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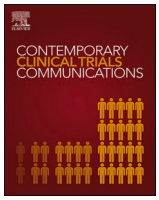

Design of an adaptive randomized clinical trial of intravenous citrulline for sickle cell pain crisis in the emergency department

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A B S T R A C T

Background: Vaso-occlusive pain crisis (VOC) is the most frequent cause for Emergency Department (ED) visits and hospital admissions for patients with sickle cell disease (SCD). Nitric oxide plays a critical role in the pathogenesis of vaso-occlusion. The amino acid, citrulline, is the main endothelial nitric oxide booster that offers the potential to ameliorate vaso-occlusion and decrease the risk of hospitalization.

Objective: In this two-part study, the goal of the first part is to determine the pharmacokinetic profile of intravenous (IV) L-citrulline and optimal dose for the second part of the study, which is to determine the efficacy and tolerability of the intervention in patients with SCD.

Design: A phase I/IIA open-label dose-finding study with subsequent double-blind, placebo-controlled, randomized Study of L-citrulline in children and adolescents with SCD presenting to the ED in VOC.

Methods: Part 1: Subjects experiencing VOC are enrolled in an open-label, ascending dose of IV L-citrulline to identify the optimum dose with endpoints of pharmacokinetic parameters, pain scores, reduction of opioid use, quality of life, proportion admitted to the hospital for treatment of pain, readmission rates, and assessment of adverse events. Part 2 of the trial is a double-blind, placebo-controlled adaptive "pick-the-winner" design to evaluate the efficacy and tolerability of IV L-citrulline in patients with SCD while receiving standard of care therapy for VOC.

Summary: This ED based sickle cell adaptive trial will determine the optimal dose for IV citrulline and whether the intervention improves outcome as a potential novel therapy for VOC in SCD.

1. Introduction

Sickle cell disease (SCD) is an autosomal recessive disorder caused by a mutation in the β-chain of hemoglobin (Hb) that leads to production of sickle hemoglobin (HbS). When deoxygenated, HbS polymerizes and deforms red blood cells (RBCs) into a sickle shape in the microcirculation, leading to painful vaso-occlusive crisis (VOC) and end organ damage [1,2]. SCD affects approximately 100,000 people in the United States and millions worldwide, most of whom are in sub-Saharan Africa. Acute pain from VOC in SCD is the most frequent cause of emergency department (ED) visits and hospital admissions, largely driving the high burden of health care costs and impaired quality of life found in SCD [3,4].

The current mainstay of treatment for VOC is opioids such as morphine. However, opioids have significant side effects related to constipation, itching, and respiratory depression which can increase the risk of acute chest syndrome, a common lung complication in sickle cell disease. Furthermore, even with escalating doses of opioids many patients may not achieve analgesia. The need for alternative therapy in this high-risk population is especially apparent when considered in the broader context of the national opioid crisis, with opioid misuse contributing to preventable death in the general population.

Nitric oxide is a powerful vasodilator and depletion of the molecule has been shown to play a critical role in the pathogenesis of vaso-occlusion in sickle cell pain crisis [5,6]. As the main supplier of endothelial nitric oxide production, the amino acid citrulline has the potential to relieve vaso-occlusion and improve outcomes for VOC [7–9]. The aim of the first part of this intravenous (IV) citrulline clinical trial is to identify the optimum dose regimen for part 2 of the trial which is a double-blind, placebo-controlled adaptive clinical trial design. This study will allow assignment of more subjects to the better treatment arm/s based on emerging data. The study will evaluate efficacy and

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tolerability of incremental doses of intravenous (IV) L-citrulline in patients with SCD while receiving standard of care therapy for VOC.

2. Methods

2.1. Overall study design

This is a study in children, adolescents, and young adults (6–21 years) with SCD presenting to the ED with VOC. VOC is defined as a painful episode without other apparent causes of pain.

This study will be conducted in 2 parts. The aim of Part 1 is to identify the optimum dose regimens for Part 2 of the trial, which is a double-blind, placebo-controlled adaptive 'pick-the-winner' design. This study will allow assignment of more subjects to the better treatment arm/s based on emerging data. The study will evaluate efficacy and tolerability of incremental doses of intravenous (IV) L-citrulline in patients with SCD while receiving standard of care therapy for VOC. The primary objectives and corresponding endpoints of the clinical trial are listed in Table 1.

2.2. Inclusion/exclusion criteria

Inclusion criteria are.

(1) Sickle cell disease (all genotypes);
(2) Children, adolescents and young adults between ages 6–21 years;
(3) Not in the midst of any acute complication other than VOC due to sickle cell disease at study entry;
(4) For females of childbearing potential, a negative urine pregnancy test and use of an adequate method of contraception or denial of sexual activity;
(5) Subjects or parents or legal guardian of the subject who are willing and able to sign and provide consent and assent (where appropriate for the age of the child).

Exclusion criteria are.

(1) Current pain lasting >3 days;
(2) >6 hospital admissions in the prior year;
(3) History of opioid dependence/substance abuse;
(4) Subject has been on a clinical trial of a new therapy for sickle cell disease within the last 3 months;
(5) Presence of any other complication related to sickle cell disease such as splenic sequestration, hepatic sequestration, stroke, avascular necrosis of the hip/shoulder, acute priapism, renal dysfunction, dactylitis, acute chest syndrome and other major medical conditions or organ dysfunction;
(6) Severe anemia (hemoglobin <6 g/dL);
(7) History of red blood cell transfusion within the last 30 days;
(8) Systemic steroid therapy within the last 48 h;
(9) Pregnancy or lactation (subjects must have a negative urine pregnancy test);
(10) Serum creatinine levels: a) Age 6–13 years > 0.9 mg/dL; b) Age 14–17 years > 1.0 mg/dL and c) age >18 years > 1.5 mg/dL;
(11) Report of fever (>38 °C) within last 48 h;
(12) Presence of acute chest syndrome, sepsis, bacterial infection, hemodynamic instability;
(13) Subjects with inability to have parental consent given (ages 6–17 years) or consent themselves (ages 18 through 21 years). Note: Parents or legal guardians can provide consent for subjects who are unable to provide assent (e.g., sleepy or preoccupied by their pain);
(14) History of allergic reaction to L-citrulline product;
(15) Medications that are known to be contra-indicated with use of L-citrulline;
(16) History of diabetes;
(17) Received any blood products within 3 weeks of the screening visit;
(18) Unreliable venous access;
(19) The PI considers that the subject will be unable to comply with the study requirements.

Table 1
Showing the objectives and endpoints of Part 1 and Part 2 of the clinical trial.

Primary objectives and corresponding endpoints:		
Part	Objectives	Endpoints
1	1 To determine optimal dose regimens for Part 2 of the study	Optimal doses for Part 2 will be determined by review of data from the following endpoints
	2 To determine the pharmacokinetic profile within and across dosing regimens of Part 1	Determination pharmacokinetic parameters (peak, trough, steady state concentration, C_{max} and area under the plasma concentration-time curve [AUC] range), dose proportionality and time-dependence of PK within and across dosing regimens of Part 1
	3 Initial evaluation of efficacy and tolerability of incremental doses of intravenous L-citrulline in patients with SCD while receiving standard of care therapy for VOC	a VAS pain scores: at least 2-point decrease or 30% reduction in VAS or FACES Pain Scale score (for subjects 6–7 years old) when compared with baseline value; assessed every 15 min b At least 25% reduction from baseline in amount of overall opioid use; assessed every 1 h c Discharge from ED/hospital within 7 h or discharge from hospital within 24 h from start of study drug d Assessment of safety parameters e Readmission within 48 h for VOC f Assessment of trial emergent adverse events (AEs) using modified Common Terminology Criteria for Adverse Events (CTCAE) g Incidence of acute chest syndrome h Assessment of Pediatric Quality of Life (PedsQL™ SCD)
2	1 To determine the pharmacokinetic profile within and across dosing regimens of Part 2	Determination pharmacokinetic parameters (peak, trough, steady state concentration, C_{max} and AUC range), dose proportionality and time-dependence of PK within and across dosing regimens of Part 2
	2 To determine the efficacy and tolerability of intravenous Lcitrulline in patients with SCD while receiving standard of care therapy for VOC	a VAS pain scores: at least 2-point decrease or 30% reduction in VAS or FACES Pain Scale score (for subjects 6–7 years old) when compared with baseline value; assessed every 15 min b At least 25% reduction from baseline in amount of overall opioid use; assessed every 1 h c Discharge from ED/hospital within 7 h or discharge from hospital within 24 h from start of study drug d Assessment of safety parameters e Readmission within 48 h for VOC f Assessment of trial emergent AEs using modified CTCAE g Incidence of acute chest syndrome h Assessment of Pediatric Quality of Life (PedsQL™ SCD)

2.3. Screening

Patients aged 6–21 years old will be screened in the ED. For patients and families who are interested in participating, the following assessments will take place during screening: Assessment for eligibility against the inclusion and exclusion criteria, demographic data (age, sex, race and ethnicity), review of medical history documentation (including substance abuse), family history, and a physical examination of skin, neck, eyes, oral mucosa, nail beds, chest, abdomen, edema, joints, and lymph nodes, concurrent medical conditions and medications, urine pregnancy test, laboratory tests (this includes complete blood picture including hemoglobin, reticulocyte count, LDH, CRP, liver enzymes, renal function, thyroid panel, urine drug screen and urinalysis) and a 12-Lead electrocardiogram (ECG). If the SCD genotype is not reliably documented in the patient's records, a sample may be taken to determine genotype during screening or at follow-up.

2.3.1. Methods

The study schema for Part 1 and Part 2 is presented in Fig. 1a and b, respectively.

Part 1: This is an open-label, ascending dose part of the study. After obtaining the informed consent/assent, the enrolled subjects will receive IV L-citrulline in the ED as bolus followed by a continuous infusion, for up to 7 h. Each dose will be administered sequentially to subjects until 5 subjects are accrued per dose cohort. Dosing levels for each dose cohort are depicted in the dosing panel below. Part 1 of the study is intended to select 2 effective and tolerated dosing levels for Part 2 of the study.

Dosing panel in Part 1.

- Dosing Level I: 25 mg/kg bolus +9 mg/kg/hr infusion
- Dosing Level II: 50 mg/kg bolus +9 mg/kg/hr infusion
- Dosing Level III: 100 mg/kg bolus +9 mg/kg/hr infusion
- Dosing Level IV: 100 mg/kg bolus +11 mg/kg/hr infusion

After each panel of 5 have been dosed, the response to a dosing level is determined by the internal review committee, based primarily on analgesic effects assessed by review of Visual Analogue Scale (VAS) score, opioid dosing, and tolerability. If the dosing level is assessed safe and 3 of 5 subjects in a cohort respond to the treatment, the current dosing level will continue to accrue a total of 10 subjects. However, if the dosing level is assessed as safe but fewer than 3 of the 5 subjects respond, the current dosing level will be stopped, and the subjects will be recruited to the next dosing level. Two additional panels of 5 subjects may be accrued at the request of the internal review committee if determined necessary to achieve the study's objectives. As long as supported by emerging data, the subjects in these additional panels may receive a lower or higher bolus injection. Up to 60 subjects may be enrolled in Part 1 of the study with a likely sample size of approximately 40 subjects.

Part 2: The optimal dose regimen/s (possibly 1 but most likely 2) selected from Part 1 will be analyzed in a randomized, double-blind, placebo-controlled adaptive "pick-the-winner" design. This will allow assignment of more subjects to the better treatment arm/s based on emerging data. Efficacy will be based primarily on analgesic effect assessed by VAS/opioid dose composite, hospital admission, and tolerability based on adverse drug reactions by the internal review committee. A total of 60–90 subjects will take part in Part 2. Subjects will be randomized to one of 2 dosing levels, selected from Part 1, or randomized to placebo (5% Dextrose in 0.45% NaCl) in a 1:1:1 ratio. Subjects, investigators, and the clinical team will all be blinded as to the study arm. After 30 subjects (10 per treatment arm) have been randomized, an interim analysis will be performed. If the higher dose is providing markedly more pain relief than the lower dose - with no safety issues - then the lower dose will be dropped. Otherwise, if efficacy and safety look similar, the lower dose will be retained, and the higher dose dropped. In other words, the minimal effective dose will be chosen for further study versus placebo. Subsequently, 30 more patients will be randomized in a 1:1 fashion in the remaining 2 study arms (15 patients each). Hence, a total of 60 subjects will be projected to take part in Part 2. In the unlikely case that the placebo arm demonstrates superior response to both active treatment arms, the interval review committee will recommend discontinuing the study. If after 30 subjects, it is determined by the internal review committee that 1 of the 2 doses is significantly more effective in subjects with particular characteristics

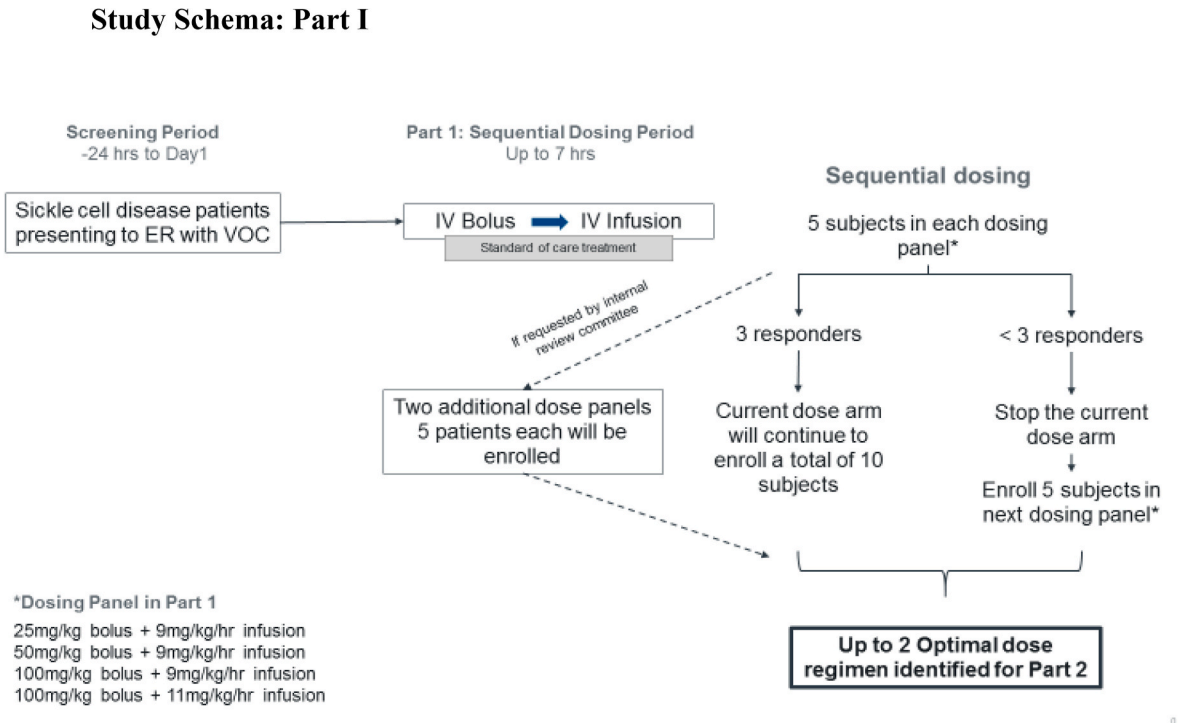

Fig. 1a. showing the adaptive design sequential dosing schema for part 1 of the study.

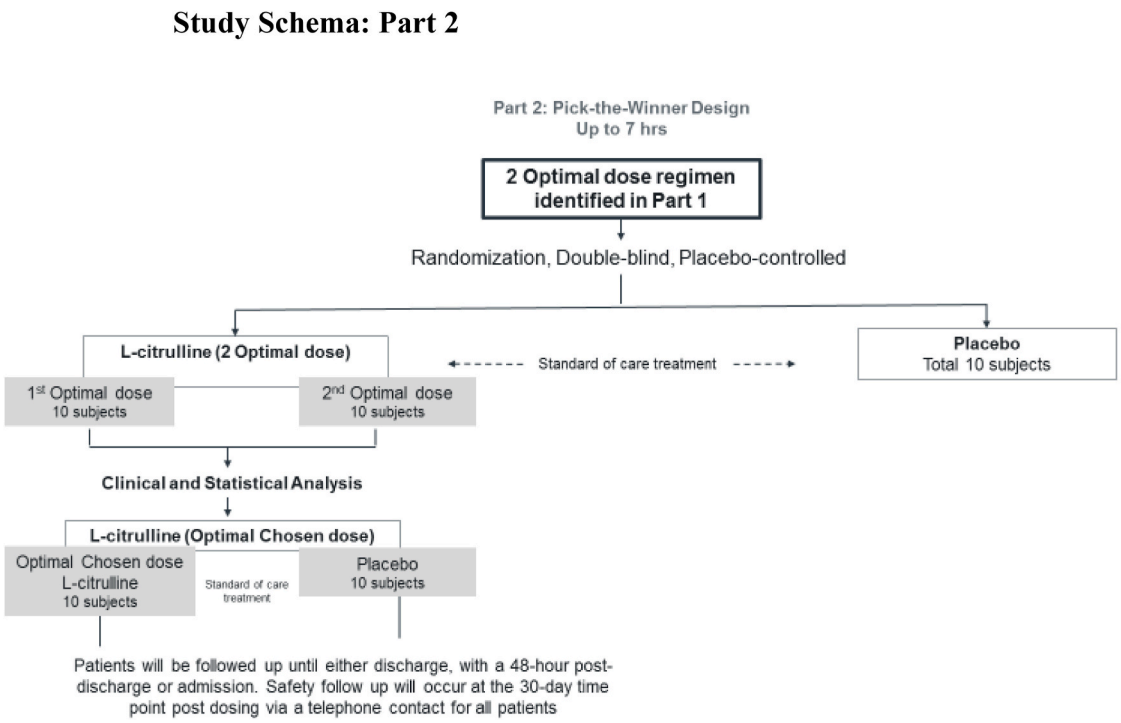

Fig. 1b. showing the schema for the drug dose chosen for the randomized, placebo controlled in part 2 of the study. Of note, subjects in both parts will also receive standard of care treatment for vaso-occlusive pain crisis (VOC).

(especially focusing on pain severity and hydroxyurea use), the less effective L-citrulline dose regimen can be dropped for those subjects. For example, if subjects with more severe VOC only respond to the higher dose but subjects with less severe VOC respond equally well to either dose, patients with severe VOC will be adaptively allocated into a separate stratum and randomized only to the higher dose or placebo. Irrespective of randomization to study treatment or placebo, subjects in Part 1 and Part 2 will receive standard of care for analgesia per our ED SCD pain management protocol and guided by individualized care plans..

2.4. Outcome evaluation

1). Pain scores will be performed at baseline (immediately before dosing) and then every 15 min for the duration of the infusion by the Visual Analogue Scale (VAS) or FACES Pain Scale. The VAS is a validated, subjective measure for acute and chronic pain. The VAS is commonly used as the outcome measure used in studies and consists of a straight line with the endpoints defining extreme limits such as 'no pain at all' and 'pain as bad as it could be'. FACES Pain Scale: The FACES pain scale is a self-report measure of pain intensity developed for children. FACES pain scales consist of a series of line diagrams of faces with expressions of increasing distress and the child has to choose according to the intensity of pain felt.

2). Opioid Use will be assessed by:
- Type and route of opioid: Fentanyl (IV, nasal) vs morphine (IV) vs hydromorphone (IV),
- Amount of opioid use: Fentanyl/morphine/hydromorphone (converted to morphine equivalent) amount; assessed every 1 h, and
- Frequency of opioid injection:
- All the opioid medications will be quantified and assessed on cumulative use across arms in Part 1 and compared with placebo in Part 2.

3). Pharmacokinetic profile will be performed at baseline and after starting study infusion, every 15 min for the first hour and then hourly until the end of the infusion.

4). Pediatric Quality of Life (PedsQL™ SCD Module) will be obtained at baseline and at a 48 h follow up visit. The PedsQL™ SCD Module is a 43-item module encompasses nine scales: 1) Pain and Hurt (9 items), 2) Pain Impact (10 items), 3) Pain Management and Control (2 items), 4) Worry I (5 items), 5) Worry II (2 items), 6) Emotions (2 items), 7) Treatment (7 items), 8) Communication I (3 items), 9) Communication II (3 items). The format, instructions, Likert response scale, and scoring method for the PedsQL™ SCD Module are identical to the PedsQL™ 4.0 Generic Core Scales, with higher scores indicating better HRQOL and lower SCD symptoms/problems. The Module Scales are comprised of parallel child self-report and parent proxy-report formats for children ages 5–18 years, and a parent proxy-report format for children ages 2–4 years. Child self-report forms are specific for ages 5 to 7, 8 to 12, and 13–18 years. Parent proxy-report forms are specific for children ages 2 to 4 (toddler), 5 to 7 (young child), 8 to 12 (child), and 13 to 18 (adolescent), and assess parents' perceptions of their child's HRQOL. The instructions ask how much of a problem each item has been during the past month. Forms are self-administered by the parent or child ages 8–18 years. For children 5–7 years of age, forms are interviewer-administered. Items are reverse-scored and linearly transformed to a 0–100 scale (0 = 100, 1 = 75, 2 = 50, 3 = 25, 4 = 0), so that higher scores indicate better HRQOL. To create the PedsQL™ SCD Module Total Scale Score (43 items), the mean is computed as the sum of the items divided by the number of items answered.

5). Safety Assessments: In addition to the vital signs (pulse, respiratory rate, systolic, diastolic and mean arterial blood pressure), a Riker score will be collected to measure sedation. The Riker scale is a valid and reliable tool which has been used in different studies as sedation-agitation scale in patients hospitalized in Intensive Care Unit. Research staff will record the Riker score and

vital signs at baseline and then every 15 min for the full duration of the infusion, and then at 48-h follow up visit in the outpatient clinic or inpatient hospital if the subject was admitted from the ED. Markers of Hemolysis will collected by research staff at the start of study drug infusion, at 15 min, 60 min and then hourly until a final sample is collected at the end of the infusion. During the infusion, follow up EKG will be monitored for any rhythm changes or prolonged QTc. In addition, laboratory tests will be repeated at 48 h from study infusion. Subjects will have their safety evaluation by history taking and/or physical examination during the study infusion, 48-h follow-up clinic visit and 30 day follow up phone call visit with adverse event reporting according to the NCI Common Terminology Criteria for Adverse Events (CTCAE).

2.5. Statistical analysis

For the primary endpoint of PK parameters, descriptive statistical methods will be used.

Concentration at each measured time point will be plotted for each subject. The mean value of concentration at each time point will be plotted by treatment group. Mean values of the parameters; peak (Cmax), trough, AUC and steady state concentrations will be computed for each dose. Descriptive statistics will be used to describe the safety profile of L-citrulline. The exploratory endpoints will be analyzed using descriptive statistics. For continuous variables such as VAS pain scores, percentage reduction of pain symptoms, length of hospital stay, time to clinical resolution of VOC, and safety assessments, means and standard deviations will be used, while proportions and percentages will be used for categorical variables, such as requirement for opioids and percentage of hospital admission. For sample size determination, power is presented for the adaptive portion, Part 2, the response to pain endpoint. Responders will have at least a 2-point decrease or 30% reduction in VAS or FACES Pain Scale score (for subjects 6–7 years old) when compared with baseline value. Assuming a two-sided significance level of 0.05, the study will have 80% power to detect a difference in proportion of subjects meeting pain response criteria of 60% in the L-citrulline group compared with 21% in the placebo group with $N = 25$ subjects per arm. Similar power (80%) is obtained with $N = 25$ subjects per arm if the L-citrulline pain response rate is 80% compared with 42% in the placebo group. Although these effect sizes may appear large at first glance, the sample size is typical for studies of this nature and can inform the planned endpoints and dosing for further Phase 2 studies.

2.6. Recruitment strategy

Given the acute care setting in the ED and limited time to consent/assent participants, a comprehensive strategy will be employed that includes pre-consenting participants in the outpatient setting: An IRB approved excel spreadsheet has been created which contains name, medical record number, primary hematologist name, consenting physician and date of consent discussion, and date of next clinic visit. The excel spreadsheet is color coded: green to indicate that the participant agreed to consent, yellow to indicate that the participant was interested but undecided, and red to indicate that they were not interested or would not meet inclusion criteria. The follow up date of next clinic is set as a reminder to the Hematology provider to re-review the consent/assent forms, to evaluate whether the participants/family are ready to sign. Of note, since IRB approval of the clinical trial, consent/assent forms have been reviewed in 238 subjects within a 4-month period.

3. Discussion

Since the discovery of SCD in modern history in 1910, there are only four FDA approved drugs for the prevention of pain in SCD, three of which received approval only in the last 5 years [10–12]. Currently, there is no FDA approved drug for the treatment of acute VOC, although a few drugs have been tested in clinical trials and without successful results [13–15]. The conduct of clinical trials in SCD has been challenging in this minority population for an otherwise complicated clinical outcome of pain. In the last several years, there has been an exponential growth in the number of clinical trials in SCD, made possible due to close partnerships between the pharmaceutical industry and academia [16]. However, the design of the clinical trial in SCD is critically important for the successful outcome; for example, the poloxamer 188 trial for VOC showed significant and positive findings initially, but unfortunately failed to achieve FDA approval because of negative results in the final phase 3 trial [15,17]. This is partly because the clinical endpoints were different between the different phases of the trials [18]. In addition, the landmark phase 3 trial of hydroxyurea for the prevention of pain in SCD showed that hydroxyurea at maximum tolerated dose significantly decreases the frequency of pain crises although debate on use of low dose use hydroxurea with reduced toxicity led to further confirmatory trials [19,20]. The history of unsuccessful trials could complicate the challenging economics associated with funding studies for diseases with limited market value [21]. Our principal method of avoiding losing funds to another unsuccessful clinical trial was to consider an adaptive trial design, especially during the earlier phases of the trial, which can facilitate planning for subsequent pain intervention research trials in SCD.

Our adaptive clinical trial design makes our sickle cell trial flexible, which can allow for analysis of accumulating results in the ongoing trial to better inform us on modification of the trial's course accordingly. Pallmann et al., highlight that compared to a traditional fixed design trial, an adaptive design is often more efficient, informative and ethical [22]. In addition, adaptive trials might also require fewer participants with better use of resources such as time and money. We designed our trial so that pain scores are collected every 15 min for the full duration of the study drug infusion. The pharmacokinetic testing coincides with the pain scores which will allow a comprehensive assessment of pharmacokinetic-pharmacodynamic (PK-PD) modeling to determine the effect of the study drug dose on pain response. Scheduled interim review of the clinical efficacy/adverse event findings of part 1 of the trial gives an opportunity to determine whether to drop or continue a particular study dose or increase it accordingly. Moreover, in part 2 of the study, which can include 3 arms (placebo, low dose and high dose study drug), interim reviews will help to determine if one of the study drug arms needs to be dropped with further participant enrollments. The overall impact of an adaptive trial on participants is that fewer participants will be exposed to the dose levels which are least likely to be safe or effective.

Adequate recruitment for clinical trials in the minority population such as SCD has been proven to be challenging [23,24]; Masese et al. found that recruitment at community events, emergency departments and pain centers had the lowest yield [25]. An ED-based study is especially difficult because it is a busy and chaotic environment with significant time constraints, which create challenges to starting the study drug in a timely fashion to meet study endpoints. Furthermore, participants will be hesitant to hear about a trial and sign a consent form when they are experiencing pain and may have impaired decision making after receiving high doses of opioids. As a result, discussing the trial in the outpatient clinic setting with trusted hematology providers, prior to presentation to the ED for VOC, will be critical to the success of the trial.

Finally, some individual with SCD have chronic pain and may present with acute-on-chronic VOC, which can likely affect the trial endpoint pain outcome. As a result, strict inclusion and exclusion criteria will be followed to exclude such subjects with a history of chronic pain considering their ED and hospital course may be different. Specifically, we will adhere to criteria that dictate exclusion of patients with characteristics of chronic pain: current pain episode is > 3 days; >6 hospital admissions in the prior year; or a history of opioid dependence. While this may adversely affect target enrollment of the study, it is

important in this early phase 1/2A trial that the cohort and phenotype of participants' is kept consistent, so that the study drug intervention results are clear and more easily interpretable.

In summary, to the best of our knowledge, this is the first study employing an adaptive clinical trial model for acute VOC in the ED setting, which will provide important information for a possible future phase 3 trial of intravenous citrulline and may ultimately provide an additional, nonopioid option for patients with SCD who require management of acute pain.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SM and MS are co-patent holders for the indication of intravenous citrulline for sickle cell pain crisis. GK is an employee of Asklepion Pharmaceuticals.

Data availability

No data was used for the research described in the article.

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