 105.0 mg [range 0.170–755.4] vs 96.2 mg [6.0–768.5]; two patients were excluded from the analysis because of missing data and two patients for lack of opioids dosed after investigational product start; appendix p 9).

Results of other related secondary endpoints (clinician and patient global impression of change; time to discharge and readiness for discharge; time to discontinuation of opioids; proportion of patients with vaso-occlusive crisis resolution at 24, 48, 72, 96 and 120 h; cumulative pain intensity difference; mean daily opioids until readiness for discharge) are listed in the appendix (pp 10–14). Additionally, acute chest syndrome occurred in six (9%) of 68 patients in the sevuparin group and six (8%) of 76 patients in the placebo group. Blood transfusions happened in 20 (29%) of 68 patients in the sevuparin

group and 24 (32%) of 76 patients in the placebo group. Re-admissions due to acute complications occurred in two (3%) of 68 patients in the sevuparin group and one (1%) of 76 patients in the placebo group. One (2%) of 58 patients in the sevuparin group and three (5%) of 62 patients in the placebo group were re-hospitalised for a vaso-occlusive crisis within 3 days of discharge and eight (14%) of 58 patients in the sevuparin group and eight (13%) of 62 patients in the placebo group after 28 days of discharge (the denominators used define the patients who could be assessed for a vaso-occlusive crisis in the first place). Re-admission for a vaso-occlusive crisis aims to assess the patients who had a vaso-occlusive crisis and then are readmitted with a new vaso-occlusive crisis; appendix p 14). Pharmacokinetic results are shown in the appendix (p 10).

Sevuparin was well tolerated by patients with sickle cell disease. There were no notable differences in the number of patients reporting treatment-emergent adverse events between study groups (appendix p 15). Most of the reported adverse events were mild to moderate and transient. Serious adverse events occurred in 15 (22%) of 68 patients in the sevuparin group and 17 (22%) of 76 patients in the placebo group and none were judged to be related to the study drug (appendix p 15). Seven (10%) patients had a treatment-emergent adverse event leading to treatment discontinuation in the sevuparin group and five (7%) in the placebo group (appendix p 16). In the sevuparin group, prolonged aPTT was the most frequent of this type of adverse event, whereas several different kinds of adverse event leading to treatment discontinuation occurred in the placebo group (appendix p 16).

The most commonly reported treatment-emergent adverse events in the overall population (occurring in  $\geq 10\%$  of patients in both groups) were pyrexia, constipation, and haemoglobin decrease (table 2). The

	Sevuparin (n=68)		Placebo (n=76)		Total (N=144)	
	Number of people with adverse events*	Number of adverse events	Number of people with adverse events*	Number of adverse events	Number of people with adverse events*	Number of adverse events
Any treatment-emergent adverse event	60 (88%)	310	67 (88%)	263	127 (88%)	573
Pyrexia	17 (25%)	20	17 (22%)	17	34 (24%)	37
Constipation	12 (18%)	13	17 (22%)	17	29 (20%)	30
Haemoglobin decrease†	18 (26%)	20	9 (12%)	9	27 (19%)	29
C-reactive protein increased†	11 (16%)	11	12 (16%)	12	23 (16%)	23
Nausea	8 (12%)	10	13 (17%)	15	21 (15%)	25
Sickle cell anaemia with crisis	9 (13%)	13	10 (13%)	13	19 (13%)	26
Vomiting	6 (9%)	7	10 (13%)	10	16 (11%)	17
Acute chest syndrome	7 (10%)	7	6 (8%)	8	13 (9%)	15
Prolonged aPTT	8 (12%)	8	0	0	8 (6%)	8

Data are n or n (%). Treatment-emergent adverse events that occurred in 10% or more of patients in the safety population for each group are shown. aPTT=activated partial thromboplastin time. \*Each patient was counted only once within each category. †Defined by a central laboratory but was judged by individual investigators taking into account local circumstances.

**Table 2: Most common treatment-emergent adverse events**

treatment-emergent adverse events (occurring in >10% of patients in any group) which differed the most between the sevuparin group and placebo group were nausea (eight [12%] vs 13 [17%]), prolonged aPTT (eight [12%] vs none), and reduced haemoglobin concentration (18 [27%] vs nine [12%]; table 2).

The aPTT elevations were expected and none were considered clinically significant by the investigator. Exclusion of the 31 (45%) of 69 patients in the intention-to-treat population for the sevuparin group who had dose reductions due to aPTT did not change the primary endpoint analysis (appendix p 8). The difference between the groups in terms of the number of patients who had decreases of haemoglobin was not mirrored in the mean haemoglobin data or the haemoglobin values reported as clinically significant by the investigators (appendix p 19). 15 (22%) of 68 patients in the sevuparin group had treatment-emergent adverse events judged to be related to study drug versus nine (12%) of 76 patients in the placebo group (appendix p 17). This difference in frequency between the groups was primarily caused by more than 10% of patients (eight of 68) having prolonged aPTT in the sevuparin group compared with none in the placebo group. Epistaxis was also reported in more than 5% of patients (four of 68) in the sevuparin group versus none in the placebo group. No other treatment-emergent adverse events judged to be related to study drug occurred in 3% or more of patients in either group (appendix p 17).

Five (7%) patients in the sevuparin group had possible bleeding events (appendix p 19). During the entirety of the study and follow-up period, there were no reported instances of heparin-induced thrombocytopenia (appendix p 19). There were no deaths in the sevuparin group and one (1%) death in the placebo group after a hyper-haemolytic episode due to alloimmunisation. aPTT was monitored based on the known profile of sevuparin and prolongations in the expected range were observed in the sevuparin group whereas no apparent differences were observed for international normalised ratio (appendix p 18). In contrast to the haemoglobin decreases reported as adverse events in the sevuparin treated patients no apparent differences in patterns were observed between treatment groups on measured haemoglobin (appendix p 18). Vaso-occlusive crisis gives rise to ongoing organ stress as shown by the liver enzyme profiles over time in the placebo group (appendix p 20).

## Discussion

In this multicentre, global, randomised, controlled study, no significant difference in the time to vaso-occlusive crisis resolution was shown between patients randomly assigned to sevuparin or placebo. The results for all key secondary endpoints (such as pain severity and cumulative opioid use) and exploratory endpoints were also not significantly different between the groups.

Additionally, no significant differences between treatment groups were seen in the frequency of blood transfusions, acute chest syndrome development, or readmissions, suggesting that sevuparin did not prevent complications secondary to vaso-occlusive crisis when compared with placebo. Because transfusions during admission are mostly given for the treatment of acute chest syndrome, the similar transfusion requirements are probably explained by the fact that no difference in acute chest syndrome incidence was observed.

With regards to safety, there were no notable differences in the number of patients reporting one or more treatment-emergent adverse events, or in the severity of the treatment-emergent adverse events between the groups. The only anticipated sevuparin specific side-effect was aPTT prolongation with no effect on INR. Because sevuparin has no anti-IIa or Xa activity due to the inactivation of the anti-thrombin domain, this prolongation is likely to be caused by the residual heparin cofactor II activity at high doses of sevuparin.<sup>23</sup> There were four instances of study drug-related epistaxis in four patients given sevuparin whereas none were observed in the placebo group. In these patients, aPTT prolongation was within the expected range for the sevuparin-treated group. Although unlikely based on these findings, a treatment-related effect on these events cannot be completely ruled out.

Our findings differ from the findings of a small randomised phase 2 study,<sup>25</sup> in which the pan-selectin inhibitor rivipansel was shown to significantly reduce cumulative opioid use with 83% ( $p=0.01$ ) as compared with placebo. However, similar to what we found, no reduction in duration of vaso-occlusive crisis was shown. In a phase 3 trial<sup>26</sup> in patients with sickle cell disease and vaso-occlusive crisis, no difference in time to resolution was found between patients given rivipansel and patients given placebo (HR 0.97). However, in a post-hoc analysis of a subset of the patients randomly assigned that received the study drug within 26.4 h of pain onset, a reduction in time to vaso-occlusive crisis resolution was shown (HR 0.58 [0.35–0.96]).<sup>26</sup>

In comparison with a randomised controlled trial<sup>8</sup> that showed efficacy in vaso-occlusive crisis prevention by monthly administration of a p-selectin neutralising monoclonal antibody, it might, from a pathophysiological perspective, not be surprising that anti-adhesive therapies are not effective in the acute treatment of vaso-occlusive crisis because the vaso-occlusive insult has already taken place. Additionally, most published preclinical studies in different murine sickle cell disease models (except for the study by Chang and colleagues)<sup>19</sup> were designed to prevent rather than treat vaso-occlusion.<sup>16–18</sup> However, we hypothesised that vaso-occlusive crisis is a dynamic process in which vaso-occlusion is not restricted to the vascular beds in which the painful crisis started, but also arises due to the occlusion of new vascular beds. This model is supported by the clinical observation that

most patients develop alternating areas of pain during vaso-occlusive crisis hospitalisation, as well as transient biomarker readouts indicating ongoing organ stress as indicated by liver enzyme values in patients over time from our trial (appendix p 20). Our findings show that an established vaso-occlusive crisis caused by a complex process of cellular adhesion and neutrophil and platelet activation, followed by vaso-occlusion and tissue ischaemia, cannot be overcome by primarily anti-adhesive strategies. Whether anti-adhesive strategies in combination with other mechanisms can be efficacious is not known and should be studied in future trials. Although sevuparin was not beneficial in acute vaso-occlusive crisis, the drug's promising safety profile, broad mode of action (eg, P-selectin inhibition), and the positive result of P-selectin inhibition in a preventive study design,<sup>8</sup> suggest that further research could be done on this drug in the prodromal setting, before the vaso-occlusive crisis manifests.

With respect to drug dosing, steady-state sevuparin concentrations were somewhat lower than the target concentration of 20 µg/mL, which was probably caused by a higher clearance rate in patients with sickle cell disease because of glomerular hyperfiltration related to sickle cell disease and aggressive fluid replacement therapy upon hospital admission with vaso-occlusive crisis. However, the drug concentrations attained were above the target range for effective anti-adhesive properties seen in preclinical studies and in ex-vivo studies using blood of patients with sickle cell disease.<sup>23,24</sup>

Although low molecular weight heparin has shown effects in the treatment of vaso-occlusive crisis in a previous randomised controlled study,<sup>20</sup> so far no other studies, have confirmed that anticoagulant therapy can change the natural course of a vaso-occlusive crisis.<sup>27</sup> Furthermore, in preclinical evaluation, sevuparin was equipotent to tinzaparin in a mouse vaso-occlusive crisis model.<sup>25</sup> Whether the presence of anticoagulant activity might be the biggest clinical difference between sevuparin and tinzaparin in established vaso-occlusive crisis is not known.

The strengths of our study were the study design (ie, randomised placebo-controlled design) to assess the time to resolution in an unbiased way and on the setting and the setting in which we carried out the trial because we picked regions where sickle cell disease constitutes a real and present health-care problem. However, our study did have limitations. First, there could have been potential subjectivity of the primary endpoint because readiness for discharge was judged by the patient or physician. Although readiness to be discharged has subjective traits, it can be counterbalanced by the freedom of parenteral opioid use criterion, which is based on time since the patient stopped the self-administered medication via the patient controlled analgesia-pump. Furthermore, the readiness for discharge criteria is not hampered by logistical matters

such as for time to discharge. Second, the actual onset time of the vaso-occlusive crisis as compared with the start of study treatment in the clinic might have led to a missed opportunity to treat in the early phase of a vaso-occlusive crisis when anti-adhesive mechanisms might be more effective. Future studies should determine whether early administration of anti-adhesive strategies can result in early resolution or even prevention of a full vaso-occlusive crisis and hospital admission when started at home.

In conclusion, the findings in this study suggest that anti-adhesive strategies antagonising selectins such as non-anticoagulant heparinoids are not beneficial when administered in the context of an already established vaso-occlusive crisis and suggest strategies to treat vaso-occlusive crises in sickle cell disease might differ from strategies to prevent this complication.

#### Contributors

All authors participated in drafting and revising the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. BJB, JKr, and JO designed the research methods. BJB, JKr, JO, AT, YK, MA-K, MA, MN, AI, and YW did the research. BJB, JKr, JO, JKO, and ED analysed data. BJB, JKO, and JO accessed and verified the trial data. BJB, JKr, JKO, ED, JO, AT, YK, MA-K, MA, MN, AI, and YW wrote and reviewed the manuscript.

#### Declaration of interests

BJB received research support of Sanquin, Global Blood Therapeutics, and Novartis; speaker honoraria from Global Blood Therapeutics and Novartis; and participated in advisory boards of Novartis, Celgene, and Global Blood Therapeutics. MA-K received speaker honoraria and participated in advisory boards of Novartis, Amgen, AbbVie, Swedish Orphan Biovitrum, Takeda, and Novo Nordisk. MA received research support from Novartis, AstraZeneca, and Global Blood Therapeutics; honoraria from Novartis and Novo Nordisk; and travel support from Novo Nordisk and Roche. AI received research support from AstraZeneca, Global Blood Therapeutics, and Cycleron; received honoraria from Pfizer, Roche, Novartis, Novo Nordisk, and Sanofi; travel support from Novartis; is member of a steering committee of Novartis; and is an advisory board member for Novartis and Cycleron. YW received research support and travel support from Novartis, Pfizer, Swedish Orphan Biovitrum, Novo Nordisk, and Modus; and participated in advisory boards for Novartis, Pfizer, and Swedish Orphan Biovitrum. JKr is a consultant to Modus Therapeutics. ED and JO were full-time employees of Modus at the time of study conduct. The rest of the authors declare no competing interests.

#### Data sharing

Individual participant data will not be shared.

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#### References

- 1 Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. *Lancet* 2017; **390**: 311–23.
- 2 Houwing ME, de Pagter PJ, van Beers EJ, et al. Sickle cell disease: clinical presentation and management of a global health challenge. *Blood Rev* 2019; **37**: 100580.
- 3 Hamideh D, Alvarez O. Sickle cell disease related mortality in the United States (1999–2009). *Pediatr Blood Cancer* 2013; **60**: 1482–86.
- 4 Gardner K, Douiri A, Drasar E, et al. Survival in adults with sickle cell disease in a high-income setting. *Blood* 2016; **128**: 1436–38.
- 5 Brousseau DC, Owens PL, Mosso AL, Panepinto JA, Steiner CA. Acute care utilization and rehospitalizations for sickle cell disease. *JAMA* 2010; **303**: 1288–94.
- 6 Novelli EM, Gladwin MT. Crises in sickle cell disease. *Chest* 2016; **149**: 1082–93.

- 7 Steinberg MH. Sickle cell disease. *Ann Intern Med* 2011; **55**: ITC31–15.
- 8 Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. *N Eng J Med* 2017; **376**: 429–39. 9 Niihara Y, Miller ST, Kanter J, et al. A phase 3 trial of L-glutamine in sickle cell disease. *N Eng J Med* 2018; **379**: 226–235.
- 10 Vichinsky E, Hoppe CC, Ataga KI, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. *N Engl J Med* 2019; **381**: 509–19.
- 11 McClish DK, Smith WR, Levenson JL, et al. Comorbidity, pain, utilization, and psychosocial outcomes in older versus younger sickle cell adults: the PiSCES project. *BioMed Res Int* 2017; **2017**: 4070547.
- 12 Palermo TM, Riley CA, Mitchell BA. Daily functioning and quality of life in children with sickle cell disease pain: relationship with family and neighborhood socioeconomic distress. *J Pain* 2008; **9**: 833–40.
- 13 van Tuijn CF, van Beers EJ, Schnog JJ, Biemond BJ. Pain rate and social circumstances rather than cumulative organ damage determine the quality of life in adults with sickle cell disease. *Am J Hematol* 2010; **85**: 532–35.
- 14 Hebbel RP. Adhesive interactions of sickle erythrocytes with endothelium. *J Clin Invest* 1997; **100** (suppl): S83–86.
- 15 Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. *Blood* 2016; **127**: 801–09.
- 16 Matsui NM, Varki A, Embury SH. Heparin inhibits the flow adhesion of sickle red blood cells to P-selectin. *Blood* 2002; **100**: 3790–96.
- 17 Embury SH, Matsui NM, Ramanujam S, et al. The contribution of endothelial cell P-selectin to the microvascular flow of mouse sickle erythrocytes in vivo. *Blood* 2004; **104**: 3378–85.
- 18 Gutsaeva DR, Parkerson JB, Yerigenahally SD, et al. Inhibition of cell adhesion by anti-P-selectin aptamer: a new potential therapeutic agent for sickle cell disease. *Blood* 2011; **117**: 727–35.
- 19 Chang J, Patton JT, Sarkar A, Ernst B, Magnani JL, Frenette PS. GMI-1070, a novel pan-selectin antagonist, reverses acute vascular occlusions in sickle cell mice. *Blood* 2010; **116**: 1779–86.
- 20 Qari MH, Aljaouni SK, Alardawi MS, et al. Reduction of painful vaso-occlusive crisis of sickle cell anaemia by tinzaparin in a double-blind randomised trial. *Thromb Haemost* 2007; **98**: 392–96.
- 21 Poterucha TJ, Libby P, Goldhaber SZ. More than an anticoagulant: do heparins have direct anti-inflammatory effects? *Thromb Haemost* 2017; **117**: 437–44.
- 22 Lindgren M, Meijers JCM, Biemond BJ, et al. Sevuparin; effects on hemostasis of a novel polysaccharide drug derived from heparin. International Society on Thrombosis and Haemostasis Congress 2015; Toronto, ON; June 20–25, 2015 (poster PO294-MON).
- 23 White J, Lindgren M, Liu K, Gao X, Jendeborg L, Hines P. Sevuparin blocks sickle blood cell adhesion and sickle-leucocyte rolling on immobilized L-selectin in a dose dependent manner. *Br J Haematol* 2019; **184**: 873–76.
- 24 Telen MJ, Batchvarova M, Shan S, et al. Sevuparin binds to multiple adhesive ligands and reduces sickle red blood cell-induced vaso-occlusion. *Br J Haematol* 2016; **175**: 935–48.
- 25 Telen MJ, Wun T, McCavit TL, et al. Randomized phase 2 study of GMI-1070 in SCD: reduction in time to resolution of vaso-occlusive events and decreased opioid use. *Blood* 2015; **125**: 2656–64.
- 26 Dampier Carlton D. Early initiation of treatment with rivipansel for acute vaso-occlusive crisis in sickle cell disease (SCD) achieves earlier discontinuation of IV opioids and shorter hospital stay: reset clinical trial analysis. *Blood* 2020; **136** (suppl 1): 18–19 (abstr).
- 27 van Zuuren EJ, Fedorowicz Z. Low-molecular-weight heparins for managing vaso-occlusive crises in people with sickle cell disease. *Cochrane Database Syst Rev* 2015; **12**: CD010155.