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Gene Transfer for Patients With Sickle Cell Disease Using a Gamma Globin Lentivirus Vector: An Open-Label Phase 1/2 Pilot Study

PROTOCOL NO.: ARU-1801_Ph1_01

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Version 2.4 (03 March 2015)
Version 2.3 (31 December 2014)
Version 2.2 (25 September 2014)
Version 2.1 (10 July 2014)
Version 2.0 (21 April 2014)
Version 1.0 (30 January 2014)

Aruvant Sciences, GmbH (ASG), a Swiss limited liability company, is the Sponsor of this study. Aruvant Sciences, Inc, a Delaware corporation and an affiliate of ASG, has been engaged by ASG to manage the day-to-day operations of the study. All references to Sponsor, as defined herein, shall refer to Aruvant Sciences, Inc, acting pursuant to a services agreement with ASG.

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All financial and nonfinancial support for this study will be provided by Aruvant Sciences. The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without the expressed, written consent of Aruvant Sciences.

The study will be conducted according to the International Council for Harmonisation harmonised tripartite guideline E6: Good Clinical Practice.

Protocol Approval – Sponsor Signatory

Study Title	Gene Transfer for Patients With Sickle Cell Disease Using a Gamma Globin Lentivirus Vector: An Open-Label Phase 1/2 Pilot Study
Protocol Number	ARU-1801_Ph1_01
Protocol Date	13 Aug 2021

Protocol accepted and approved by a representative of Aruvant Sciences, GmbH:

Will Chou, MD
Chief Executive Officer
Aruvant Sciences
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8/20/2021



Signature Date

Declaration of Investigator

I have read and understood all sections of the protocol entitled "Gene Transfer for Patients With Sickle Cell Disease Using a Gamma Globin Lentivirus Vector: An Open-Label Phase 1/2 Pilot Study" and the accompanying investigator's brochure.

I agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the Final Protocol Version 13.0, 13 Aug 2021, the International Council for Harmonisation harmonised tripartite guideline E6: Good Clinical Practice and all applicable government regulations. I will not make changes to the protocol before consulting with Aruvant Sciences or implement protocol changes without independent ethics committee approval except to eliminate an immediate risk to subjects. I agree to administer study treatment only to subjects under my personal supervision or the supervision of a subinvestigator.

I will not supply the investigational drug to any person not authorized to receive it. Confidentiality will be protected. Subject identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorization from Aruvant Sciences.

Signature of Principal Investigator	Date

Printed Name of Principal Investigator

Protocol Amendment Summary of Changes

Protocol Version 13.0 (13 Aug 2021)

Summary of Change	Rationale	Protocol Sections Affected
Test Melphalan Dose		
• Removed optional test dose of melphalan.	• The test dose is no longer needed to inform melphalan pharmacokinetics because predictive modeling will now be used.	Sections 2.1.3, 3.1, 6.1, 6.6, 7.5, 7.6.3, and the Schedule of Events (Table 13-1 and Table 13-1 footnote x)
Exploratory Objectives		
• Clarified the objective regarding melphalan pharmacokinetics in patients with SCD.	• The wording of this objective was revised to reflect removal of melphalan test dose.	Section 2.1.3
Melphalan Dosing		
• For all subjects receiving melphalan, changed melphalan dosing from a dose of 140 mg/m^2 of body surface area to model-informed precise dosing for subjects with a predicted melphalan AUC <8 µg.h/mL and added supportive rationale.	• Patients with SCD may have higher than normal renal GFR, which can result in suboptimal exposure to melphalan conditioning. Proposed model informed dosing is based on a published model, as well as melphalan PK, and engraftment data from the first 4 subjects treated with ARU-1801.	Sections 3.1, 3.1.1, 5.5.3, 6.6 and the Schedule of Events (Table 13-1), References, and Appendices 5 and 6
CD34+ Kinetics Visit		
• The CD34+ Kinetics Clearance and Kinetics visits have been removed	• The plerixafor mobilization without apheresis is no longer necessary to understand subject's mobilization pattern. CD34+ counts from peripheral blood will continue to be measured as part of Days 1 and 2 of HSC Collection	Section 3.1.1, 5.5.1, 6.1, 6.3.3, and 6.5.3 and the Schedule of Events (Table 13-1)
Neutrophil and Platelet Recovery		
• Adjusted the definition of neutrophil and platelet recovery	• Modified to align with more broadly accepted definitions.	Section 2.2.1
Feasibility Endpoints		
• Clarified that optional leukopack collection for research purposes is not included in the feasibility endpoint on CD34+ cell collection.	• Administrative clarifications.	Section 2.2.1

Summary of Change	Rationale	Protocol Sections Affected
• Clarified that subjects must have an average VCN of >0.01 copies per cell for the feasibility endpoint on gene-marked cells		
Proportion Anti-sickling Hemoglobin		
• Changed the endpoint on proportion anti-sickling hemoglobin from Month 6-12 to Month 6-24	• Sustained levels of anti-sickling hemoglobin are of clinical relevance	Sections 2.2.2 and 7.2
Opioid Use		
• Removed secondary endpoint of change in disease severity from baseline as measured by opioid use • Added an exploratory endpoint on change in VOEs requiring parenteral opoids • Updated wording “opiate” to “opioid” for consistent word use throughout	• At home opioid use has proven difficult to accurately capture. • Data were captured in different ways in the pre-treatment and post-treatment periods, resulting in the information being captured not being comparable. • VOEs requiring parenteral opioid use can be captured accurately and are of clinical relevance.	Sections 2.2.2, 2.2.3, 6.5.3, 7.2, 7.3, 7.6.2, 7.6.4, and the Schedule of Events (Table 13-1)
Breastfeeding and Pregnancy		
• Clarified that subjects should not become pregnant or breastfeed from the time of consent through 1 year after ARU-1801	• The previous exclusion criteria did not specify restrictions in the pre-treatment period.	Section 4.1.2
Transfusion Withdrawal		
• Revised guidance on transfusion withdrawal to wean transfusions beginning at Month 3 and to conclude transfusion support between Months 4 and 5	• Revised to permit earlier cessation of transfusions.	Section 5.7
Prior Medications and Procedures		
• Clarified that all disease-modifying therapies should be discontinued when the subject initiates HbS reductive transfusions at least 2 months before HSC collection, not just hydroxyurea.	• Clarification was needed to address newly approved medications.	Section 5.10.1
Enrollment Definition and Number of Subjects		
• Provided a clear definition of “Enrolled”.	• Enrollment was not previously defined, and this is needed to	Sections 6.1 and 7

Summary of Change	Rationale	Protocol Sections Affected
	avoid any confusion in providing study results.	
Laboratory Tests		
• Added blood smear to list of laboratory tests.	• This is an added safety test.	Section 6.3.2
Efficacy Assessments		
• Clarified wording on efficacy assessments.	• Revised to more clearly describe the tests being performed.	Section 6.5.2
Bone Marrow Gene Transfer Testing		
• Removed burst forming unit erythroid (pooled) for RNA from bone marrow gene transfer testing.	• This test is no longer needed to evaluate ARU-1801.	Section 6.5.2.3
Vaso-occlusive Episode Definition		
• Removed myocardial infarction, renal infarction, bone infarction, and venous thromboembolism from the definition of vaso-occlusive episode. • Provided definition of splenic sequestration.	• The listed events can be the result of different etiologies than vaso-occlusion due to sickling. • Splenic sequestration was previously not defined and open for interpretation.	Section 6.5.3

Protocol Version 12.0 (16 May 2020)

Summary of Change	Rationale	Protocol Sections Affected
Withdrawal of Subjects From Study Treatment and/or the Study		
• Added the specific chromosomal abnormalities or genetic mutations that would result in subject withdrawal. • Deleted text about the evaluation of somatic mutations.	• To clarify the course of action for any positive findings of chromosomal abnormalities or genetic mutations.	Section 4.2 and Section 6.1
Study Visits		
• Added that the baseline bone marrow collection and testing must be completed after enrollment and prior to initiation of additional research procedures.	• To clarify the timing of the baseline bone marrow collection, to ensure genetic testing is completed before the subjects receive any study intervention.	Section 6.1 and the Schedule of Events (Table 13-1 and Table 13-1 footnote v)

Summary of Change	Rationale	Protocol Sections Affected
• In the Schedule of Events, added an "X" in the column for the HbS Reductive Visit and deleted the "(X)" in the columns for CD34+ Kinetics Clearance Visit and Stem Cell Clearance Visit, for bone marrow morphology, flow cytometry, genomic evaluation for myeloid malignancy, and sample for gene transfer testing • Added footnote v to the "X" in the column for the HbS Reductive Visit. Footnote v states that the baseline bone marrow testing must be completed after enrollment and prior to initiation of additional research procedures such as central line placement or RBC transfusions.		
• Added subject counseling for the baseline bone marrow genomic evaluation.	• To ensure subjects are informed of any positive genetic findings and are offered genetic counseling to understand the implications of these findings.	Section 6.1

Protocol Version 11.0 (10 March 2021)

Summary of Change	Rationale	Protocol Sections Affected
Introduction		
• Provided most up to date information on clinical experience with lentiviral gene therapy • Added section on risk of hematologic malignancy	• Information updated to reflect most up-to-date safety information in trials of lentiviral gene therapy for SCD.	Sections 1.2, 1.3 and 1.4
Withdrawal of Subjects From Study Treatment and/or the Study		
• Added increased risk for hematologic malignancy based upon baseline NGS or cytogenetic findings	• Prevents additional risk to subjects with cytogenetic abnormalities or mutations that are associated with a high risk for development of malignancy.	Section 4.2

Summary of Change	Rationale	Protocol Sections Affected
Study Procedures		
• Revised schedule and timepoints for peripheral blood CD34+ kinetics	• Data for first 2 days of HSC collection is required to understand CD34+ kinetics • CD34+ kinetics from subsequent collection days are not required as they are no longer informative. o The 10-12 and 24 hour timepoints are not needed to understand plerixafor mobilization kinetics.	Section 5.5.1.2
• Removed bone marrow harvest as source for CD34+ cells to manufacture ARU-1801	• Peripheral blood stem cell collection is the safest and most feasible method for collecting CD34+ cells.	Synopsis and Sections 1.3.2, 3, 5.5.1 5.5.2, 5.10.2, and 6.1
• Added radioactive GFR to be done within 30 days of planned chemotherapy	• This test measures true renal filtration, which is impaired in SCD. This will generate data to evaluate the relationship between renal filtration and exposure to melphalan.	Section 5.5.3 and Section 6.3.6.3
• Added NGS and cytogenetic evaluation of bone marrow as safety test	• NGS and cytogenetics will be done on all collected bone marrow samples to more closely evaluate for possible indicators of potential hematologic malignancy.	Sections 6.1, 6.3.2, and 6.4.2.1
Schedule of Events		
• Added genetic evaluation of bone marrow for myeloid malignancy to all timepoints when bone marrow is collected: pre-ARU-1801, Months 6, 12, 18, 24 and Year 3 visits • Revised CD34+ kinetics schedule • Modified pregnancy testing schedule so that repeat testing is not needed if test result is available within 48 hours; reduced testing timepoints in follow-up • Added radioactive GFR to chemotherapy clearance visit with footnote that may be performed within 30 days of chemotherapy		Appendix 1. Tables 13-1, 13-2 and 13-3

Protocol Version 10.0 (21 September 2020)

Summary of Change	Rationale	Protocol Sections Affected
Schedule of Events		
• Additional timepoints were added for the following assessments: F-RBCs and F-retics, Complete Blood Count w/reticulocytes, and Hemoglobin Electrophoresis		Appendix 1. Tables 13-2 and 13-3

Protocol Version 9.0 (11 August 2020)

Summary of Change	Rationale	Protocol Sections Affected
Hematopoietic Stem and Progenitor Cell Collection		
Add an additional optional HSC collection visit and remove the total Hb requirements for the Stem Cell Collection Visit	HSC are required for research purposes to develop ARU-1801 drug product manufacturing process	Section 3.1.1, Section 5.5.1 and Section 5.5.1.2
Exclusion Criteria		
Add wording on male contraception requirements	As melphalan is teratogenic, contraception is required for male subjects for 1-year post- infusion	Section 4.1.2
Pregnancy		
Add wording on collection of pregnancy safety information on partners of male subjects	To ensure safety information is collected from pregnant partners of male subjects	Section 6.7
Administrative		
• Clarify language around Cohort 2 enrollment and dosing • Clarification of types of research material to include manufacturing intermediates and RBCs		Section 3.1 Section 6.8

Protocol Version 8.0 (10 October 2019)

Summary of Change	Rationale	Protocol Sections Affected
Endpoints		
Added primary safety endpoints for the time required for neutrophil and platelet recovery	Added safety endpoints for laboratory parameters already being measured	Synopsis; Sections 2.2.1 and 7

Summary of Change	Rationale	Protocol Sections Affected
Inclusion/Exclusion Criteria		
Revised upper age limit from 35 to 45 years old	Broaden pool of eligible subjects with adequate functional status and organ function	Synopsis; Sections 3.1, 4.1.1, 7, and 7.6
Revised exclusion criterion to exclude subjects with a history of stroke or at moderate to high risk of stroke	Exclude subjects who may be candidates for alternative therapies (eg, hydroxyurea or chronic treatment with blood transfusions) for the primary or secondary prevention of stroke	Synopsis, Section 4.1.2
Study Procedures		
• Added screening electrocardiogram (ECG) • Added screening brain magnetic resonance imaging and angiography • Added new study visit to assess CD34+ kinetics after plerixafor injection without stem cell collection • Modified language related to hematopoietic stem cell (HSC) collection	• Evaluate for baseline ECG abnormalities • Assess risk of stroke • Identify time window for subsequent HSC collection to minimize the number of apheresis procedures • Provide flexibility to alter apheresis parameters to improve HSC collection	Synopsis; Sections 3.1, 3.1.1, 5.5.1, 5.5.1.1 (new), 5.5.1.2, 6.1, 6.2, 6.3.4, and 6.3.6; Table 13-1
• Removed option for ARU-1801 administration via intra-bone marrow infusion	• ARU-1801 will be administered intravenously. Current data do not support improved efficacy with intra-bone marrow infusion.	Synopsis; Sections 3.1, 5.2, 5.5.3, 5.5.5, and 5.10.2
• Adjusted melphalan dose calculation for subjects weighing >130% of ideal body weight (rather than >125%) • Updated pharmacokinetic (PK) endpoint analysis, including PK parameters	• Updated per current prescribing information • Allow for comprehensive characterization of melphalan PK	Synopsis; Sections 5.5.3, 6.6, 7.3, 7.5, and 7.7.3; Table 13-1

Summary of Change	Rationale	Protocol Sections Affected
Adverse Event Monitoring and Reporting		
• Revised adverse event (AE) collection to include all AEs up to 2 years after ARU-1801 infusion and a more limited collection from Years 2 through 15 • Provided additional clarifications on AE and sickle cell disease (SCD)-related event definitions • Added retrospective AE and SCD-related event collection (only for subjects who enrolled before protocol Version 8.0)	• Allow for a more comprehensive collection of safety data • Provide additional investigator guidance for AE monitoring, • Allow capture of all AEs recorded on source documentation	Sections 6.3.1.1, 6.3.1.2, 6.3.1.3, 6.3.1.4, 6.3.1.6, 6.3.1.8, 6.3.1.10, and 6.5.3; Tables 13-2 and 13-3
Other Study Changes		
Allow for outpatient monitoring after ARU-1801 infusion for compliant subjects and per investigator's discretion	Provide flexibility to investigator to individualize subject monitoring plan after chemotherapy	Figure 1; Section 5.5.3; Table 13-1
Clarified study duration and transition to separate long-term follow-up (LTFU) study	Provide information on transition to anticipated LTFU study after Year 2 once study is active and has received site IRB/IEC/REB approval	Synopsis; Sections 3.1 and 6.1; Tables 13-1, 13-2, and 13-3
Revised ARU-1801 description, identification, packaging, and storage	Align with 2019 Investigator's Brochure	Sections 5.2, 5.3, and 5.4.1
Modified language regarding subject withdrawal	Provide guidance to investigator to encourage subject follow-up	Section 4.2.2
Added reproductive health history collection	Data needed to understand effects of treatment on reproductive health	Sections 6.2.1 and 6.3.4; Tables 13-1, 13-2, and 13-3
Removed standard of care imaging assessments	Remove collection of data not used for study analyses	Synopsis; Sections 6.3 and 6.3.6; Tables 13-2 and 13-3
Added future testing of blood samples	Allow collection of additional plasma and serum for future biomedical research purposes	Section 6.8; Tables 13-1 and 13-2
Minor revisions to schedules of events	Align with modifications in study procedures	Tables 13-1, 13-2, and 13-3

Summary of Change	Rationale	Protocol Sections Affected
Administrative		
The following changes were made for administrative purposes: • Definition of Sponsor • Updates to introduction per 2019 investigator's brochure • Principal investigator roles and responsibilities • Study schematic revisions • Listing screening procedures • Central laboratory responsibilities • Rearranging safety versus efficacy gene therapy testing and assessments for feasibility		Title page; Protocol Approval – Sponsor Signatory page; Synopsis Sections 1.3, 3.1, 6.2, 6.3, 6.3.2, 6.3.3 (new), 6.5.1, 6.5.2, 6.5.2.3, 9.1, and 10

Protocol Version 7.0 (01 October 2019)

Summary of Change	Rationale	Protocol Sections Affected
Study Population		
• Age range for subject enrollment changed to 18 to 35 years • Reference to future enrollment of pediatric subjects was removed	Provide clarification to investigators that subject enrollment is limited to adult subjects	Synopsis; Sections 2.2.2, 3.1, 3.1.1, 4.1.1, 4.1.2, 4.2.1, 4.2.2, 5.4.3, 5.5.5, 5.10.1, 6, 6.3.1.3, 6.4.3, 6.5.5, 6.5.6, 7, 7.2, 7.4, 7.6.1, 7.6.2, 7.6.4, 9.1, 9.3, 10.1, and 10.3; Tables 13-1, 13-2, and 13-3; Appendix 2 and 4

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Protocol Synopsis

Protocol Number:	ARU-1801_Ph1_01
Title:	Gene Transfer for Patients With Sickle Cell Disease Using a Gamma Globin Lentivirus Vector: An Open-Label Phase 1/2 Pilot Study
Sponsor:	Aruvant Sciences, GmbH Viaduktstrasse 8, 4051 Basel, Switzerland
Study Phase:	½
Indication:	Sickle cell disease (SCD)
Rationale:	This Phase 1/2 study will use a novel therapy, ARU-1801, for SCD using gene transfer of recombinant γ-globin lentivirus (LV) vector (pseudotyped with a vesicular stomatitis virus G [VSV-G]) with a point mutation at codon 16 ($sGbG^{M}$) into CD34+ cells (from human bone marrow [BM] or mobilized peripheral blood) followed by autologous transplant after reduced intensity conditioning with single-dose melphalan. Current therapies for symptomatic and supportive care for patients with SCD include anti-inflammatories and analgesics to treat pain from episodic sickling, iron chelation to mitigate iron overload from frequent transfusions, infection prevention using penicillin prophylaxis, early immunizations against encapsulated bacteria, and aggressive parenteral antibiotic therapy for presumed bacteremia. Efficacy of these therapies is variable, and indefinite treatment is needed. Hydroxyurea, an approved treatment for SCD, induces fetal hemoglobin (HbF) and reduces SCD complications; however, close medical monitoring and daily lifelong intake and compliance are required, which are the main deterrents to its effectiveness. Another approved treatment, L-glutamine, significantly reduced the number of pain crises and hospitalizations compared with the control group. L-glutamine treatment also requires daily lifelong intake and is limited by cost and availability and therefore has not been widely used. Patients with severe SCD may be offered an allogeneic hematopoietic stem cell (HSC) transplant, which is curative but requires a matched related donor, is often limited to children due to increased toxicities in older SCD patients, and is associated with complications, such as graft-versus-host disease, graft

rejection, and late effects, such as sterility and secondary malignancy from high-dose chemotherapy.

There continues to be an unmet need for SCD therapy that has durable effects, would not rely on donor availability, has fewer complications than are associated with allogeneic, unrelated, or haplo-identical HSC transplant, and would not require intense induction.

This Phase 1/2 gene transfer study is designed to evaluate the safety, feasibility, and efficacy of 1) obtaining sufficient CD34+ cells via plerixafor-mobilized peripheral blood (P-MPB) in subjects with SCD; 2) ex vivo gene transfer of the γ-globin LV vector into the sickle CD34+ cells; 3) reduced intensity conditioning; and 4) engraftment and efficacy of autologous gene-modified CD34+ cells, when transplanted back into subjects with SCD.

Objectives:

Primary Objectives:

The following are the primary objectives:

- To evaluate the safety of:
  - Stem cell collection from subjects with SCD
  - Infusion of CD34+ cells transduced with sGbG^{M} vector into subjects with SCD
  - Chemotherapy conditioning with melphalan in subjects with SCD
- To evaluate the feasibility of:
  - Obtaining clinically sufficient number of CD34+ cells for gene transfer
  - Ex vivo manipulation of CD34+ cells and gene transfer
  - Engraftment of ex vivo manipulated gene-modified stem cells

Secondary Objectives:

The following are the secondary objectives:

- To evaluate the efficacy of genetic correction via determination of the following:
  - Percentage of gene-marked cells
  - Contribution of gene-modified cells to multilineage hematopoiesis and circulating red blood cells (RBCs)
  - Hematological and functional correction of sickling in SCD

- To evaluate subjects for change in disease severity from baseline
- To evaluate subjects for change in quality of life from baseline

Exploratory Objectives:

The following is the exploratory objective:

- To characterize the pharmacokinetics (PK) of melphalan in patients with SCD
- To characterize the relationship between the PK of melphalan and engraftment of ARU-1801

Study Population:

Subjects enrolled in this study must be from 18 to 45 years of age with a confirmed diagnosis of severe SCD (as defined in protocol), have adequate functional status and organ function, and either failed, were unable to tolerate, or chose not to have hydroxyurea therapy. Subjects must be capable of understanding the written informed consent, provide signed and witnessed written informed consent, and agree to comply with protocol requirements.

Subjects will not be allowed to enroll if they have active malignant disease; have a current diagnosis of human immunodeficiency virus; have abnormal pulmonary function tests; have evidence of uncontrolled bacterial, viral, or fungal infections; have a history of stroke or are at moderate to high risk of primary stroke; have αthalassemia (2 or more gene deletions or any αglobin structural variants) SCD; have previous documented liver biopsy result showing cirrhosis, bridging hepatic fibrosis, or active hepatitis; have previous documented evidence of iron overload; have documented matched sibling donors and have not declined HSC transplant; have a medical history of pulmonary hypertension; or have any condition, screening laboratory or diagnostic test, or chronic physical or mental illness that, in the opinion of the investigator, makes participation ill advised. Female subjects cannot be pregnant or lactating/breastfeeding and must be either surgically sterile, postmenopausal (no menses for the previous 12 months), or must agree to practice an effective method of birth control as determined by the investigator from time of consent through at least 1 year post ARU-1801 infusion. Male subjects must agree to practice an effective method of birth control as determined by the investigator for a minimum of 1 year after ARU-1801 infusion.

Study Design:

The study is an open-label Phase 1/2 study in adult subjects aged 18 to 45 years with severe SCD completed in 2 stages. In the first stage, a cohort of subjects will be enrolled in a staggered manner until a total of 6 subjects are enrolled. Specifically, in the first stage, approximately 30 days must elapse after the infusion of 1 subject before the next subject can undergo infusion of ARU-1801. The independent Data Safety Monitoring Board (DSMB) will review the safety data from each subject before treating the next subject, although the DSMB may propose to alter this requirement as detailed in the DSMB charter.

After 6 subjects in the first stage are evaluable, the DSMB will review the safety and feasibility of the procedure in this cohort and make a recommendation regarding dosing 4 additional subjects, for a total enrollment of 10 subjects in this study.

After providing informed consent, potential subjects will undergo screening procedures and be evaluated for inclusion into the study. If all inclusion and no exclusion criteria are met, the subject will be allowed to enroll in the study. The principal investigator at each site will be responsible for all treatment and care the subject receives at the site during the study. Enrolled subjects will have sickle hemoglobin (HbS) reduced via erythrocytapheresis or transfusions with a goal of achieving a HbS level of ≤30% for at least 2 months before HSC collection and ≤20% within 7 days before HSC collection by P-MPB. Chronic transfusions are continued through treatment with ARU-1801 and should begin to be weaned starting at Month 3 after infusion. The overall goal of HSC collection is to collect ≥8×10^6 CD34+ cells/kg body weight. At least 2 weeks before HSC collection, serial sampling of peripheral blood CD34+ concentration will be assessed after plerixafor administration but without apheresis to understand CD34+ kinetics and guide the subsequent timing and duration of apheresis for each individual subject. Collection of HSCs via PMPB may be repeated to obtain sufficient HSCs. For subjects who provide separate consent, additional HSC collection will be performed to obtain additional HSCs for research purposes. The HSCs are enriched for CD34+ cells and then transduced ex vivo with the $sGbG^M$ LV vector. Subjects will undergo chemotherapy conditioning with a single intravenous (IV) dose of melphalan. The melphalan dose will be identified via predictive modeling but will not exceed 200 mg/m^2 of body surface area. All subjects will have

	melphalan PK evaluated after administration of the conditioning dose. At least 36 hours after the melphalan conditioning dose, ARU-1801 (the transduced CD34+ cells) are prepared for autologous IV infusion. The goal is to infuse 4×10^6 CD34+ cells/kg body weight or higher to the subject. Subjects will return to the study site at regular intervals for follow-up for 2 years after the ARU-1801 infusion. It is anticipated that a separate long-term follow-up (LTFU) clinical study will be initiated. Once the LTFU study is approved at the site, all subjects who complete the Year 2 study visit in this study will be asked to consent and enroll into the LTFU study and will be followed for a total of 15 years after the ARU-1801 infusion. Long-term follow-up after Year 2 will continue in this Phase 1/2 study until subjects have transitioned into the LTFU study. The independent DSMB will review safety data on an ongoing basis throughout the study. Predefined safety criteria toxicities, feasibility criteria, and early study stopping criteria will be monitored by the investigator(s) and Sponsor and reviewed on an ongoing basis by the DSMB. Subjects are free to withdraw from the study at any time. At the time of consent withdrawal, the subject will be asked to provide permission for collection of relevant source documentation from clinical records to ascertain the subject's health status. The duration of the study is defined for each subject as the date signed written informed consent is provided through the last follow-up visit.
Estimated Study Duration:	Before subjects receive ARU-1801 infusion on Day 0, subjects will undergo screening and have visits for HbS reduction (in the months before stem cell collection), stem cell clearance, stem cell collection, chemotherapy clearance, and chemotherapy (at least 36 hours before infusion). Subjects will be followed in this study and/or in the LTFU study for a total follow-up period of 15 years after the ARU-1801 infusion.

Safety Assessments: Safety will be assessed in this study through the assessment of adverse events (AEs) and serious AEs (SAEs), chronic conditions related to SCD, laboratory test results (including assessment of neutrophil and platelet recovery), physical examination findings, and vital sign measurements. Pulmonary function tests will be performed at screening. Peripheral blood gene transfer safety testing will include replication competent LV assay and vector insertion site analysis, and BM samples will be tested for standard morphology, flow cytometry for leukemia panel, genomic evaluation, and vector insertion site analysis.

Efficacy Assessments: Peripheral blood testing will include the following:

- Vector copy number analysis (VCN) analysis by quantitative polymerase chain reaction (qPCR) on DNA from white blood cell (WBC) fraction
- VCN analysis (qPCR) on DNA from sorted cell subpopulations of erythroid, myeloid and B and T cells
- HbF content in RBC (F-RBC) and in reticulocytes (F-retics) by flow cytometry
- RBC testing (P50 [partial pressure of oxygen in the blood at which hemoglobin is 50% saturated] and ektacytometry with Oxygenscan)
- Mononuclear cells and plasma for archiving
- Capillary zone electrophoresis (CZE) analysis for HbF and variants

Bone marrow aspirates will be performed at baseline (at some timepoint prior to ARU-1801 infusion), and then at Months 6, 12, 18, 24 and Year 3 and will include the following (if there is clinical or laboratory indication of a clonal dominance or oligoclonality, BM aspirates may also be obtained during LTFU as indicated):

- VCN analysis by qPCR on DNA isolated from BM mononuclear cells and sorted myeloid, T, B and precursor erythroid hematopoietic lineages
- Colony-forming unit cells (CFU-c) assay for vector copies by qPCR on individual CFU-c

Disease severity will be assessed through the incidence of vaso-occlusive episodes (pre-transplant versus post-transplant).

Patient-reported outcomes will be assessed using the adult sickle cell quality of life measurement (ASCQ-Me®). A modified Karnofsky Performance Status Scale, designed for recipients

	aged ≥10 years will used to determine the score (10-100) that best represents the subject's activity status at the requested time point.
Pharmacokinetic Assessments:	Blood samples will be collected for analysis of melphalan PK for all subjects. Blood samples for quantification of melphalan concentration in plasma will be collected at pre-dose (within 60 minutes before the start of the melphalan infusion), immediately at the end of the melphalan infusion, and approximately 5 minutes, 15 minutes, 1 hour, 2 hours, 4 hours, and 6 hours after the end of the infusion.
Study Drug, Dosage, and Route of Administration:	The investigational product, ARU-1801, consists of the CD34+ cells transduced with the γ-globin ($sGbG^M$) LV vector. It may be given as a fresh infusion or cryopreserved for later administration. The product for fresh infusion is formulated in <10 mL/kg volume of Plasmalyte-A® supplemented with 1% human serum albumin and filled into a syringe. The cryopreserved product is formulated in a cryopreservation medium containing 10% dimethyl sulfoxide and filled into a bag. The investigational product, ARU-1801, is administered via IV infusion. Cells will be suspended and intravenously infused over a period no longer than 15 minutes, followed by an IV infusion of 10 mL/kg bolus of saline over approximately 30 minutes. The goal is to infuse the subject with 4×10^6 CD34+ cells/kg body weight or higher.
Sample Size:	A sample size of 10 subjects is planned to obtain initial data on the safety and feasibility of γ-globin gene transfer in subjects with SCD. Early stopping rules are in place for this study. Accrual of subjects will be suspended pending DSMB review if 1 or more of the first 3 subjects do not meet the safety criteria or 3 or more of 6 subjects do not meet either the safety or the feasibility criteria. The study will be stopped if 2 of the first 3 subjects fail to meet the safety criteria or at least 4 subjects (of 6 subjects) fail to meet both the safety and feasibility criteria. According to the study stopping criteria and assuming the probability of being both safe and feasible to be 0.64 (64%), the study will terminate at or before 3 subjects with a probability of 0.104, and at or before 6 subjects with a probability of 0.122.
Statistical Methods:	Statistical analysis will be performed using SAS software (SAS Institute, Inc, Cary, North Carolina) Version 9.3 or later.

Continuous variables (absolute values and change from baseline values) will be summarized using the mean, standard deviation, median, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages. Shift tables from baseline to post-infusion visits may be provided. Data will be listed in data listings.

Given the small sample size and single-arm design of the study, the focus of the statistical analysis will be on the provision of point estimates with 2-sided 95% confidence intervals. In addition, some exploratory informal testing analyses will be performed, using a 2-sided significance level of 0.15 (15%) for informational purposes only.

Primary Safety and Feasibility

Summary statistics of the number of subjects that failed the safety, feasibility, or both safety and feasibility criteria will be provided. For each of the safety and feasibility endpoints, summary statistics will be provided. The incidence of AEs and SAEs will also be provided.

Moreover, the summary of the safety endpoints will be broken down into the following 3 distinct parts:

- Part 1 will include those events from study entry to the recovery from the stem cell collections.
- Part 2 will include those events from immediately before administration (baseline) through 30 days after gene transfer infusion.
- Part 3 will include capturing subject status at Day +30 post infusion, defined as baseline through the remainder of the study.

Secondary Efficacy

The secondary efficacy endpoints related to gene transfer will be measured by the following:

- Quantification of HbF^{G16D} (γ-globin gene with point mutation at codon 16) and other hemoglobin subtypes, including HbF (endogenous), HbS, adult Hb (HbA), HbA2 and, if applicable, HbC and HbE by CZE
- Change in the proportion of anti-sickling/sickling hemoglobin ($HbF+HbF^{G16D}$+HbA2/HbS) in Months 6-24 post-transplantation compared with baseline by CZE
- Flow cytometry for percentage of F-RBC and F-retics in peripheral blood

- Determining vector copies in the WBC fraction by qPCR
- Determining gene transfer in BM based on the following:
  - Vector copies by qPCR
  - CFU-c analysis for gene-marked CFU-c by qPCR

For each of the secondary gene transfer efficacy endpoints, summary statistics will be provided.

Summary statistics will be presented for changes from baseline in disease severity (eg, annualized vaso-occlusive episode pre-transplant versus post-transplant). Baseline disease severity will be determined from a retrospective review of the subject's medical records for the last 2 years before the screening visit. Summary statistics will be presented for ASCQ-Me scores at baseline and by post-baseline time points.

Exploratory PK

Individual plasma concentration of melphalan will be listed for each sampling time point and summarized using descriptive statistics. Plasma PK parameters for melphalan including, but not limited to, area under the plasma concentration versus time curve (AUC) from time zero (pre-dose) to time of last quantifiable concentration (AUC[0-t]), AUC from time zero (pre-dose) extrapolated to infinite time (AUC[0-∞]), maximum observed plasma concentration, clearance, and volume of distribution will be listed individually for each subject and summarized using descriptive statistics. Individual and mean plasma concentration versus time profiles for melphalan will be plotted.

The relationship between melphalan AUC and safety and efficacy endpoints will be explored using descriptive statistics. Correlation coefficients will be generated between PK versus pharmacodynamic parameters.

Version and Date of Protocol: Version 13 (13 Aug 2021)

List of Abbreviations

Abbreviation	Definition
ACS	acute chest syndrome
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ASCQ-Me®	adult sickle cell quality-of-life measurement
AST	aspartate aminotransferase
AUC	area under the plasma concentration versus time curve
AUC(0-t)	area under the plasma concentration versus time curve from time zero (pre-dose) to time of last quantifiable concentration
AUC(0-∞)	area under the plasma concentration versus time curve from time zero (pre-dose) extrapolated to infinite time
BM	bone marrow
BMH	bone marrow harvest
CBC	complete blood count
CFR	Code of Federal Regulations
CFU-c	colony-forming unit cells
C_{max}	maximum observed plasma concentration
CTCAE v5.0	Common Terminology Criteria for Adverse Events Version 5.0
DLCO	diffusing capacity of carbon monoxide
DMSO	dimethyl sulfoxide
DP	drug product
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
FAS	full-analysis set
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
F-RBC	HbF content in RBC
F-retics	HbF content in reticulocytes
FVC	forced vital capacity

Abbreviation	Definition
GCP	Good Clinical Practice
G-CSF	granulocyte-colony stimulating factor
GFR	Glomerular filtration rate
GI	Gastrointestinal
Hb	Hemoglobin
HbA	adult hemoglobin
HbA2	adult hemoglobin 2
HbF	fetal hemoglobin
HbF^{G16D}	γ-globin gene with point mutation at codon 16
HbS	sickle hemoglobin
HIV	human immunodeficiency virus
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplant
IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IRB	institutional review board
IV	Intravenous
LTFU	long-term follow-up
LTR	long terminal repeat
LV	Lentivirus
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
NGS	Next Generation Sequencing
PFT	pulmonary function tests
PK	pharmacokinetic(s)
PKS	pharmacokinetic set
P-MPB	plerixafor-mobilized peripheral blood
PRBC	packed red blood cells
PRO	patient-reported outcome
QoL	quality of life

Abbreviation	Definition
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
RCL	replication competent lentivirus
REB	research ethics board
RIC	reduced intensity conditioning
SAE	serious adverse event
SAP	statistical analysis plan
SCD	sickle cell disease
$sGbG^{M}$	vesicular stomatitis virus G with a point mutation at codon 16
SUSAR	suspected unexpected serious adverse reaction
ULN	upper limit of normal
VCN	vector copy number
VOE	vaso-occlusive episode
VSV-G	vesicular stomatitis virus G
WBC	white blood cell
WHODrug	World Health Organization Drug Dictionary

1 Introduction

1.1 Sickle Cell Disease

Sickle cell disease (SCD) is a common monogenic blood disorder caused by an A→T transversion in the sixth codon of the β-globin gene that affects approximately 100,000 Americans (NHGRI 2016). Because of the abnormality, hemoglobin forms long fibers upon deoxygenation (sickle hemoglobin [HbS]), resulting in red blood cells (RBCs) that are rigid and sickle-shaped rather than flexible and disc-shaped. This is reversible upon reoxygenation, but repeated cycles of sickling cause damage to RBC membranes and results in dense, poorly deformable RBCs that have difficulty traversing the microvasculature and are hemolyzed or removed by the reticuloendothelial system. Chronic hemolytic anemia and shortened RBC lifespan are hallmarks of the disease; the lifespan of a sickle RBC is approximately 14 days (as compared with approximately 120 days in a normal RBC). Patients with SCD have accelerated erythropoiesis and reticulocyte counts elevated to nearly 10 times normal.

The clinical consequences of these RBC abnormalities include chronic anemia, severe pain, and organ damage caused by occlusion of veins. Episodic vascular occlusions can severely diminish quality of life (QoL) and life expectancy (Platt et al 1994), and cumulative organ damage eventually results in organ failure. Health care costs for treating patients with SCD exceed 1.1 billion dollars annually in the United States alone (Kauf et al 2009). In addition, despite the genetic homogeneity of the SCD mutation, there is substantial phenotypic diversity and research is ongoing into how to predict disease severity so that early aggressive therapeutic modalities might be applied to the appropriate population.

1.2 Current Therapies for Sickle Cell Disease

Current therapies for symptomatic and supportive care for patients with SCD include anti-inflammatories and analgesics to treat pain from episodic sickling, iron chelation to mitigate iron overload from frequent transfusions, infection prevention using penicillin prophylaxis, early immunizations against encapsulated bacteria, and aggressive parenteral antibiotic therapy for presumed bacteremia. Efficacy of these therapies is variable and indefinite treatment is needed.

Hydroxyurea, an approved therapy for SCD, induces fetal hemoglobin (HbF), which is more effective than adult hemoglobin in binding and transporting oxygen. The therapy reduces but does not eliminate complications from SCD, requires close monitoring, and is associated

with side effects from marrow suppression (Charache et al 1995; Steinberg et al 2003; Brawley et al 2008). Additionally, SCD patients are reluctant to choose hydroxyurea therapy due to required daily, lifelong compliance and close monitoring (Brawley et al 2008).

In 2018 the US Food and Drug Administration (FDA) approved L-glutamine for the treatment of SCD. Patients receiving L-glutamine showed a significant reduction in the number of pain crises and hospitalizations compared with the control group (Niihara et al 2018). L-glutamine treatment also requires daily lifelong intake and is limited by cost and availability and therefore has not been widely used.

Crizanlizumab was approved by the US FDA in 2019 to reduce the frequency of vaso-occlusive crises in adults and pediatric patients aged 16 years and older with SCD. Crizanlizumab does not treat the underlying cause of SCD but rather the mechanism in which vaso-occlusive crises occur, which is ischemia through vascular occlusion caused by sickled RBC adhesion to endothelial cells mediated through p-selection. Crizanlizumab must be administered IV every 4 weeks, therefore this treatment is not accessible to all patients.

Voxelotor was approved by the US FDA in 2019 in adults and pediatric patients 12 years of age and older with SCD. Voxelotor is an HbS polymerization inhibitor, which works by increasing the oxygen affinity of Hb and stabilizing RBCs in an oxygenated stage, and thus preventing Hb polymerization of HbS. The efficacy of voxelotor was demonstrated in a randomized, double-blind, placebo-controlled, multicenter study that included 2 doses of voxelotor (1500 mg and 900 mg) taken once daily. The study showed statistically significant improvements in total Hb and markers of hemolysis.

Despite voxelotor's clinically significant improvement in Hb and hemolysis, no meaningful decrease in vaso-occlusive crises was observed. Thus, voxelotor treatment response is limited to partial correction of anemia and hemolysis in SCD, and voxelotor does not address the clinical manifestation of vaso-occlusive crises, which is arguably the most clinically important outcome in SCD.

Patients with severe SCD may be offered an allogeneic hematopoietic stem cell (HSC) transplant (HSCT), which is curative but requires a suitable donor. The outcomes of HSCT are heavily dependent upon the degree of human leukocyte antigen (HLA) matching between the donor and the patient. Event-free survival (survival without graft failure) is highest in patients who receive HSCT from a matched sibling donor and is significantly worse in

patients who receive an HSCT from a matched unrelated donor or haploidentical donor (Eapen et al 2019). Myeloablative conditioning regimens are common before HSCT (Brazauskas et al 2020) and carry their own safety risks such as prolonged neutropenia and thrombocytopenia, organ toxicity, and infertility. HSCT carries long-term risks of graft versus host disease (GVHD), which greatly contributes to the morbidity of HSCT. Acute GVHD occurs in 10% to 21% of patients with SCD after allogeneic HSCT, and chronic GVHD occurs in 5% to 20% (Gluckman 2013, Walters 2015, Gluckman et al 2017). Prophylaxis for GVHD can cause additional adverse effects. HSCT has a significant mortality risk. HSCT for SCD is a viable treatment option for the few patients who have a matched sibling donor where outcomes are improved and mortality is as low as 5% (Khoury and Abboud 2011). However, at increasing ages, and with less optimal HLA-matched graft sources, the risks of HSCT begin to increase, with mortality as high as 29% (Brazauskas et al 2020).

There continues to be an unmet need for SCD therapy that has durable effects, is not limited by donor availability, has fewer complications than are associated with allogeneic, unrelated, or haplo-identical HSCT, and does not require intense induction. The experience with hydroxyurea for SCD has shown the beneficial effects of increased HbF. Increased endogenous levels of HbF can ameliorate the clinical course of inherited disorders of β-globin gene expression, such as β-thalassemia and sickle cell anemia (Forget 1998). The experience with HSCT for SCD has shown that eliminating the β^S-globin gene and replacing sickle RBC with those that produce normal β-globin is curative when the graft is stable, Furthermore, it has been shown that a limited number of engrafted normal (or genetically corrected) donor stem cells can repopulate nearly the entire peripheral RBC compartment. Prevention of sickling with the addition of an anti-sickling γ-globin gene to autologous HSCs is possible via gene therapy, which eliminates the need for a matched donor, eliminates the high risk of graft rejection, and allows the use of reduced intensity conditioning (RIC) and thus reducing morbidity related to immunosuppression and also preserving fertility. Unlike hydroxyurea and L-glutamine treatment, gene therapy could be a one-time treatment.

1.3 Study Intervention

The Sponsor proposes ARU-1801 as a novel therapy for SCD using gene transfer of recombinant γ-globin lentivirus (LV) vector (pseudotyped with a vesicular stomatitis virus G [VSV-G]) with a point mutation at codon 16 ($sGbG^M$) into CD34+ cells (from human bone

marrow [BM] or mobilized peripheral blood) followed by autologous transplant after RIC with single-dose melphalan.

The transgene is a recombinant human β-globin gene with human Aγ-globin exons and a point mutation in the γ-globin exon to improve affinity to α-globin. The transgene is driven by the β-globin gene promoter and carries the β-globin 3' enhancer and the hypersensitive sites 2, 3, and 4 of the locus control region of the β-globin locus, an erythroid-specific enhancer. Further details about the gene mutation are provided in the investigator's brochure (IB).

Lentiviral vectors based on human immunodeficiency virus type 1 (HIV-1) are retroviruses that share the long terminal repeat (LTR)-Gag-Pol-Env-LTR genome common to all retroviruses but have additional genes that modulate viral replication and facilitate entry through the intact nuclear membrane of nondividing cells; LV vectors integrate into nondividing HSCs with high efficiency (Naldini et al 1996; Naldini and Verma 2000; Case et al 1999). This allows transduction in as little as 18 to 20 hours while achieving a high level of correction of human hematopoietic stem and progenitor cells. The HIV-based LV vectors confer several advantages over conventional LTR-driven γ-retroviral vectors. While γ-retroviral vectors have shown success in X-linked severe combined immunodeficiency (Gaspar et al 2004; Cavazzana-Calvo et al 2000), chronic granulomatous disease (Ott et al 2006), adenosine deaminase deficiency (Aiuti et al 2009), and Wiscott-Aldrich syndrome (Aiuti et al 2013), they have been associated with severe adverse events (AEs) in X-linked severe combined immunodeficiency and chronic granulomatous disease, including T-cell acute lymphoblastic and T-cell leukemia as well as myelodysplastic syndrome and monosomy 7 (Thrasher et al 2006; Hacein-Bey-Abina et al 2008; Howe et al 2008). The self-inactivating design of LV vectors mitigates such risks and allows for efficient concentration without significant loss in titers, which is important for globin vectors that are typically produced at very low titers. In addition, development of LV vectors has minimized the possibility that replication competent LV could be generated (Recombinant DNA Advisory Committee, 2015). The integration profile of LV vectors is different from γ-retroviral vectors, which improves their safety. In addition, because the LV vector can accommodate the large globin gene and associated regulatory elements, the sGbG^{M} LV vector expresses a high level of HbF in postnatal RBC after gene transfer into HSCs. The safety of vectors derived from the HIV virus has been rigorously demonstrated in preclinical studies (Kaner et al 2016). Furthermore, studies in over 250 patients who have received gene

therapy using LV vectors have been published to date, with no evidence of insertional oncogenesis (Tucci et al 2020). Details of vector development are provided in the IB.

The sGbG^{M} LV vector is pseudotyped with the VSV-G envelope generated for the transduction of HSCs from patients with hemoglobinopathies. The virus reverse transcribes and integrates into the CD34+ cell genome following cell entry. The final product consists of autologous CD34+ cells, from peripheral blood or BM, transduced with the sGbG^{M} LV vector; the final product is referred to as ARU-1801.

Development of ARU-1801 began at Cincinnati Children's Hospital Medical Center, and an investigator-initiated US investigational new drug application was submitted to the FDA in November 2013. This study originally began as Protocol 2010-2588. In February 2019, the Sponsor (Aruvant) submitted documentation to the FDA initiating the transfer of the investigational new drug from Cincinnati Children's Hospital Medical Center to Aruvant. As of April 2019, 4 subjects with severe SCD have been enrolled in the study. Two subjects were withdrawn from the study before administration of the gene-modified cell product (1 withdrew consent during screening and 1 was withdrawn due to inability to comply with study procedure), and 2 subjects had completed treatment under the protocol and at least 15 months of follow-up.

1.3.1 Nonclinical Experience

A number of nonclinical studies have been undertaken in mice to explore the pharmacology and toxicity of the prototype sGbG LV vector. A safety study was also conducted in macaques with this vector. During the nonclinical development, the HbF gene was modified to optimize oxygen carrying capacity and anti-sickling properties. Corroborative studies using an in vitro immortalization assay further confirmed the safety of the test vector, which showed a nearly 200-fold lower genotoxicity of the sGbG vector backbone. A subsequent toxicology study was performed with the sGbG^{M} LV vector in mice. The results of these studies demonstrated the long-term safety of the sGbG and sGbG^{M} LV vectors and did not provide any indication of insertional mutagenesis in vivo or in vitro or evidence of donor-derived tumors.

In vivo nonclinical studies using the prototype sGbG LV vector demonstrated complete correction of the hematological, and functional RBC parameters and inflammation and organ pathology were seen in sickle mice following myeloablative-conditioning and transplant.

Correction was sustained long term in primary and secondary transplant recipients with a 6-month survival rate of 100% in genetically corrected mice compared with a 20% survival rate in mock-transplanted mice.

Comparative studies between sGbG and sGbG^{M} LV vector have been performed in 2 sickle mouse models that demonstrated the superior ability of the sGbG^{M} LV vector to form HbF compared with sGbG, resulting in correction of sickling, a superior reduction in reticulocytosis, and a rise in Hb in both types of sickle mice.

Refer to the ARU-1801 IB for detailed summaries of nonclinical studies.

1.3.2 Clinical Experience

The use of LV vectors in humans has not been associated with adverse effects from gene transfer or from the viral vector in HIV-positive subjects (Humeau et al 2004; Manilla et al 2005; Levine et al 2006; Wang et al 2009), subjects with β-thalassemia (Cavazzana-Calvo et al 2010; Thompson et al 2018), subjects with adrenoleukodystrophy (Cartier et al 2009; Eichler et al 2017), and in other indications. Potential safety concerns with genotoxicity are well documented in the literature.

The proposed LV gene therapeutic approach has been used in at least 50 patients with SCD and over 100 patients with transfusion-dependent beta thalassemia. These trials have shown significant clinical improvement and promising expression of the target globin (Thompson et al 2018; Thompson et al 2020).

This Phase 1/2 gene transfer study is designed to evaluate the safety, feasibility, and efficacy of 1) obtaining sufficient CD34+ cells via plerixafor-mobilized peripheral blood (P-MPB) in subjects with SCD; 2) ex vivo gene transfer of the γ-globin LV vector into the sickle CD34+ cells; 3) reduced intensity of conditioning; and 4) engraftment and efficacy of autologous gene-modified CD34+ cells, when transplanted back into subjects with SCD.

1.4 Benefit-Risk Assessment

The potential benefits of gene therapy for SCD include the possibility of cure with a single treatment using autologous HSC, which obviates the need for a matched related donor, reduces the risk of graft rejection even with reduced-intensity myeloablative conditioning, reduces immunological toxicities, and may preserve fertility.

The potential risks of this therapy include inadvertent generation of replication competent LV (of which there are no reports to date) and insertional mutagenesis. Insertional mutagenesis is less likely with the proposed LV vector than with retrovirus vectors (Montini et al 2006; Arumugam et al 2009; Modlich et al 2009; Montini et al 2009; Milone and O'Doherty 2018 [review]). In addition, the LV vector used for the proposed procedure has the following characteristics to enhance safety: a self-inactivating vector design, a relatively lineage-restricted enhancer that is transcriptionally inactive, and a transgene (γ-globin) that cannot alter cellular proliferation. Nonclinical testing of the vector has not shown evidence of γ-globin vector-derived malignancy in any animal or development of clonal dominance attributable to the γ-globin vector. Furthermore, there are no reported cases of leukemogenesis in gene therapy trials that involve modification of HSCs or nondividing T cells (Milone and O'Doherty 2018 [review]).

Immune responses to the transgene may be minimal with the approach of expressing the naturally occurring HbF via gene transfer into HSC (Perumbeti et al 2009). While the immunological effects of expressing altered β-globin or γ-globin chains in humans cannot be predicted, the long history of chronic transfusions in patients with SCD and thalassemia suggests that globin proteins have low immunogenicity.

Potential procedural risks include infusion-related toxicity, the risks of stem cell harvesting, and the toxicity of chemotherapy conditioning. The potential for infusion-related toxicity is reduced by procedures to minimize contamination and by pre-infusion testing for microbial contamination and endotoxin. Furthermore, initial nonclinical and clinical experience has not shown significant reactions from cultured- and gene-altered autologous blood progenitors. The recommended procedure for stem cell harvesting is P-MPB, which can be performed without anesthesia and is associated with less morbidity than bone marrow harvest (BMH) of the pelvis, which also entails the risks of general anesthesia. Adverse effects of melphalan conditioning are well known and include anorexia, vomiting, hyponatremia, myelosuppression, mucositis, diarrhea, alopecia, and cytopenias. Blood counts will be monitored.

1.4.1 Hematologic Malignancy Risks

Secondary malignancy is a known risk of both allogeneic and autologous HSCT, even for non-malignant hemoglobinopathies such as SCD. The pathogenesis of secondary malignancy is multifactorial, including host susceptibility and damage to residual and persistent HSCs

from alkylating chemotherapy, which is necessary to clear the native stem cell niche and allow engraftment of the therapeutic cells.

Risk of Hematologic Malignancy in SCD

Multiple studies have concluded that patients with SCD have an underlying increased risk of leukemia compared with the general population. A population-based retrospective study of patients from England concluded that patients with SCD have 11 times higher rates of developing acute myelogenous leukemia (AML) than patients hospitalized for other conditions (Seminog et al 2016). Another study performed in California analyzed 27 years of population-based data and found a 3.37 times higher incidence of leukemia in patients with SCD aged 15-39 years and a 2.6 times higher incidence in patients with SCD over 40 years of age. In this study, the median age of first primary cancer was 46 years (n=115 patients with malignancy in the SCD group) (Brunson et al 2017). Similar results were found in a study at University of Illinois at Chicago, which identified 8 cases of hematologic malignancies in 1,327 patients with SCD from 2010 to 2015 by manually verifying cases per ICD-9-CM codes (0.12% per patient-year) versus 12 cases in 5,295 matched control individuals, which is a 2.66 times higher incidence in patients with SCD (Jain et al 2018).

While these studies were limited by retrospective design, all three have concluded that patients with SCD carry an increased burden of risk for the development of hematologic malignancies, likely due to chronic lifelong stress erythropoiesis and inflammation which could lead to development of genetic aberrations within HSCs and premature stem cell aging.

In the general population, the median age at diagnosis of myelodysplastic syndrome (MDS) is >70 years (Sallman and Padron 2017), and only 6% of cases are diagnosed in persons younger than 50 years old (Ma 2012). Incidence rates of MDS/AML in the general population are provided by SEER, which shows rates increase with age (National Cancer Institute). The findings of the UK and California studies of hematologic malignancies in adolescents and young adults with SCD demonstrate a much earlier median age of onset of malignancy than the general population. These data validate the idea that BM in SCD patients sustains more damage, experiencing premature aging, which results in a higher and earlier risk of malignancy than the general population.

Risk of Secondary Malignancy in Autologous Gene Therapy for SCD

As of March 2021, 4 subjects have undergone melphalan conditioning and ARU-1801 infusion, with a median follow-up of 26.5 months (the shortest follow-up is 6 months). There have been no reported cases of hematologic malignancy in the ARU-1801_Ph1_01 trial.

The most extensive experience of autologous gene therapy for SCD is with BB1111, a LV gene therapy approach being developed by Bluebird Bio. BB1111 has been administered to 47 patients with SCD since approximately 2015. To date, a total of 3 patients with SCD who have received BB1111 have developed leukemia or MDS. The first case was a patient who developed MDS 36 months after BB1111 infusion, which later evolved into AML. After extensive evaluation, the investigators were able to exclude insertional oncogenesis as the cause and attributed the malignancy to the combination of underlying patient predisposition and exposure to myeloablative chemotherapy (Hseih et al 2020). Bluebird Bio has recently reported 2 additional cases of hematologic malignancy within the SCD cohort: one case of AML 5.5 years after drug product (DP) infusion and one case of suspected MDS 6 months after DP infusion. Bluebird Bio has performed an extensive evaluation in the case of AML and was able to conclude that the vector was not integrated into a gene that affects cellular proliferation and had not impacted expression of the surrounding genes. The case of suspected MDS was reported based on clinical evidence of prolonged anemia after conditioning and presence of trisomy 8 in a small fraction of cells in the BM. It is unclear if the trisomy 8 existed before therapy. Of note, this patient has only 6 months of follow-up. In all the known cases of leukemia after gene therapy with more oncogenic gamma retroviral vectors, onset of malignancy occurred from 16 to 60 months (Hacein-Bey-Abina et al 2003; Hacein-Bey-Abina et al 2008; Howe et al 2008; Braun et al 2014; Stein et al 2010; Siler et al 2015; Uchiyama et al 2019; Gaspar 2011). Furthermore, a case report of trisomy 8-related pancytopenia and MDS after gene therapy for ADA-SCID showed that trisomy 8 was pre-existing, having predated gene therapy infusion based on cryopreserved BM and fluorescence in situ hybridization analysis (Engel et al 2007). Another study of patients with ADA-SCID later demonstrated dysplasia in patients' marrow before chemotherapy and peripheral blood abnormalities in 7 patients who had undergone neither chemotherapy nor gene therapy, indicating that the risk for MDS is often a primary problem of the underlying disease (ADA-SCID), unrelated to chemotherapy or gene therapy (Sokolic et al 2011). Given that the suspected MDS case occurred within 6 months of treatment and was preceded by prolonged anemia after busulfan, there is a good possibility that this patient had pre-existing risk factors

for developing MDS at baseline, which highlights the need for more thorough screening for MDS risk factors before treatment.

There have been no reported cases of hematologic malignancy in over 100 patients treated with transfusion-dependent beta thalassemia (Hseih et al 2020). Notably, all trials of BB1111 utilize myeloablative doses of busulfan administered IV over 4 days as the stem cell conditioning agent before DP infusion. While this is not dissimilar from conditioning regimens used for allogeneic HSCT in SCD patients, the relative rate of malignancy in Bluebird's trials is higher than expected (3 of 47 patients or 6.4% compared with approximately 1% in allogeneic HSCT) (Eapen et al 2019; Kahn et al 2020).

Risk of Secondary Malignancy in Allogeneic HSCT for SCD

The overall incidence of secondary malignancy after allogeneic HSCT for SCD is low across a variety of different conditioning regimens. In one large study that included 294 patients with SCD, 3 patients developed malignancy after HSCT, representing an incidence of 1% (Kahn et al 2020). In another large study that included 910 patients with SCD, 6 developed malignancy after HSCT, for an incidence of 0.7% (Eapen et al 2019). This evidence establishes that the only currently available curative therapy for SCD has a 1% risk of secondary malignancy.

A RIC regimen has been studied in allogeneic HSCT for SCD with good results. In a study of 43 patients treated, no cases of hematologic malignancy have been reported after a median follow-up of 3.4 years (King et al 2015). These findings suggest that the risk of secondary malignancy might be lower in the setting of RIC with a melphalan-based regimen.

Given the potential for curative therapy, the favorable preliminary experience with gene therapeutic approaches to hematologic diseases, and the known, monitorable, and manageable potential risks of the therapeutic procedure, the benefit-risk assessment supports the conduct of this study.

2 Study Objectives and Endpoints

2.1 Objectives

2.1.1 Primary Objectives

The following are the primary objectives:

- To evaluate the safety of:
  - Stem cell collection from subjects with SCD
  - Infusion of CD34+ cells transduced with sGbG^{M} vector into subjects with SCD
  - Chemotherapy conditioning with melphalan in subjects with SCD
- To evaluate the feasibility of:
  - Obtaining clinically sufficient number of CD34+ cells for gene transfer
  - Ex vivo manipulation of CD34+ cells and gene transfer
  - Engraftment of ex vivo manipulated gene-modified stem cells

2.1.2 Secondary Objectives

The following are the secondary objectives:

- To evaluate the efficacy of genetic correction via determination of the following:
  - Percentage of gene-marked cells
  - Contribution of gene-modified cells to multilineage hematopoiesis and circulating RBCs
  - Hematological and functional correction of sickling in SCD
- To evaluate subjects for change in disease severity from baseline
- To evaluate subjects for change in QoL from baseline

2.1.3 Exploratory Objectives

The following is the exploratory objective:

- To characterize the pharmacokinetics (PK) of melphalan in patients with SCD

- To characterize the relationship between the PK of melphalan and engraftment of ARU-1801.

2.2 Endpoints

2.2.1 Primary Endpoints

The following are the primary safety endpoints:

- The incidence of:
  - Grade 3 allergic reaction associated with infusion of the transduced cell product
  - Grade 4 infection following infusion of the transduced cell product uncontrolled for >14 days
  - Grade 4 neutropenia lasting >1 month following melphalan
  - Grade 3 or 4 irreversible organ toxicity–neurologic, pulmonary, cardiac, gastrointestinal (GI), genitourinary, hepatic, or cutaneous–that is attributable to the study procedures
  - AEs and serious adverse events (SAEs) graded per the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (v5.0)
  - Death due to study procedures
  - Hematological malignancy due to vector insertion
  - Hematological cancer related to the investigational product or study medications/procedures
- Duration of neutropenia following conditioning (defined as the time from first measurement where absolute neutrophil count (ANC) < 500 cells/µL to first of 3 consecutive absolute neutrophil count measurements on 3 consecutive days with ANC ≥500 cells/µL)
- Duration of thrombocytopenia following conditioning (defined as the time from first measurement where platelet count < 50,000 cells/µL to the first of 3 consecutive platelet counts on 3 consecutive days ≥50,000 cells/µL and independent of platelet transfusion for ≥7 days consecutive days)

The following are the primary feasibility endpoints:

- Number of subjects with a total number of CD34+ cells recovered from all collections combined (mobilized peripheral blood and BM) of at least 8×10^6/kg viable CD34+ cells (excluding the optional leukopack collection for research purposes)
- Proportion of subjects for which a minimum of 4×10^6 CD34+ cells/kg body weight from all collections combined have been successfully transduced
- Number of subjects with BM aspirates at 1-year post-infusion with ≥1% gene-marked cells (with an average vector copy number [VCN] of > 0.01 copies per cell).

2.2.2 Secondary Endpoints

The following are the secondary efficacy endpoints:

- Quantification of HbF^{G16D} (γ-globin gene with point mutation at codon 16) and other Hb subtypes, including HbF (endogenous), HbS, adult Hb (HbA), HbA2 and, if applicable, HbC and HbE by capillary zone electrophoresis (CZE)
- Change in the proportion of antisickling/sickling hemoglobin ($HbF+HbF^{G16D}$+HbA2/HbS) in Months 6-24 post-transplantation compared with baseline by CZE
- Flow cytometry for percentage of HbF-containing RBC (F-RBC) and HbF content in reticulocytes (F-retics) in peripheral blood
- Determining vector copies in the white blood cell (WBC) fraction
- Determining gene transfer in BM based on the following:
  - Vector copies
  - Colony-forming unit cells (CFU-c) analysis for gene-marked CFU-c
- Change in disease severity from baseline as measured by:
  - Annualized vaso-occlusive episodes (VOEs) pre-transplant versus post-transplant
- Change in QoL from baseline as measured by the adult sickle cell quality-of-life measurement (ASCQ-Me®)

2.2.3 Exploratory Endpoints

The following are the exploratory endpoints:

- Plasma PK parameters of melphalan including, but not limited to, area under the plasma concentration versus time curve (AUC), maximum observed plasma concentration (C_{max}), clearance, and volume of distribution
- Change in VOEs requiring parenteral opioids

3 Investigational Plan

3.1 Study Design

The study is an open-label Phase 1/2 study in adult subjects aged 18 to 45 years with severe SCD completed in 2 stages. In the first stage, a cohort of subjects will be enrolled in a staggered manner until a total of 6 subjects are enrolled. Specifically, approximately 30 days must elapse after the infusion of 1 subject before the next subject can undergo infusion of ARU-1801. The independent Data Safety Monitoring Board (DSMB) will review the safety data from each subject before treating the next subject, although the DSMB may propose to alter this requirement as detailed in the DSMB charter.

After 6 subjects in the first stage are evaluable, the DSMB will review the safety and feasibility of the procedure in this cohort and make a recommendation regarding treating 4 additional subjects, for a total enrollment of 10 evaluable subjects in this study.

After providing informed consent, potential subjects will undergo screening procedures and be evaluated for inclusion into the study. If all inclusion and no exclusion criteria are met, the subject will be allowed to enroll in the study. The principal investigator at each site will be responsible for all treatment and care the subject receives at the site during the study (Section 10). Enrolled subjects will have HbS reduced via erythrocytapheresis or transfusions with a goal of achieving a HbS ≤30% for at least 2 months before HSC collection and ≤20% within 7 days before HSC collection by P-MPB. Minor deviations of these HbS target goals for a given subject may be made at the investigator's discretion based upon consideration of safety and feasibility. The minimum 2-month requirement for HbS reduction may incorporate time required to receive erythrocytapheresis or transfusions just before screening as long as a HbS ≤30% over time is documented in the subject's medical records. Subjects may be treated with erythrocytapheresis or transfusions to maintain a HbS ≤30% for longer periods of time (eg, 6 months) before HSC collection at the investigator's discretion. The overall goal of HSC collection is to collect $\geq 8\times10^6$ CD34+ cells/kg body weight. Collection of HSCs via P-MPB may be repeated to obtain sufficient HSCs. For subjects who provide separate consent, additional HSC collection will be performed to obtain HSCs for research purposes. These will be primarily used for ARU-1801 DP manufacturing process improvements.

The HSCs are enriched for CD34+ cells and then transduced ex vivo with the sGbG^M LV vector. Subjects will undergo chemotherapy conditioning with RIC melphalan. Melphalan

dosing details are provided in Section 5.5.3.At least 36 hours after melphalan conditioning, ARU-1801 (the transduced CD34+ cells) are prepared for autologous IV infusion.

ARU-1801 (the gene-modified CD34+ cells) are suspended in <10 mL/kg volume of Plasmalyte-A® supplemented with 1% human serum albumin and given to the subject as a single IV infusion and followed by a normal saline bolus (Section 5.2). The goal is to infuse 4×10^6 CD34+ cells/kg body weight or higher to the subject.

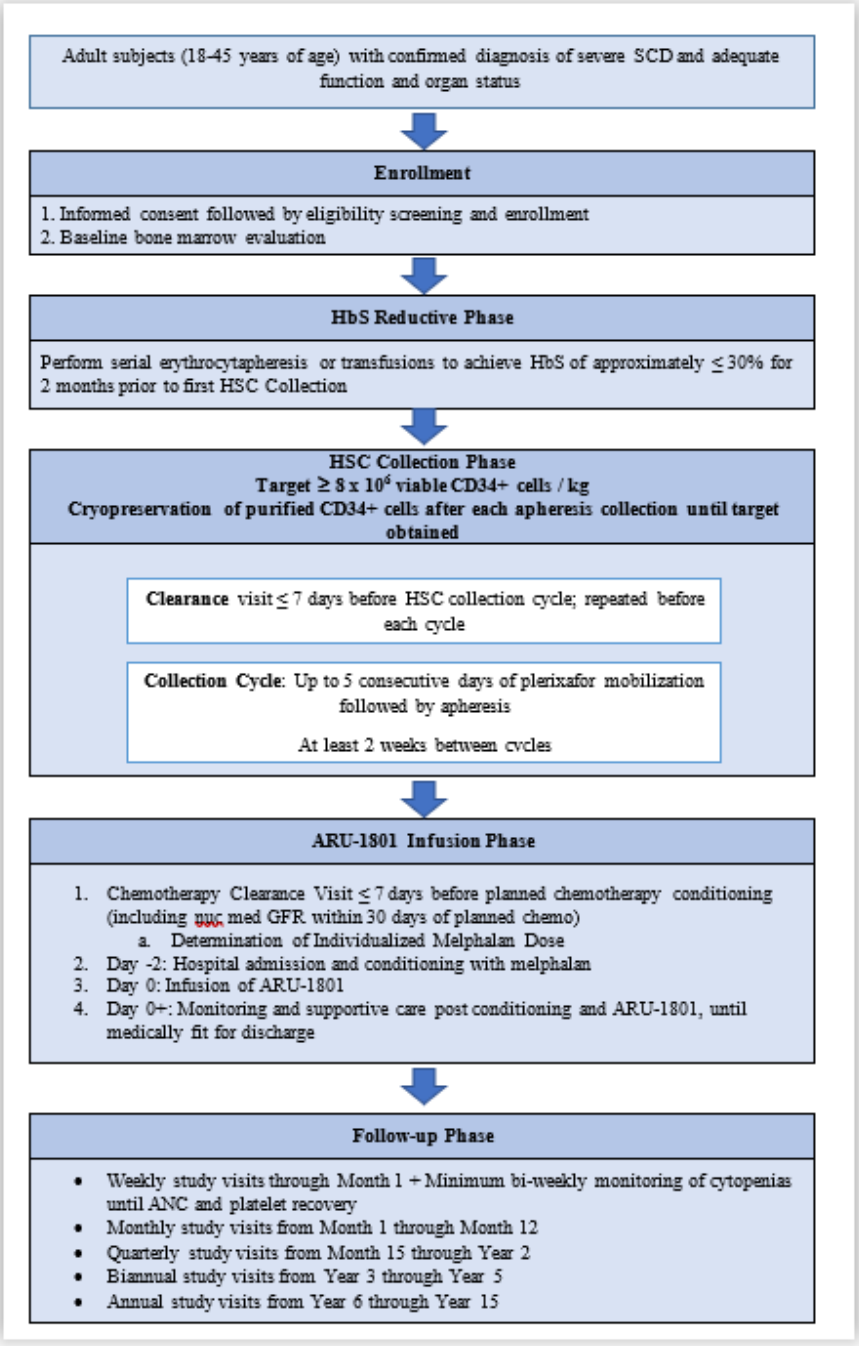
Subjects will return to the study site at regular intervals for follow-up for 2 years after the ARU-1801 infusion. It is anticipated that a separate long-term follow-up (LTFU) clinical study will be initiated. Once the LTFU study is approved at the site, all subjects who complete the Year 2 study visit in this study will be asked to consent and enroll into the LTFU study and will be followed for a total of 15 years after the ARU-1801 infusion. Long-term follow-up after Year 2 will continue in this Phase 1/2 study until subjects have transitioned into the LTFU study.

Patient reported outcomes (PROs) will be assessed using the ASCQ-Me. The independent DSMB will monitor safety data throughout the study (Section 6.4.1). Predefined safety criteria toxicities (Section 6.4.2.1), feasibility criteria (Section 6.4.2.2), and early study stopping criteria (Section 6.4.3) will be monitored by the investigator(s) and Sponsor and reviewed on an ongoing basis by the DSMB. Subjects are free to withdraw from the study at any time (Section 4.2). At the time of consent withdrawal, the subject will be asked to provide permission for collection of relevant source documentation from clinical records to ascertain the subject's health status (Section 4.2.2).

The duration of the study is defined for each subject as the date signed written informed consent is provided through the last follow-up visit.

The study design schema is presented in Figure 1.

Figure 1 Study Design Schema



Abbreviations: HbS, sickle hemoglobin; HSC, hematopoietic stem cell; PK, pharmacokinetic; P-MPB, plerixafor-mobilized peripheral blood; SCD, sickle cell disease.

a. At investigator's discretion and based on the subject's compliance, subjects may complete clinical monitoring assessments as an outpatient (Section 5.5.3).

3.1.1 Rationale of Study Design

The scientific grounds for important aspects of the study design are described as follows:

- **Subject Population**: Although gene therapy is predicted to have greatest efficacy when given to younger patients (before the onset of organ damage), safety and feasibility will be assessed in adult subjects in this study.
- **HSC Collection**: The planned method for HSC collection is plerixafor followed by apheresis. Plerixafor is approved for use in the stimulation of stem cell release from the BM in preparation for autologous transplant in subjects with lymphoma and multiple myeloma. Plerixafor has been used successfully in gene therapy protocols for thalassemia and SCD (bluebirdbio Protocols HGB-205 and HGB-206). Plerixafor has been administered to patients with SCD in dose-escalation studies, without gene therapy, and to assess the kinetics of CD34+ mobilization into peripheral blood in settings when apheresis is not subsequently performed (Boulad et al 2018; Esrick et al 2018; King AA, Kamani N, Bunin N, et al. Successful matched sibling donor marrow transplantation following reduced intensity conditioning in children with hemoglobinopathies. Am J Hematol. 2015;90(12):1093-8.
- Lagresle-Peyrou et al 2018). The use of plerixafor with apheresis is preferred because it may supplant multiple BMH procedures and avoid the accompanying pain, risks, and morbidity associated with multiple surgical procedures under anesthesia.
- There is limited information on the kinetics of CD34+ mobilization and the optimal time window for CD34+ apheresis collection among individual subjects with SCD (Boulad et al 2018; Esrick et al 2018; King AA, Kamani N, Bunin N, et al. Successful matched sibling donor marrow transplantation following reduced intensity conditioning in children with hemoglobinopathies. Am J Hematol. 2015;90(12):1093-8.

- Lagresle-Peyrou et al 2018). Based upon published data on CD34+ kinetics after plerixafor administration in a small number of subjects with SCD, the peak concentration of peripheral blood CD34+ cells appears to occur within the first 6 hours after plerixafor administration and with considerable variability in peak CD34 concentration and area under the time-concentration curve (Boulad et al 2018; Esrick et al 2018; King AA, Kamani N, Bunin N, et al. Successful matched sibling donor marrow transplantation following reduced intensity conditioning in children with hemoglobinopathies. Am J Hematol. 2015;90(12):1093-8.

  Lagresle-Peyrou et al 2018). Therefore, in the current study, serial sampling of peripheral blood CD34+ concentration will be assessed after plerixafor administration and the individualized mobilization pattern may be used to guide the subsequent timing and duration of apheresis for each individual subject.

- **Conditioning Regimen**: The benefit-risk profile of melphalan for pretransplant conditioning has been well characterized after clinical experience with transplantation and with gene therapy for adenosine deaminase deficiency (Gaspar et al 2006) and for SCD (Shenoy 2007). Based on previous clinical experience with melphalan, this study will include RIC with a single dose of melphalan. An advantage of RIC is that autologous recovery occurs within 3 to 4 weeks of the conditioning regimen, which means that it is not necessary to collect and store BM as a backup for potential reinfusion. In the present study, this means that after adequate CD34+ cells have been collected for gene therapy, no further HSC collection is needed. This RIC has not been associated with ovarian failure, delayed puberty, or other endocrine dysfunction (Blood and Marrow Transplant Clinical Trials Network Protocol 0601).

  Patients with SCD typically have altered kidney function, including renal hyperfiltration. The median glomerular filtration rate (GFR) in patients with SCD is 137-154 mL/min with ranges reported of 132-176 mL/min (95% CI). (Marouf et al 2006; Niss et al 2020). Because of the renal contribution to melphalan clearance, renal hyperfiltration can result in reduced melphalan exposure in some patients with SCD. Successful engraftment of ARU-1801 is key to ensure sustained production of HbF^{G16D} and continued clinical benefits. Reduced melphalan AUC and subsequent lower ARU-1801 engraftment were observed in one of the first 4 subjects treated with ARU-1801. Before chemotherapy, this subject had renal hyperfiltration according to

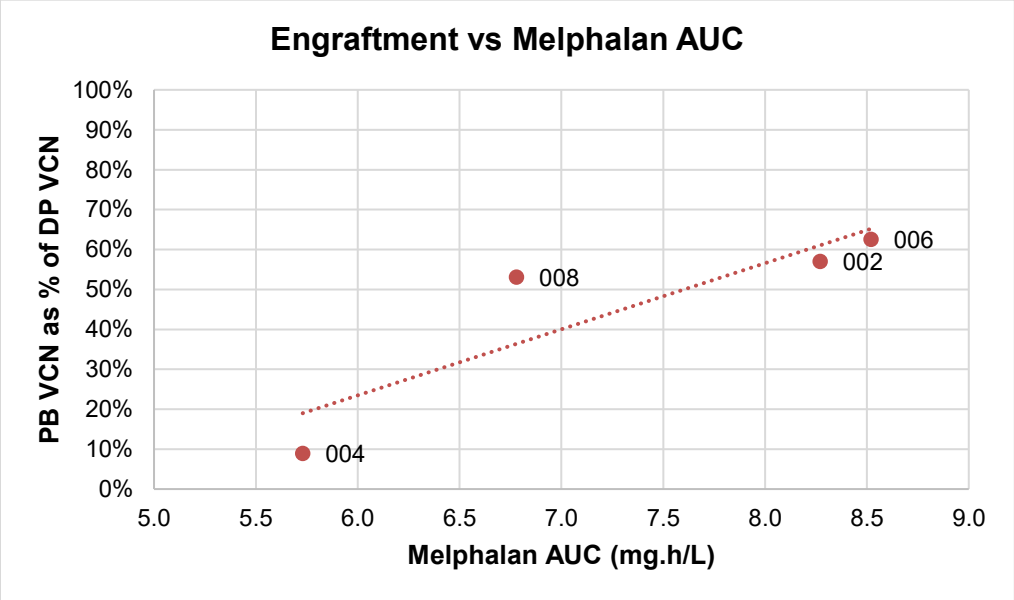
cystatin C estimated glomerular filtration rate (eGFR) (Subject 004, Table 3-1, Figure 2).

Table 3-1 Cystatin C eGFR and Melphalan AUC in Subjects Treated with ARU-1801

	Subject			
Parameter	**002**	**004**	**006**	**008**
Cystatin C eGFR (mL/min/1.73m^2)	78	200	108	126
Melphalan AUC (µg.h/mL)	8.27	5.73	8.52	6.78

Abbreviations: AUC, area under the plasma concentration versus time curve; eGFR, estimated glomerular filtration rate.

Figure 2 Engraftment versus AUC

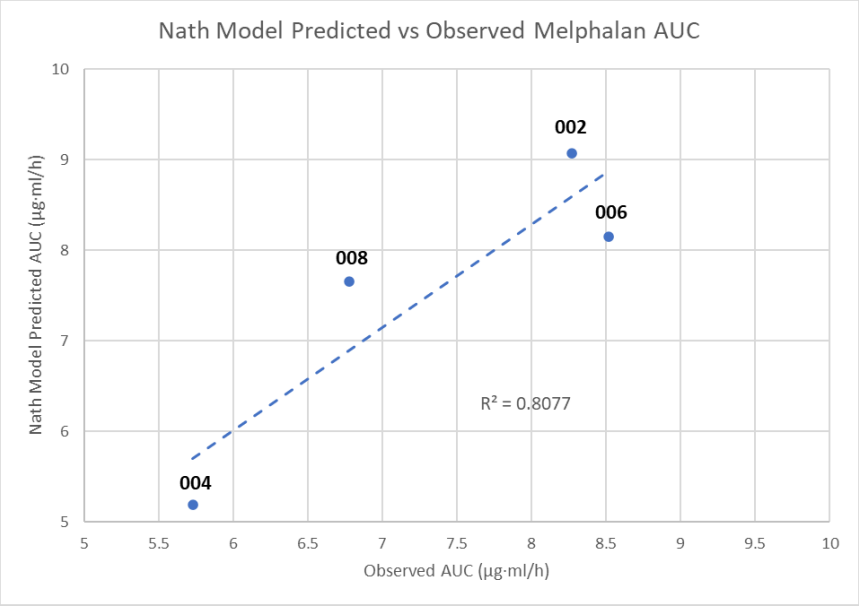


Abbreviations: AUC, area under the plasma concentration versus time curve; DP, drug product; PB, peripheral blood; VCN, vector copy number.

To lower the probability for reduced engraftment of ARU-1801 in subsequent subjects enrolled in this study, the exact dose of melphalan given to a subject will be based on predictive modeling (Section 5.5.3).

- Based on a study of 100 patients treated with melphalan prior to stem cell transplant for multiple myeloma, Nath et al in 2010 developed a predictive model for melphalan AUC based on the 3 covariates GFR, lean body mass, and hematocrit. Using the Nath model, simulations comparing patients with multiple myeloma and patients with SCD predict a shift towards lower exposures for patients with SCD, with an AUC <7 µg.h/mL for approximately 1 in 4 patients with SCD (vs 1 in 10 patients with multiple myeloma) (InsightRx).
- Individual PK results from the first 4 subjects treated with ARU-1801 were compared with modeled PK using each subject's individual covariates. The modeled exposure was calculated using a modified version of that published by Nath et al in 2010 (substituting estimated eGFR using Cystatin C for creatinine clearance) and highly correlated to the measured exposure via PK in all 4 subjects (Figure 3). Therefore, the modified Nath model will be used to predict melphalan AUC in all subjects.

Figure 3 Observed and Predicted AUC



Abbreviation: AUC, area under the plasma concentration versus time curve.

- The target range for melphalan AUC will be 7.0 – 13.0 µg.h/mL.

- The lower bound of the AUC target range of 7 µg.h/mL was selected based on analysis of the relationship between melphalan AUC and ARU-1801 engraftment (as measured by ratio of most recent peripheral blood VCN/ Drug Product VCN) in the first 4 subjects with SCD treated with ARU-1801. This analysis demonstrated that the 3 subjects with a melphalan AUC > 6.7 µg.h/mL had peripheral blood VCN / Drug Product VCN ratios >50%. The one subject with an AUC < 6.7 µg.h/mL had a peripheral blood/DP VCN of only 10%, evidence of poor engraftment (Figure 2).
- The upper bound of the AUC target range of 13.0 µg.h/mL was selected based on a simulation of predicted AUC in 10,000 patients with multiple myeloma given a conventional BSA-based dose of melphalan 140 mg/m^2, using the Nath model and model covariates based on NHANES 2017-18 and Nath et al 2010. In this simulation, the 25th percentile AUC was 8.6 µg.h/mL, and the 75th percentile AUC was 13.0 µg.h/mL (InsightRx). The results of this simulation are similar to the actual values found in patients with multiple myeloma who received a median dose of melphalan 192 mg/m^2 and had an AUC range of 10.8 - 15.1 µg.h/mL (25th, 75th percentiles). The goal of selecting an upper bound of a target AUC range that is at the 75th percentile of the expected AUC for a reduced intensity melphalan dose of 140 mg/m^2 is to maintain the expected adverse event profile of a RIC dose of melphalan. Melphalan exposures with median AUCs of 13.4 and 14.1 µg.h/mL have been associated with either no or low-grade AEs (Nath et al 2010; Cho et al 2017).
- Subjects with a model-predicted melphalan AUC ≥ 8.0 µg.h/mL for a 140 mg/m^2 melphalan dose will receive a conventional body-surface area-based dose of melphalan 140 mg/m^2.
- Subjects with a model-predicted melphalan AUC < 8.0 µg.h/mL for a 140 mg/m^2 melphalan dose will receive a model-informed dose of melphalan to target achieving a melphalan AUC of 10.4 µg.h/mL. The model-informed dose to achieve a target AUC of 10.4 µg.h/mL will be limited to a maximum of 200 mg/m^2, which was chosen to minimize toxicity.
- This dosing strategy balanced the percentage of subjects below and above the target melphalan AUC (7.0– 13.0 µg.h/mL), with simulations showing 11% above target and 17% below target (Insight Rx). Previous studies have shown

that the toxicity of melphalan is related to the AUC achieved (Shaw et al 2014 Table 2). In one study, Grade 3-4 mucositis was associated with a median melphalan AUC of 16.9 ug.h/mL, whereas Grade 0-2 mucositis was associated with a median AUC of 13.4 ug.h/mL (Nath et al 2010 Table 9). Similarly, in a different study, Grade 2-3 mucositis was associated with an AUC of 16.1 ug.h/mL, whereas Grade 0-1 mucositis was associated with an AUC of 14.1 ug.h/mL (Cho et al 2017). Accordingly, the upper AUC limit represents a low risk of melphalan toxicity, and the selective dose increase is not expected to significantly increase subject exposure to this low risk.

  - The higher doses administered to these subjects (many of whom will have renal hyperfiltration) will achieve an AUC that is similar to an expected AUC with a 140 mg/m^2 melphalan dose in patients with normal renal function. Thus, in these subjects, the toxicity profile of higher doses of melphalan is expected to mirror a RIC 140 mg/m^2 dose.

- **Premedication**: Subjects will receive premedication with acetaminophen, diphenhydramine, and hydrocortisone before infusion of ARU-1801 to prevent or mitigate systemic reactions to infusions (see Section 5.4.3).
- **Long-term Toxicity Monitoring Related to Gene Transfer:** Peripheral blood samples from enrolled subjects will be analyzed regularly to monitor for abnormal morphology, replication competent LV, and for clonality of insertion sites as evidence of a monoclonal population indicating malignant transformation. This is consistent with regulatory guidelines for viral vector-based human gene therapy products.

4 Subject Selection and Withdrawal Criteria

4.1 Selection of Study Population

Adult subjects with severe SCD will be enrolled until a total of 10 subjects have received ARU-1801. Subjects will receive study treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

Deviations from the inclusion and exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

4.1.1 Inclusion Criteria

Each subject must meet all of the following criteria to be enrolled in this study:

1. Is 18 to 45 years old.
2. Is capable of understanding the written informed consent, provides signed and witnessed written informed consent, and agrees to comply with protocol requirements (Section 9.3).
3. Has confirmed diagnosis of SCD (genotype HbS-β^0-thalassemia or HbS-β+ thalassemia disease or HbSS, confirmed by β-globin gene analysis and a hemoglobin separation method).
4. Has severe SCD, defined as 1 or more of the following:
    a. Minimum of 2 episodes of clinically diagnosed acute chest syndrome (ACS) requiring hospital admission, or 1 life-threatening episode of ACS requiring intensive care unit admission for exchange transfusion and/or intubation, or frequent ACS episodes, which necessitate treatment with chronic transfusion therapy.
    b. Frequent painful VOEs that significantly interfere with normal life activities, defined as a history of 2 or more severe acute sickle pain events per year requiring additional treatment at a medical facility outside of home pain management over the preceding 2-year period prior to study enrollment, or that necessitate chronic transfusion therapy.

c. Subjects on chronic transfusion therapy for severe disease symptoms other than those previously listed, and which interfere with normal life activities.

5. Has failed hydroxyurea therapy, was unable to tolerate hydroxyurea therapy, or has actively made the choice to not take the recommended daily hydroxyurea advised for severe disease (note: must be off hydroxyurea therapy for 2 months prior to stem cell collection). If refusing hydroxyurea, the subject must document that he/she has been educated about the benefits and continues to refuse the treatment. Subjects who were placed on chronic transfusion therapy instead of hydroxyurea for severe disease are eligible. Subjects who are unable to take hydroxyurea due to financial or safety monitoring constraints are eligible.
6. Has adequate functional status and organ function as defined by the following:
   a. Karnofsky performance score ≥60.
   b. Ability to undergo general or regional (spinal or epidural) anesthesia as per standard institutional practice.
   c. Adequate renal function: defined as a cystatin C estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m^2.
   d. Adequate liver function as defined by a serum conjugated (direct) bilirubin <2.5× the upper limit of normal (ULN) for age; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <5×ULN for age as per local laboratory.
   e. Adequate cardiac function, defined as left ventricular ejection fraction >40%.

4.1.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. All participants of reproductive potential must agree to use an effective method of birth control as determined by the investigator for a period of no less than 1 year after the ARU-1801 infusion.
   a. Female subjects cannot be pregnant or lactating/breastfeeding and must be either surgically sterile, postmenopausal (no menses for the previous 12 months),or must be practicing an effective method of birth control as determined by the investigator (eg, oral contraceptives, double barrier methods, hormonal injectable or implanted contraceptives, tubal ligation, or partner with vasectomy). Women

must agree not to breastfeed from consent through at least 1 year after the ARU-1801 infusion as stipulated in the informed consent document.

b. Male subjects agree to use an effective method of birth control as determined by the investigator for a minimum of 1 year after ARU-1801 infusion.

2. Has active malignant disease or is receiving treatment for any type of cancer (except squamous cell carcinoma, basal cell carcinoma, or carcinoma in situ of the skin).
3. Has a current diagnosis or history of hepatitis B (positive for surface hepatitis B antigen), hepatitis C, or HIV.
4. Has had treatment with any other investigational drug within 30 days or within 5 half lives of the last dose (whichever is longer) prior to screening.
5. Has abnormal pulmonary function tests (PFT) as defined below. Most adults with SCD will have compromised PFTs; therefore, subjects who have mild or moderate obstruction or restriction or diffusion defects will be allowed to participate in the study. However, subject with severe obstruction, restriction, or diffusion defects will be excluded, as follows:

   a. Moderate obstruction is defined per American Thoracic Society criteria as a forced expiratory volume in 1 second (FEV1) down to 60% of predicted, associated with a FEV1/forced vital capacity (FVC) ratio below the 95% confidence intervals. Moderate restriction is defined as a total lung capacity down to 60% of predicted. Moderate diffusion defect is defined as a diffusing capacity of carbon monoxide (DLCO)-corrected for hemoglobin down to 40% of predicted.

   b. Subjects will be ineligible for this study if their lung function falls below any of the following 3 criteria: i) FEV1<60% predicted with a FEV1/FVC ratio <95% confidence interval; ii) total lung capacity <60% of predicted; and iii) DLCO-corrected for hemoglobin <40%.

6. Has evidence of uncontrolled bacterial, viral, or fungal infections (currently taking medication and progression of clinical symptoms) within 1 month prior to starting the conditioning regimen. Subjects with fever should await resolution of symptoms before starting the conditioning regimen.

7. Has a history of stroke or is at moderate to high risk of primary stroke (eg, receiving chronic transfusions or hydroxyurea for primary prevention of stroke; has severe cerebral vasculopathy defined as moderate stenosis in >2 arterial segments; and/or has severe stenosis/occlusion in ≤2 segments in the polygon of Willis or presence of Moyamoya-like disease).
8. Has α-thalassemia (2 or more gene deletions or any α-globin structural variants) SCD.
9. Has previous documented liver biopsy result performed before screening that shows cirrhosis, bridging hepatic fibrosis, or active hepatitis or subject has received chronic transfusions (>200 mL/kg body weight) and has previous documented evidence of iron overload (a serum ferritin level of >1000 ng/mL and a magnetic resonance imaging [MRI; T2* or FerriScan] estimated liver iron >15 mg/g dry weight) and previous documented evidence of liver fibrosis by noninvasive liver imaging.
10. Has documented matched sibling donor, unless subject was fully informed about the benefits and risks of HSCT and has declined this option or this option is not feasible due to comorbidities or financial or logistical constraints. In order for subject to be considered eligible, documentation must be included as part of the informed consent process for subjects who decline this option.
11. Has medical history of pulmonary hypertension documented as a tricuspid regurgitation jet velocity >3 m/s by transthoracic echocardiography and/or clinical signs and symptoms of pulmonary hypertension (mean pulmonary artery pressures >25 mm Hg).
12. Has any condition, screening laboratory or diagnostic test result, or chronic physical or mental illness that, in the opinion of the investigator, makes participation ill advised.
13. Has a known hypersensitivity to any study treatments (eg, melphalan, plerixafor).
14. Is an employee or family member of the investigator or study site personnel.

4.2 Withdrawal of Subjects From Study Treatment and/or the Study

4.2.1 Reasons for Withdrawal/Discontinuation

Subjects may withdraw from the study at any time and for any reason without prejudice to their future medical care by the investigator or at the study site. Every effort should be made

to keep subjects in the study. The reasons for subjects not completing the study will be recorded (see below).

Given the nature of the therapeutic intervention explored in this study and the intensity and duration of the preparations required before infusion of ARU-1801, subjects may be withdrawn from the study before receiving study treatment. Such subjects may be replaced (Section 4.2.3). In addition, since the therapy consists of a 1-time infusion, discontinuation of study treatment is not relevant to this study, except in the event that a subject does not tolerate the infusion of ARU-1801 and the procedure is interrupted before the intended volume has been administered.

Before receiving the ARU-1801 infusion, a subject may be withdrawn from the study for any of the following reasons:

- Subject is unable to tolerate HbS reduction to ≤30% via erythrocytapheresis or transfusions before HSC collection.
- Baseline genetic evaluation of BM demonstrates an increased risk to development of hematologic malignancy. Identification of any of the following chromosomal abnormalities or genetic mutations must result in subject withdrawal and the subject should not undergo any further research procedures:
    - Cytogenetic chromosomal abnormalities, identified by karyotype evaluation, such as monosomy 7 or trisomy 8.
    - Chromosomal abnormalities associated with MDS, identified by FISH evaluation: RPN1, MECOM (3q21, 3q26.2) | 5q-, -5 (5p15, 5q31, 5q33) | 7q-, -7 (Cen 7, 7q22, 7q31) | Trisomy 8 (Cen 8) MLL (11q23) | ETV6 (12p13) | 17p- (TP53 17p13.1, NF1 17q11.2) | +19 (19p13.2, 19q13) | 20q-(20q12, 20qter).
    - Genetic mutations in the Neotype Myeloid Disorders Profile identified by Next Generation Sequencing (NGS) as part of the Neotype Myeloid Disorders Panel (ML/MPN/MDS/MDS-MPN). Currently these mutations include: ABL1, ASXL1, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CSF3R, CUX1, DDX41, DNMT3A, ETNK1, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, GNB1, HRAS, IDH1, IDH2, IKZF1, JAK2 including V617F and Exons 12+14, JAK3, KDM6A, KIT, KRAS, MLL, MPL, MYD88, NF1, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PML, PPM1D,

PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5B, TET2, TP53, U2AF1, WT1, ZRSR2.

- Harvest of CD34+ cells is inadequate despite multiple harvest attempts.
- Subject cannot tolerate further CD34+ harvest attempts in order to obtain adequate CD34+ cells.
- Preparation of gene-modified cells is unsuccessful for any reason, including failure of transduction or tests for contamination, viability, and concentration.
- Subject no longer meets the protocol inclusion or exclusion criteria.
- Subject is noncompliant with the protocol.
- Subject has a serious or intolerable AE that in the investigator's opinion requires withdrawal from the study.
- Subject has laboratory safety results that reveal clinically significant hematological or biochemical changes from the baseline values.
- Subject has symptoms or an intercurrent illness not consistent with the protocol requirements or that justify withdrawal.
- Subject becomes pregnant before receiving ARU-1801; if subject becomes pregnant after infusion of ARU-1801, she may remain in the study (see Section 6.7).
- Sponsor terminates the study (see Section 11.3).

All subjects who receive the ARU-1801 infusion will be encouraged to complete all follow-up visits. However, a subject may voluntarily withdraw from study participation at any time.

After administration of the ARU-1801 infusion, a subject may be withdrawn from the study for the following reasons:

- Subject is lost to follow-up.

Upon occurrence of a serious or intolerable AE, whether before or after the ARU-1801 infusion, the investigator will confer with the Sponsor. If a subject is withdrawn because of an AE, the event will be followed until it is resolved. Any subject may withdraw his or her consent at any time. At the time of consent withdrawal, the subject will be asked to provide

permission for collection of relevant source documentation from clinical records to ascertain the subject's health status (Section 4.2.2).

4.2.2 Handling of Withdrawals

Subjects are free to withdraw from the study at any time upon request. Subject participation in the study may be stopped at any time at the discretion of the investigator or at the request of the Sponsor.

Subjects who no longer want to participate in the study should NOT be considered withdrawn from the study UNLESS consent is withdrawn. When possible, these subjects should return for protocol-scheduled visits as indicated in Appendix 1. If a subject fails to return for site visits for unknown reasons, the investigator or designee should make every effort to contact the subject. This contact should preferably be performed according to the schedules of events. If the subject cannot or is unwilling to attend any visit(s), the site staff should maintain regular telephone contact with the subject. Telephone contact should preferably be completed per the schedules of events (Appendix 1). At a minimum, in abbreviated visits, the following data should be collected at site visits or via telephone (per schedules of events):

- Concomitant medications (including opioids)
- AEs and SAEs
- Survival status

For subjects who withdraw consent, the reason(s) for withdrawal shall be recorded by the investigator on the relevant page of the electronic case report form (eCRF). Whenever possible, all subjects who withdraw from the study prematurely after receiving the ARU-1801 infusion will undergo safety procedures before withdrawal. In addition, every effort will be made to undertake protocol-specified, safety follow-up procedures, in particular for subjects who withdraw because of an AE or serious AE. For subjects who withdraw and are unwilling to return for a site visit, the investigator or designee may conduct a follow-up visit via telephone contact. Study sites should make every effort to re-establish contact with subjects who repeatedly fail to return for scheduled visits and who they are unable to contact, including searching public records (court, property, mortality). Subjects who fail to return for

final assessments after 2 documented telephone contact attempts and a registered letter will be considered lost to follow-up.

Subjects who withdraw consent at any time will be asked at the time of withdrawal to provide permission for collection of relevant source documentation from clinical records to monitor any AEs. The permission request and response will be recorded in the appropriate eCRF. For subjects who withdraw before ARU-1801 infusion, any AE will be attributable to study procedures only and not to the gene product, which would not have been received. Any SAEs will be reported according to Section 6.3.1.5.

4.2.3 Replacements

Any subject who withdraws from the study before or after receiving the ARU-1801 infusion may be replaced.

5 Study Treatments

5.1 Method of Assigning Subjects to Treatment Groups

This is an open-label study. No randomization or blinding will be performed.

5.2 Treatments Administered

The investigational product, ARU-1801, consists of the CD34+ cells transduced with the γ-globin (sGbG^M) LV vector. It may be given as a fresh infusion or cryopreserved for later administration. The product for fresh infusion is formulated in <10 mL/kg volume of Plasmalyte-A® supplemented with 1% human serum albumin and filled into a syringe. The cryopreserved product is formulated in a cryopreservation medium containing 10% dimethyl sulfoxide (DMSO) and filled into a bag.

The investigational product, ARU-1801, will be administered via IV infusion over a period no longer than 15 minutes, followed by an IV infusion of 10 mL/kg bolus of saline over approximately 30 minutes. The goal is to infuse the subject with 4×10^6 CD34+ cells/kg body weight or higher.

Detailed instruction for preparation and administration of autologous CD34+ cells transduced with the sGbG^M LV vector are provided in the Pharmacy Manual.

5.3 Identity of Investigational Product

The investigational product, ARU-1801, consists of the CD34+ cells transduced with the γ-globin (sGbG^M) LV vector.

Refer to the IB for further details related to the sGbG^M LV vector.

5.4 Management of Clinical Supplies

5.4.1 Investigational Product Packaging and Storage

The formulated subject ARU-1801 product for fresh infusion is placed in a 60-cc syringe contained in a sterile, reclosable, plastic overwrap and stored at 2°C to 8°C for up to 8 hours until the initial testing (final cell count, Gram stain, and endotoxin) is complete and it is released for infusion. The infusion of the released product must be complete within 8 hours,

although the Sponsor recommends that the cell product (ARU-1801) be infused as soon as possible.

Alternatively, the CD34+ drug substance will be resuspended at a concentration of 1 to 10×10^6 cells/mL in CryoStor® CS10 cryopreservation media containing 10% DMSO and transferred to a 250-mL cryopreservation bag. ARU-1801 cryopreserved product will be stored at <-140°C in the vapor phase of liquid nitrogen. Upon thaw, the ARU-1801 DP should be administered by infusion from the bag immediately.

5.4.2 Test Article Accountability

The investigator or designee is responsible for the following:

- Logging receipt of each delivery of investigational product (ARU-1801)
- Confirming the actual contents of delivery
- Verifying receipt of the investigational product by signing the appropriate documentation provided by the Sponsor or designee
- Indicating the status of each sterile tube or cryovial received

The investigator will maintain accurate records of receipt of all test articles, including dates of receipt. In addition, accurate records will be kept regarding when and how much test article is dispensed and used for each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. At the completion of the study, regulatory requirements regarding drug accountability will be followed. Guidance for investigational product accountability will be provided in the Pharmacy Manual.

5.4.3 Other Supplies

The following drug supplies will be supplied by the site:

- Melphalan (Evomela® [New Drug Application: 207155] or if not available a therapeutic equivalent melphalan will be used)
- Plerixafor
- Acetaminophen, diphenhydramine, hydrocortisone, and any other premedications given per institutional policy

Melphalan for injection is supplied as a dry powder. Detailed instructions for melphalan preparation and administration are provided in the Pharmacy Manual.

Plerixafor, a hematopoietic stem cell mobilizer, will be used for peripheral blood mobilization. Drug preparation and administration should adhere to institutional standard practice and product prescribing information.

As premedication before infusion of ARU-1801, subjects will receive oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg). The route of administration for acetaminophen and diphenhydramine is at the investigator's discretion; hydrocortisone should be administered intravenously.

5.5 Study Treatment Procedures and Administration

Study treatment procedures are HSC collection (Section 5.5.1), CD34+ cells transduction with the γ-globin LV vector (Section 5.5.4), subject conditioning with melphalan (Section 5.5.3), and infusion of transduced CD34+ cells back to the subject (Section 5.5.5).

5.5.1 Hematopoietic Stem and Progenitor Cell Collection

After completing the assessment for the Stem Cell Collection Clearance Visit (Table 13-1), HSCs will be collected from P-MPB (Section 5.5.1.1) (Section 5.5.1.2). The use of P-MPB is at the discretion of the investigator. The target for HSC collection is $\geq 8\times10^6$ CD34+ cells/kg body weight (see Section 5.5.1.2).

Because P-MPB procedures are not routinely performed in patients with SCD, it is difficult to estimate the amount of CD34+ cells that will be obtained from 1 sample collection. For this reason, serial sampling of peripheral blood CD34+ concentration will be assessed to understand the kinetics of CD34+ mobilization after plerixafor administration and guide the subsequent timing and duration of apheresis for each individual subject (Section 5.5.1.1). Overall, it is anticipated that multiple P-MPB procedures will be needed to achieve a minimum target CD34+ cell collection of $\geq 8\times10^6$ CD34+ cells/kg body weight. For subjects who provide separate consent, additional HSC collection will be performed to obtain HSCs for research purposes.

Target HbS and Hemoglobin Criteria

Target HbS and hemoglobin values will be achieved using standard procedures, including serial simple transfusions done a few weeks apart, partial exchange transfusions (each a few weeks apart), or by using erythrocytapheresis; the methodology is at the discretion of the hematologist.

After enrollment on study, subjects will begin the HbS reduction phase. The goal is to reduce HbS to ≤30% via erythrocytapheresis or transfusions for a period of 2 to 3 months. The frequency of required procedures and visits in order to achieve the target HbS at least 2 months before HSC collection will be at the investigator's discretion. The 2-month minimum requirement for HbS reduction may incorporate time required to receive erythrocytapheresis or transfusions just before screening as long as HbS ≤30% over time is documented in the subject's medical records. Subjects may be treated with erythrocytapheresis or transfusions to maintain a HbS ≤30% for longer periods of time (eg, 6 months) before HSC collection at the investigator's discretion.

Within 7 days prior to plerixafor administrations at the Stem Cell Collection Visit, the subject should have documented HbS ≤20% or have completed erythrocytapheresis to the goal HbS of ≤20%. Fluctuations in HbS outside of this range will be permitted as long as the subject meets the criteria at any 1 time point during the 7-day period. Minor deviations of these HbS target goals for a given subject may be made at the investigator's discretion based upon consideration of safety and feasibility.

5.5.1.1 Plerixafor-Mobilized Peripheral Blood Collection

Subjects will receive a 240 µg/kg subcutaneous injection of plerixafor and undergo apheresis at a time point informed by the CD34+ kinetic data (Section 5.5.1.1) and for a duration designed to maximize CD34+ cell yield and maintain subject safety. During the first 2 days of collection of the first cycle, blood samples will be collected to measure peripheral blood CD34+ cell counts before administration of plerixafor (baseline) and at approximately 1, 2, 4 and 8 hours after plerixafor dosing to evaluate the stem cell mobilization kinetics of each subject. If additional collection cycles are needed, the kinetics may be omitted. The timing of apheresis initiation, duration, collection volume, interface parameters, and other apheresis parameters will also be recorded.

Based upon emerging data on CD34+ kinetics and cell yields in the study, apheresis parameters may be altered to maximize CD34+ cell yield and maintain subject safety. After consultation with the Sponsor, the collection time points for peripheral blood sampling for CD34+ cell measurements may be changed, the number of blood samples collected for peripheral blood CD34+ measurements may be reduced, and the time window for apheresis may be altered.

If needed, a second dose of 240 µg/kg of plerixafor may be given the next day, followed by a second apheresis for stem cell collection. Additional P-MPB procedures (mobilization and apheresis) using the same dose of plerixafor and schedule may be performed until sufficient stem cells are collected. For those subjects who are consented and at least 12 x10^6 CD34+ cells/kg body weight have been obtained from them and frozen for use in ARU-1801 investigational product, additional HSC collection will be performed. These HSCs will be used for research purposes to support future manufacturing process improvements.

Peripheral blood CD34+ cell count should be analyzed before and after (1, 2, 4 and 8 hours) each plerixafor administration (detailed above and in Section 5.5.1.1). Collection procedures using P-MPB may be performed on up to 5 consecutive days, if tolerable to subject or as directed by the investigator. If multiple cycles of plerixafor and apheresis are needed (ie, insufficient stem cells are collected after consecutive P-MPB procedures), at least 2 weeks should elapse between cycles.

After apheresis, a blood sample will be collected for a complete blood count (CBC). The subject may be administered packed RBC (PRBC) or a platelet transfusion, as required, to maintain target levels of hemoglobin (>10.5 g/dL) and platelets (>100K; see Section 5.6.2).

5.5.1.2 HSC Collection Target

Collected material will be evaluated for total nucleated cell count and CD34+ cell content. The overall goal of HSC collection is to collect ≥8×10^6 CD34+ cells/kg body weight before conditioning subject with melphalan.

If the subject tolerates the HSC collection procedure well and <8×10^6 CD34+ cells/kg body weight are obtained from P-MPB apheresis, the cells will be cryopreserved and additional collections will be performed until the total target viable CD34+ cells (≥8×10^6 CD34+ cells/kg body weight) are obtained and cryopreserved.

If $<8\times10^6$ CD34+ cells/kg body weight are obtained following P-MPB apheresis and no additional collections are performed due to the subject's condition or preference, it will be deemed unfeasible to obtain sufficient CD34+ cells and the subject will not receive chemotherapy (Section 4.2).

For those subjects who consented to the additional HSC collection, the HSC target will be $\geq12\times10^6$ CD34+ cells/kg body weight. If this target is obtained, excess collected HSCs will be used for research purposes. Any additional HSC collection will be done at the discretion of principal investigator and subject and will take into consideration how the subject tolerates the P-MPB procedure (mobilization and apheresis).

5.5.2 CD34+ Cell Isolation

CD34+ cells will be isolated from apheresed material. The collected cells will be RBC-depleted and enriched for CD34+ cells. The purified CD34+ cells will be resuspended in cryopreservative medium containing DMSO and cryopreserved until sufficient cells are collected.

5.5.3 Chemotherapy Conditioning With Melphalan

Once sufficient stem cells are obtained, subjects will be prepared for administration of chemotherapy and administration of ARU-1801. Subjects will undergo a Chemotherapy Clearance Visit (Table 13-1), including evaluation of renal filtration by Cystatin C eGFR, nuclear medicine GFR, hematocrit, and height and weight. Detailed instructions for collection of the covariate data needed for the melphalan model-informed dose are provided below. Sites will be required to submit the accompanying de-identified source documentation to ePip once collected.

- **Gender, height,** and **weight**: should be measured locally at the site during the Chemotherapy Clearance Visit and verified by two independent healthcare professionals.

- **Hematocrit**: Should be measured after the last planned erythrocytapheresis or therapeutic blood transfusion that is done prior to chemotherapy, and no more than 7 days before the planned administration of melphalan. Analysis at the central laboratory is preferred but may be performed locally if necessary.

- **Cystatin C eGFR:** Should be collected at the Chemotherapy Clearance Visit and analyzed at the central lab. May be analyzed at the site's local lab if necessary. Nuclear medicine GFR will not be used to inform the model-informed dose of melphalan.

Within no more than 7 days before planned administration of chemotherapy, the melphalan dose should be determined. The subject's gender, height, weight, hematocrit, and cystatin C eGFR will be used to determine the predicted melphalan AUC.

Subjects should also undergo HbS reduction to ensure the % HbS is less than 30% prior to chemotherapy. After clearance, subjects will then be admitted to the hospital in anticipation of chemotherapy administration and will remain in the hospital for close monitoring and supportive care until clinically fit for discharge after cell count recovery. Testing performed for clinical monitoring up to 30 days post-infusion should be recorded in the study database (CBC with reticulocyte and differential, clinical chemistry, and hemoglobin electrophoresis).

At the investigator's discretion, subjects who the site regards as reliable and highly compliant with the protocol and whose resource needs are met (eg, caregiver, transportation) may not require hospitalization for specific procedures and may instead return to the site at the frequency specified in the protocol to complete visits for collecting blood samples, determining cell counts, and completing other procedures. Investigators must instruct subjects and caregivers to immediately contact the site to report any AEs, including any signs or symptoms of infection (eg, fever).

A RIC dose of melphalan will be given intravenously to all subjects at the Chemotherapy Visit. The exact dose of melphalan to be given to a subject will be based on predictive modeling using the formulas from Nath et al 2010, which are provided below. First, the predicted melphalan AUC for a conventional body-surface area-based dose of 140 mg/m^2 is calculated (Equation 1). Subjects with a predicted melphalan AUC ≥8.0 µg.h/mL using this calculation will receive a dose of 140 mg/m^2. Subjects with a predicted melphalan AUC <8.0 µg.h/mL will receive a model-informed dose of melphalan to achieve a target AUC of 10.4 µg.h/mL. This dose is calculated using Equation 2. The model-informed dose of melphalan will be limited to a maximum of 200 mg/m^2. A flow chart for determining the melphalan dose is provided in Section 13.6 Appendix 6.

Equation 1. Predicted $AUC_{BSA.140}$ = 140 (mg/m^2) x BSA (m^2) / CL_{pred}

Equation 2. $MEL_{AUC10.4}$ Dose = 10.4 (mg*h/L) * CL_{pred}

$$\text{Where } CL_{predicted} = 17 \cdot \left(\frac{HCT}{34}\right)^{0.462} \cdot \left(\frac{FFM}{53}\right)^{0.75} + 11.1 \cdot \frac{eGFR_{CystC,normalized}}{5.28} \text{ [L/h]}$$

Equations for determination of eGFR, FFM, and ideal body weight are shown in Appendix 5 Section 13.5.

Abbreviations: $AUC_{BSA.140}$, area under the plasma concentration versus time curve for a body surface area-based dose of 140 mg/m^2; BSA, body surface area (calculated using ideal body weight in subjects weighing >130% of ideal body weight); CL_{pred}, predicted clearance; $eGFR_{CystC,normalized}$, estimated glomerular filtration rate based on Cystatin C normalized to 70 kg; FFM, fat free mass; HCT, hematocrit; $MEL_{AUC10.4}$ Dose, melphalan dose calculated for a target area under the plasma concentration versus time curve for a target of 10.4 µg.h/mL.

Blood samples will be collected for measurement of melphalan concentration for 6 hours after the conditioning dose. The conditioning dose should be administered at least 36 hours before the infusion of ARU-1801 on Day 0. In subjects weighing >130% of ideal body weight, body surface area will be calculated using ideal body weight. Drug preparation and administration procedures, including use of antiemetics, should adhere to institutional standard practice, and product prescribing information. The investigator or designee should monitor subjects for melphalan-related side-effects and treat per standard practice. Known melphalan side effects and associated risks are summarized in Appendix 3.

At least 36 hours after melphalan conditioning, the transduced CD34+ cells will be prepared for IV infusion.

5.5.4 Transduction of CD34+ Cells

Manufacture of ARU-1801 will be performed in a Class 7 clean room environment according to defined standard operating procedures; all open manipulations are performed in a Class 5 Biosafety Cabinet in accordance with FDA's guidance for aseptic processing. Gene transfer is performed following good manufacturing practice requirements applicable for early phase clinical studies with formal quality assurance oversight.

Purified CD34+ cells will be thawed, allowed to recover, pooled, and pre-stimulated in preparation for transduction. The pre-stimulated cells will be transduced ex vivo using the sGbG^{M-} LV vector. Transduced cells will be infused following gene transfer either as a fresh product or a thawed cryopreserved product as detailed in Section 5.4.1.

5.5.4.1 Safety and Efficacy Testing of Transduced Cells

The final product will be released for infusion based on a portion of the full testing. This testing will include evaluating the CD34+ product for viable cell count, verifying that the product is negative for microbial contamination by Gram stain evaluation, and that viability is ≥70%. Endotoxin levels of the product will also be determined before the infusion, and the infused product must have <5 EU/kg body weight. The remaining tests will be performed after the infusion and are included on the final ARU-1801 product certificate of analysis.

5.5.4.2 Positive Microbial Culture Results

A positive microbial report on a subject's harvested or CD34-enriched cells will result in the following actions.

- An antibiotic-sensitivity analysis will be performed.
- The product may be infused if all conditional release criteria are met; the Gram stain is negative; if there is no impact to the safety, identity, strength, purity, and quality; and, if in the opinion of the investigator, the product is considered safe for infusion. If the product is infused the following precautions will be taken.
- Physical examinations and evaluation for signs of infection daily for 3 days.
- A CBC with differential daily for 3 days.
- Blood and urine cultures daily for 3 days.
- Appropriate broad-spectrum antibiotics will be administered intravenously, as determined by the investigator.

An investigation as to the likely source of contamination will be conducted by the processing facility per institution's standard operating procedures to determine if the positive culture occurred during the open manipulation. Reports of the investigation and associated corrective action will be reported to the Sponsor and the investigator for review and approval.

5.5.5 Infusion of ARU-1801

At the end of transduction and at least 36 hours after the subject receives melphalan, transduced CD34+ cells will be prepared for IV infusion.

Subjects will be premedicated with acetaminophen (650 mg), diphenhydramine (50 mg), and hydrocortisone (100 mg) approximately 30 minutes prior to infusion of ARU-1801. Cells will be infused via IV infusion as detailed in Section 5.2.

Subjects will be monitored closely for possible stem cell infusion-related toxicities, including fever within 24 hours and hypertension, as detailed in Section 5.6.3. Subjects will remain hospitalized after receiving the ARU-1801 until they are ready for discharge as per institutional protocols for transplant patients but not before the last melphalan PK sample has been collected (Section 6.6).

Chronic transfusions will be continued for 3 months following transplant. Subjects will then be gradually weaned from transfusions with the goal of maintaining hemoglobin at ≥8 g/dL. The process for weaning transfusions is detailed in Section 5.7.

5.6 Other Treatments: Supportive Care

5.6.1 Infectious Disease Prophylaxis

Following receipt of melphalan, subjects will receive *Pneumocystis* prophylaxis per standard of care until adequate immune recovery is demonstrated. Antiviral and antifungal prophylaxis will be administered per standard of care when indicated.

5.6.2 Blood Product Support

Because the administration of melphalan may produce cytopenias for 2 to 3 weeks, a CBC will be performed at a minimum twice weekly until absolute neutrophil count is $>1\times10^9$/L and then weekly thereafter until the investigator considers the subject recovered. At investigator's discretion, CBCs may be performed more frequently; all test results should be recorded. Note: granulocyte-colony stimulating factor (G-CSF) is contraindicated in patients with SCD (see Section 5.10.2).

Platelet transfusions should be administered to maintain a platelet count >50,000/mm^3.

All red cell products should undergo extended RBC typing, specifically minor antigen matching for Kell, E and C blood groups for SCD identified and phenotypically matched, and transfusion of HbS negative units. Packed red cell transfusions should be given to maintain a hemoglobin at approximately 11 g/dL, and a HbS level ≤30% for the first 3 months after chemotherapy conditioning.

5.6.3 Management of ARU-1801 Infusion-Related Toxicities

After infusion of ARU-1801, the following guidelines should be followed for monitoring possible infusion-related toxicities:

- Hypertension should be strictly controlled to prevent central nervous system toxicity. Blood pressure should be monitored closely and both systolic and diastolic hypertension should be treated promptly to maintain blood pressure at the subject's pre-transplant baseline ± 20%.
- Any subject who develops a fever to >38°C orally or axillary within 24 hours of infusion of ARU-1801 will undergo the following:
  - Immediate evaluation to identify the source of the fever
  - Collection of blood and urine for microbial culture
  - Collection of blood sample for CBC with differential to determine absolute neutrophil count
  - Intravenous administration of broad-spectrum antibiotics

5.6.4 Fertility Preservation

Due to a potential risk of infertility/sterility in SCD subjects receiving melphalan conditioning, an option will be presented to each subject regarding a fertility consult and preservation. This research study will cover costs incurred up to $1000 per SCD subject. In the event that the subject's insurance does not cover additional charges, the subject will be responsible for any fertility costs that exceed $1000. These costs will cover fertility consult, sperm preservation, and transportation, if needed. Additionally, regular reproductive testing will be performed (Section 6.3.2 and Appendix 1).

5.7 Transfusion Withdrawal

In order to exert selective pressure on the gene-corrected CD34+ cells to proliferate, transfusion support after the gene transfer infusion will be slowly weaned. The projected plan for weaning transfusion support is outlined below. This plan will be implemented based on the practical capabilities of individual study sites and the health of the subjects:

- **Months 1 to 3 (inclusive) after transplant**: In the initial months after gene therapy, subjects will receive simple erythrocyte transfusions approximately every 4 weeks to maintain target hemoglobin concentration of approximately 11 g/dL and HbS ≤30%. This will suppress endogenous erythropoiesis from occurring during this time while the gene-modified stem cells engraft.

  Months 4 to 5 after transplant: After Month 3, the subject should begin to be weaned from transfusions to allow endogenous erythropoiesis, which is expected to stimulate erythropoiesis from gene-modified HSCs. At or after the Month 4 visit, the subject should be transfused if total Hb is <10 g/dL. The final transfusion should occur before the conclusion of Month 5. The investigator should use clinical judgment and may deviate from this weaning process if necessary to ensure the safe weaning of transfusions. As transfused blood washes out, subjects will return to their baseline hemoglobin (typically 7 to 9 g/dL) if gene transfer was ineffective. If gene transfer is effective, subjects may maintain their hemoglobin above baseline by increasing HbF.

- **HbF monitoring**: HbF will be monitored at the time points indicated in the schedules of events (Appendix 1).

5.8 Blinding

This is an open-label study.

5.9 Treatment Compliance

The investigator will oversee compliance with HSC collection, chemotherapy conditioning, and infusion of ARU-1801. The investigator or designee will document the success and/or completeness of treatment administration and infusion of ARU-1801 and record information in the eCRF.

5.10 Prior and Concomitant Therapy

Use of prior and concomitant medications will be recorded in the subject's eCRF. The minimum requirement is that drug name and the dates of administration should be recorded. Medications reported will include all prescription drugs, over-the-counter medications, herbal products, vitamins, and minerals. Any changes in concomitant medications also will be recorded in the subject's eCRF. The minimum information reported for concomitant procedures should be the name or description of the procedure and the date on which it was performed; the reason for the procedure should be included to the extent possible.

5.10.1 Prior Medications and Procedures

Prior medications are those the subject was taking at the time the informed consent form (ICF) was signed. Prior procedures will be captured as part of medical history and will includes procedures performed up to 2 years before screening (Section 6.2). Restrictions on prior medications and procedures are as follows:

- Disease-modifying therapies for the treatment of the subject's SCD should be discontinued at the time that the subject initiates HbS reductive transfusions and must be discontinued at least 2 months before HSC collection. Example medications include hydroxyurea, L-glutamine, voxelotor and crizanlizumab.
- Iron chelation therapy must be discontinued at least 48 hours before the start of melphalan conditioning and may not be resumed until 3 months after the ARU-1801 infusion (see Section 5.10.3).

5.10.2 Concomitant Medications and Procedures

Any concomitant medication or procedure deemed necessary for the welfare of the subject during the study may be given or performed at the investigator's discretion. Restrictions on concomitant medications and procedures are as follows:

- G-CSF is contraindicated in subjects with SCD. Increased leukocytosis from G-CSF has resulted in VOEs, ACS, and death (Fitzhugh et al 2009).
- Elective surgeries in the first 12 months after the ARU-1801 infusion.

Therapies and procedures that are permitted during study participation are as follows:

- Therapies for hemoglobinopathy, including exchange transfusions to prevent VOE, transfusions of blood or platelets to maintain appropriate cell counts, erythrocytapheresis, or any of these procedures to achieve target HbS and hemoglobin values before P-MPB.
- Medications required to manage chronic pain and other complications from SCD at the investigator's discretion.
- Protocol-specified plerixafor for mobilization of peripheral blood before HSC harvest.
- Protocol-specified premedication (acetaminophen, diphenhydramine, and hydrocortisone) before infusion of ARU-1801.
- Medications for anesthesia during required study procedures, if needed.

5.10.3 Medications and Procedures After Treatment

Restrictions and guidelines related to medications and procedures after treatment are as follows:

- Iron chelation or a program of phlebotomy may be resumed 3 months after infusion of ARU-1801 at the discretion of the investigator.
- Subjects will gradually wean use of transfusions starting 3 months after the infusion of ARU-1801 (Section 5.7).

6 Study Assessments and Procedures

Before performing any study procedures, all potential subjects will sign an ICF. Subjects will have the opportunity to have any questions answered before signing the ICF. The investigator must address all questions raised by the subject. The investigator or designee will also sign the ICF.

The schedules of events are presented by visit in Table 13-1 (Screening and Study Treatment Visits), Table 13-2 (Post-infusion Visits: Day 7 to Year 2), and Table 13-3 (Long-term Follow-up Visits).

6.1 Study Visits

Screening: Screening assessments will be performed for all potential subjects to determine study eligibility. The screening assessments are summarized in Section 6.2 and indicated in Table 13-1.

Enrollment: Following completion of all screening assessments, the investigator and Medical Monitor will independently verify eligibility. Upon final sign-off by the Medical Monitor, the subject will be 'Enrolled.'

Baseline bone marrow aspirate (BMA): The baseline BM testing must be completed after enrollment and prior to initiation of additional research procedures. Specifically, the testing should be completed before a central line is placed (if applicable) or the subject is initiated on RBC transfusions. In addition, morphological assessment, immunophenotyping for leukemia/lymphoma, and NGS results must be complete and reviewed by the investigator before the first HbS Reduction Visit.

- Subjects with cytogenetic abnormalities or genetic mutations associated with risk of hematologic malignancy will be withdrawn. See Section 4.2.1 for a full list of the chromosomal abnormalities or genetic mutations that would result in subject withdrawal.

- Subject Counseling: The baseline BM genomic evaluation is being performed out of an abundance of caution and to protect subjects who may be at increased risk of developing myeloid malignancy after exposure to alkylating chemotherapy and autologous HSCT. Neither cytogenetics nor NGS evaluation are performed routinely

as a screening test in the absence of clinical evidence of a new hematologic disorder. The sensitivity and specificity of these tests in a population with no hematologic abnormalities is unknown. The positive predictive value of these tests to detect a genetic abnormality which will ultimately become clinically relevant is unknown. Although the precise negative predictive value is also unknown, at a wide range of sensitivity and specificity assumptions, and using a wide range of prevalence estimates, the negative predictive value of these tests is high. Subjects should be informed of any positive findings from these evaluations, and will be offered consultation with a genetic counselor to more completely understand the implications of their unique status.

HbS Reductive Visit(s): Before HSC collection, subjects enrolled in this study will begin HbS reduction to ≤30% via erythrocytapheresis or transfusions over a period of 2 to 3 months. Details for HbS reduction are provided in Section 5.5.1. The frequency of required procedures and visits in order to achieve HbS reduction ≤30% at least 2 months before HSC collection will be at the investigator's discretion. Within 7 days before HSC collection, a subject's HbS target is ≤20%. The assessments performed at this visit are indicated in Table 13-1.

Stem Cell Collection Clearance Visit(s): This visit will occur within 7 days of HSC collection. This visit may be repeated, as required, if subjects require multiple cycles of P-MPB at Stem Cell Collection Visit (see Section 5.5.1.1 and Section 5.5.1.2). The assessments performed at this visit are indicated in Table 13-1.

Stem Cell Collection Visit(s): Collection of HSCs will be from P-MPB, with details for each provided in Section 5.5.1.1 and Section 5.5.1.2, respectively. The use of P-MPB is at the investigator's discretion. This visit may be repeated as necessary to achieve collection of targeted count of CD34+ cells (see Section 5.5.1.2). The assessments performed at this visit are indicated in Table 13-1.

Chemotherapy Clearance Visit: Subjects will be cleared for administration of chemotherapy treatment with melphalan within 7 days before the Chemotherapy Visit. The assessments performed at this visit are indicated in Table 13-1.

Note: The HbS Reductive Visit, Stem Cell Collection Clearance Visit, and Chemotherapy Clearance Visit can occur on the same day. If combined, the investigator will verify that the

subject has been cleared for HSC collection via P-MPB; and 3) cleared for administration of chemotherapy. Any procedures that are required for each respective visit may be completed only once when any of these visits are completed within the same 7-day time period.

Chemotherapy Visit: Melphalan will be administered to subjects at least 36 hours before the infusion of ARU-1801. Samples for melphalan PK assessment are collected during this visit. Details regarding chemotherapy administration are provided in Section 5.5.3. The assessments performed at this visit are indicated in Table 13-1.

Infusion Visit: Once the HSC transduction procedure has been completed (Section 5.5.4) and at least 36 hours after the subject has received melphalan (Section 5.5.3), subject will receive an infusion of ARU-1801 (see Section 5.5.5).

Follow-up Visits: Subjects will have follow-up assessments at the time points indicated in Table 13-2 and Table 13-3. This will include assessments at Day 7, 14, 21, 30, 60, and 90 and at Months 4 through 12; Months 15, 18, 21; and Year 2. It is anticipated that a separate LTFU clinical study will be initiated. Once the LTFU study is approved at the site, all subjects who complete the Year 2 study visit in this study will be asked to consent and enroll in the LTFU study and will be followed for a total of 15 years after the ARU-1801 infusion. Long-term follow-up after Year 2 will continue in this Phase 1/2 study until subjects have transitioned into the LTFU study.

6.2 Screening Assessments

After the subject provides written informed consent, the subject will complete the following assessments at the screening visits, as indicated in Table 13-1, to confirm eligibility (the screening result will serve as baseline for each assessment):

- Inclusion/exclusion criteria (Section 4.1)
- Demographics and medical history, including reproductive health, VOE, opioid use, and other sickle events (Section 6.2.1)
- Physical examination (complete; Section 6.3.3)
- Vital sign measurements (Section 6.3.4)
- Iron overload status (Section 6.3.6.2)

- Electrocardiogram (ECG; performed at screening only) and echocardiogram (Section 6.3.5)
- Brain MRI and magnetic resonance angiography (MRA) (performed at screening only; can be omitted if performed within 6 months of consent; Section 6.3.5)
- Concomitant medication review (Section 5.10)
- Adverse event monitoring (Section 6.3.1)
- Karnofsky performance status (Section 6.5.4)
- Pulmonary function tests (oxygen saturation, spirometry, and diffusion) (Section 6.3.6.1)
- Safety Laboratory Tests (Section 6.3.2):
  - Clinical chemistry
  - Urinalysis (macroscopic with reflex microscopic analysis)
  - Urine microalbumin
  - CBC with reticulocyte and differential (automated)
  - Serum ferritin
  - Reproductive testing
  - Globin gene mapping (and globin deletional analysis if not done previously)
  - Enzyme genetic analysis
  - Serum pregnancy test
  - Hepatitis and HIV testing
  - Cystatin C eGFR
- Hemoglobin electrophoresis (Section 6.5.2.1)

6.2.1 Demographics and Baseline Medical History

At the screening visits, subject demographics, and history of VOEs, opioid use, and other SCD events during the previous 2-year period before enrollment will be recorded. Each subject's incidence of SCD-related events and opioid use will be monitored throughout the study.

The subject's available medical records pertaining to SCD status (VOEs, organ function parameters, laboratory tests, medications, transfusions, etc) may be used to obtain history from the 2-year period before enrollment. Medical history will also include prior procedures performed up to 2 years before the screening visit. Additionally, significant surgeries and medical events that occurred longer than 2 years before screening should also be recorded. Subjects will be asked to provide a reproductive health history as part of the medical history recorded at screening. Baseline disease severity will also be determined from a retrospective review of the subject's medical records for the last 2 years before the screening visit for subjects enrolled under previous versions of the protocol.

6.3 Safety Assessments

Safety will be assessed in this study through the assessment of AEs and SAEs, chronic conditions related to SCD, laboratory test results, physical examination findings, and vital sign measurements. Pulmonary function tests will be performed at screening. Safety testing will be performed on peripheral blood and BM samples.

6.3.1 Adverse Events

6.3.1.1 Definitions of Adverse Events

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to the investigational product (ARU-1801) or study medications/procedures.

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to investigational product. Subjects will be instructed to contact the investigator at any time after enrollment if any symptoms develop. Baseline conditions at study entry will be recorded on the medical history eCRF. If the baseline conditions worsen or increase in frequency, the investigator should reassess the event and record and report as an AE.

A treatment-emergent AE is defined as any event that occurs after exposure to a study procedure, study medication, or the investigational product.

If temporally associated with the use of an investigational product or a study medication/procedure, events meeting the definition of an AE include:

- An unexpected worsening, excluding minor fluctuations, in the nature, severity, frequency, or duration of a pre-existing condition.
- A new condition detected or diagnosed after investigational product administration even though it may have been present before the start of the study.
- Injury or accidents: If a medical condition is known to have caused the injury or accident, the medical condition and the accident should be reported as 2 separate medical events (eg, for a fall secondary to dizziness, both "dizziness" and "fall" should be recorded separately).
- An investigational abnormality (eg, laboratory parameter, vital sign, ECG) only if the abnormality is considered clinically significant by the investigator based on at least 1 of the following criteria:
  - Induces clinical signs or symptoms
  - Requires active intervention
  - Requires interruption or discontinuation of the investigational product infusion
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of study medications (eg, mobilization or conditioning regimens) or a concomitant medication.

Events that do not meet the definition of an AE include:

- Medical or surgical procedure (eg, endoscopy, appendectomy); the condition that leads to the procedure is an AE
- Situations where an untoward medical occurrence did not occur (eg, planned hospitalization for an elective procedure or study-required procedure, with elective defined as known or planned at the time of signing of the informed consent; social and/or convenience admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen

6.3.1.2 Serious Adverse Events

An SAE is defined as any event that

- Results in death
- Is immediately life threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization

  Note: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

  Hospitalization for a study-required procedure or for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in persistent or significant disability/incapacity that subject had not previously experienced or aggravates a prior existing significant disability/incapacity
- Results in a congenital anomaly/birth defect in the offspring of a female subject or female partner of a subject

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include hematopoietic malignancy such as a leukemic transformation from the gene transfer, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

6.3.1.3 Eliciting and Documenting Adverse Events

All AEs will be collected and reported from the time the subject signs the ICF until 2 years after the ARU-1801 infusion. After Year 2, it is anticipated that all subjects will be

transitioned from this study to the separate LTFU study and will be followed for a total of 15 years after the ARU-1801 infusion. In the LTFU study and for subjects in this study who have not yet transitioned to the LTFU study, AE collection and reporting after the Year 2 follow-up visit in this study will be limited to the following:

- All SAEs
- Nonserious AEs leading to study withdrawal or that are considered at least possibly related to investigational product or study medications/procedures
- AEs of special interest (Section 6.3.1.7)
- SCD VOEs (Section 6.5.3)

At every study visit, subjects will be asked a standard nonleading question to elicit any medically related changes in their well-being. They will also be asked if they have been hospitalized, had any accidents, used any new medications, or changed concomitant medication regimens (both prescription and over-the-counter medications).

In addition to subject observations, AEs identified from any study data (eg, laboratory values, physical examination findings) or identified from review of other documents that are relevant to subject safety will be documented on the AE page in the eCRF.

6.3.1.4 Reporting Adverse Events

All AEs reported or observed during the study, as defined in Section 6.3.1.3, will be recorded on the AE page in the eCRF. Information to be collected includes the following:

- Drug treatment
- Dose
- Event term
- Time of onset
- Investigator-specified assessment of severity and relationship to investigational product or study medications/procedures
- Time of resolution of the event

- Seriousness
- Any required treatment or evaluations
- Outcome

Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed as described in Section 6.3.1.11. The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all AEs.

Any pre-existing medical condition that is present at the time that the subject is screened but does not deteriorate should not be reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

In general, an abnormal diagnostic or laboratory value should not be recorded as an AE unless it is associated with clinical signs or symptoms (ie, assessed as clinically significant), requires an intervention, results in an SAE, or results in study termination or interruption/discontinuation of study treatment. When recording an AE resulting from a diagnostic or laboratory abnormality, the medical condition or diagnosis rather than the abnormality itself should be recorded (eg, record “anemia” rather than “low hemoglobin”).

For subjects enrolled under previous versions of the protocol (before Version 8.0), which did not stipulate recording all AEs during the first 2 years of follow-up after the ARU-1801 infusion, the investigators or designee will query existing study source documents and medical records (if required) in a retrospective manner. Investigators or designee will record AE information in the study database for those AEs that were collected previously in source documents and/or medical records but not entered into the study database.

6.3.1.5 Reporting Serious Adverse Events

Any AE that meets SAE criteria (Section 6.3.1.1) must be reported to PPD immediately (ie, within 24 hours) after the time site personnel first learn about the event regardless of the relationship (or lack of) of the SAE to study participation. The site should report SAEs via electronic data capture.

In the event that electronic data capture entry is not possible (eg, system failure or access problems), the site staff should complete the paper SAE report form and fax the form to PPD Pharmacovigilance within 24 hours of awareness. The following contact information should be used for SAE reporting with a paper SAE form:

PPD Pharmacovigilance – North America

24 Hour Safety Hotline: +1 800-201-8725

Safety Hotline Fax: +1 888-488-9697

6.3.1.6 Sickle Cell-Related Events

Chronic conditions related to SCD are those events that are known symptoms or associated conditions related to sickle cell anemia, stroke, or iron overload or an event related to standard clinical management, as all subjects enrolled in this study will receive all therapy and monitoring considered as standard of care for subjects with SCD. Acute and chronic conditions associated with SCD are listed in Table 6-1. Further, because all subjects in this study have a severe form of SCD, it is anticipated that certain AEs will occur that are related to the progression of the disease, which will be taken into account when determining whether or not an event is related to ARU-1801 or study procedures.

Table 6-1 Acute and Chronic Conditions Associated With Sickle Cell Disease

Albuminuria	Elevated serum transaminases	Pulmonary embolism
Amenorrhea	Fever	Pulmonary hypertension
Anemia	Hematuria	Pulmonary infiltrate (new) on chest x-ray consistent with acute chest syndrome
Aplastic crisis	Hemolysis	Pyelonephritis
Arthralgia	Hepatic sequestration	Renal insufficiency
Avascular necrosis of hip/shoulder	Hepatomegaly	Renal papillary necrosis
Bacteremia	Hyperbilirubinemia (unconjugated)	Reticulocytosis
Bone infarction	Hypersplenism	Retinal hemorrhage
Cardiac arrhythmia	Hypertension	Retinopathy
Cardiomegaly	Hypocalcemia	Rhabdomyolysis
Cholelithiasis	Hyposthenuria	Seizure
Constipation	Hypoxemia (O_2 saturation <85 mm Hg)	Septicemia
Cranial nerve palsy	Ileus	Silent infarct
Dactylitis	Leukocytosis	Skin ulcer
Decreased lung function	Meningitis	Splenic sequestration
Decreased renal function	Nephropathy	Splenomegaly
Delayed growth/puberty	Osteomyelitis	Systemic Infection: bacterial, invasive catheter, viral
Depression	Pain: back, chest, joint, long bone, severe abdominal, sternal, or rib	Transfusion
Dizziness	Pneumonia	Vaso-occlusive pain
Electrolyte Imbalance	Priapism	
Elevated bilirubin	Proteinuria	

6.3.1.7 Adverse Events of Special Interest

The following AEs are of special interest for evaluating the outcomes for subjects participating in this study:

- Insertional mutagenesis related to administration of ARU-1801
- Myelodysplastic syndrome and/or any hematological malignancy related to melphalan conditioning and/or vector insertion

6.3.1.8 Suspected Unexpected Serious Adverse Reactions and Nonserious Adverse Events of Special Interest

The Sponsor will promptly evaluate all suspected unexpected serious adverse reactions (SUSARs) and nonserious AEs of special interest against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, institutional review boards (IRBs)/independent ethics committees (IECs)/research ethics boards (REBs), and applicable health authorities based on applicable legislation. The SUSAR events and AEs of special interest will also be reported expeditiously to the DSMB.

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events using the following:

- ARU-1801 IB
- Melphalan prescribing information
- Plerixafor prescribing information
- SCD-related events (Table 6-1)

The Sponsor will compare the severity of each SUSAR and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document (eg, IB, melphalan prescribing information, plerixafor prescribing information).

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

AEs will be reported to the DSMB at the frequency defined in the DSMB charter.

6.3.1.9 Assessment of Severity

The severity, or intensity, of an AE refers to the extent to which an AE affects the subject’s daily activities. The severity of all AEs should be graded according to the CTCAE v5.0. If the event is not found in the CTCAE v5.0, the event will be assessed using the grading provided in Table 6-2.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent do not require documentation of onset and duration of each episode.

Table 6-2 Severity Grading for Events not Listed in the Common Terminology Criteria for Adverse Events Version 5.0

Grade	Severity	Description
1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate	Minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[a]
3	Severe or medically significant but not immediately life threatening	Hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]
4	Life-threatening consequences	Urgent intervention indicated
5	Fatal	Death related to adverse event

Abbreviations: ADL, activities of daily living.

a. Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

b. Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

6.3.1.10 Assessment of Causality

The investigator's assessment of an AE's relationship to study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The Sponsor will make the final determination of causality.

The relationship or association of the investigational product or study medications/procedures in causing or contributing to the AE will be characterized using the following classification and criteria (Note. Study medication includes other study medications, such as mobilization and conditioning regimens):

Unrelated:	The event is unrelated to the investigational product or study medications/procedures. The event is consistent with and can be attributed to subject's disease or another cause of the event is most plausible.
Unlikely:	The event or severity of event is doubtfully related to the investigational product or study medications/procedures.
Possible:	The event or severity of event is not usually associated by the clinical condition or standard of care for the condition, but there is no strong evidence to link the event to the investigational product or study medications/procedures.
Probable:	The event or severity of event is such that it can likely be correlated to the investigational product or study medications/procedures.
Definite:	There is a strong correlation between the event and the investigational product or study medications/procedures.

6.3.1.11 Follow-up of Subjects Reporting Adverse Events

All AEs must be reported in detail on the appropriate page in the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, or until the subject is considered to be stable.

6.3.2 Safety Laboratory Tests

Samples for laboratory testing will be collected at the time points specified in the schedules of events (Appendix 1). Testing will be performed by the central laboratory, except when local laboratory results are required for safety monitoring and/or central laboratory results are not available. Processing, storage, and shipping procedures for all laboratory samples and central versus local laboratory testing are provided in the Laboratory Manual.

The following laboratory tests will be performed:

- Clinical chemistry panel: alkaline phosphatase, ALT, AST, bicarbonate, bilirubin (total and indirect), blood urea nitrogen, calcium (total), carbon dioxide, chloride, creatinine, γ-glutamyltransferase, lactate dehydrogenase, magnesium, potassium, and sodium.
- CBC with reticulocyte, differential (automated) and blood smear central and local testing (Section 5.6.3)
- CD34+ cell count (performed locally; Section 5.5.1 and Section 5.5.2)
- Urinalysis: Macroscopic analysis (with reflex microscopic analysis), albumin, and microalbumin
- Reproductive testing (performed before fertility preservation [Section 5.6.4] for males and females; at a time point after fertility preservation for females; and at Month 12, Year 2, and Year 5 for males and females):
    - Female: luteinizing hormone, follicle-stimulating hormone, estradiol, and anti-Müllerian hormone. The date of the last menstrual period should be obtained at the time of hormonal testing to analyze results based on cyclical fluctuations.
    - Male: luteinizing hormone, follicle-stimulating hormone, testosterone, and inhibin B
- Renal function: cystatin C eGFR
- Infectious disease titer: cytomegalovirus antibody (immunoglobulin M and immunoglobulin G), HIV-1 and HIV-2, acute hepatitis panel, qualitative syphilis test, human T-lymphotropic virus (1 and 2), varicella zoster virus, and parvovirus
- Pregnancy testing: Serum pregnancy test at screening; local urine pregnancy tests at all subsequent visits (Note: Test should be performed at the Chemotherapy Clearance Visit only if the last test was performed at study site >48 hours before visit.)
- Additional screening tests: serum ferritin and HIV testing

Blood samples will be collected for the following genetic analyses (at screening only):

- Globin gene mapping (and globin deletional analysis if not done previously)
- Enzyme genetic analysis. Defects in the RBC enzyme, such as G6PD deficiency, are common in the SCD population and require a genetic test, as functional defects in patients with SCD cannot be determined by an enzyme assay alone. Subjects who are found to have a mutation for G6PD deficiency will be provided with guidance on concomitant medications that must be avoided.

6.3.2.1 Gene Transfer Safety Testing

Safety testing for peripheral blood gene transfer will be performed as indicated in Table 13-1 and Table 13-2. For subjects who have not yet transitioned to the LTFU study, analysis after Year 2 will also be performed as indicated in Table 13-3. Testing will include the following:

- Replication competent LV assay: If all post-infusion assay results are negative in the first year, yearly follow-up samples may be discontinued. If any post-infusion results are positive, assay should be performed annually thereafter until Year 15
- Vector insertion site analysis

The safety tests on BM will be performed as indicated in Table 13-1 and Table 13-2. For subjects who have not yet transitioned to the LTFU study, if there is clinical or laboratory indication of a clonal dominance or oligoclonality, BM aspirates may be obtained as indicated in Table 13-3. Testing will include the following:

- Standard morphology testing
- Flow cytometry for leukemia panel
- Genomic evaluation for myeloid malignancy (such as MDS or AML) via cytogenetic evaluation and NGS panel for myeloid malignancy
- Vector insertion site analysis

6.3.3 Physical Examinations

A complete or targeted physical examination will be conducted as indicated in the schedules of events (Appendix 1).

A complete physical examination will include height (screening visit only), weight, and evaluation of body systems, including but not limited to the following: skin; head, eyes, ears, nose, and throat; respiratory system; cardiovascular system; abdomen (liver, spleen); reproductive systems; and lymph nodes. The examinations performed at the HbS Reduction Visit(s) will only be required if >30 days have elapsed since last assessment.

A targeted physical examination will include evaluation of the subject's general appearance and other components as indicated by the subject's medical history or symptoms. A targeted physical examination should be completed within 24 hours of the start of each stem cell collection cycle.

6.3.4 Vital Sign Measurements

Vital signs will be measured as indicated in the schedules of events (Appendix 1) and include systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature). When scheduled at the same visit, vital sign measurements should be performed before collection of any blood or other samples.

6.3.5 Imaging

Because plerixafor may cause splenic rupture, a baseline abdominal ultrasound for examination of the spleen will be performed before the first dose of plerixafor is at the P-MPB Stem Cell Collection Visit.

Echocardiograms will be performed at the time points indicated in the tables in Appendix 1. An ECG will be performed at screening and at other time points during the study as clinically indicated.

Brain MRI and MRA will be performed at the screening visit only in order to assess risk of stroke (per exclusion #7 [Section 4.1.2]) and to establish the subject's baseline status. If such imaging has been performed within 6 months before the date of informed consent, and the report and images are available within the medical record, there is no need to repeat the procedure at screening.

6.3.6 Other Assessments

6.3.6.1 Pulmonary Function Tests

Pulmonary function tests (oxygen saturation, spirometry, and diffusion) will be performed at screening to ensure subjects do not meet Exclusion Criterion 5 (Section 4.1.2). Subsequent PFTs will be performed at the time points indicated in Table 13-2 and Table 13-3.

6.3.6.2 Iron Overload Status

Iron overload status will be evaluated at screening (Table 13-1) and as applicable per the investigator's discretion. At screening, subjects will be evaluated for chronic transfusions >200 mL/kg and evidence of iron overload from FerriScan imaging performed as part of standard of care and hepatic fibrosis by noninvasive liver imaging obtained per the subject's standard of care.

6.3.6.3 Nuclear Medicine GFR

Nuclear medicine GFR should be completed within 30 days of administration of chemotherapy for conditioning. This will be used to evaluate the relationship between GFR, melphalan clearance and melphalan exposure.

6.4 Safety Monitoring

6.4.1 Data Safety Monitoring Board

The DSMB is an independent group of experts who are not affiliated with any study site and who will advise the Sponsor regarding the future of the study. The members of the DSMB serve in an individual capacity and provide their expertise and recommendations. The DSMB will be comprised of individuals with expertise in hematology, stem cell transplantation, and biostatistics. The DSMB will review the safety data from each subject before treating the next subject, although the DSMB may propose to alter this requirement as detailed in the DSMB charter. Details on the composition, meeting frequency, and other activities of the DSMB are outlined in the DSMB charter.

The following are the primary responsibilities of the DSMB:

- Periodically review and evaluate the accumulated study data for subject safety, study conduct, and progress.

- Make recommendations to the Sponsor concerning the continuation, modification, or termination of the study.

The DSMB reviews will include but will not be limited to the following (see the DSMB charter for details):

- SAEs, adverse events of special interest, and other specific AEs as described in DSMB charter
- Toxicities of infused product
- Toxicities related to gene transfer
- Positive microbial culture results
- Safety and feasibility criteria (Section 6.4.2)
- Early stopping rules (Section 6.4.3)

6.4.2 Safety and Feasibility Criteria

The primary toxicities for this study are those related to stem cell collection and chemotherapy and those related to the use of gene transfer.

6.4.2.1 Safety Criteria: Toxicity

Safety criterion will be defined as follows: Toxicity events will be graded using CTCAE v5.0. The investigator or designee will be responsible for determining the attribution of events to study-related procedures. Study enrollment and procedures may be suspended pending DSMB review in the event of failing to meet the safety criterion as defined below and in Section 6.4.3.

All irreversible/unresolvable Grade 3-4 toxicities that are probably or definitely attributable to the study procedure/s, and not the subject's SCD, will be considered unsafe.

The following will specifically constitute a failure to meet the safety criteria:

- Grade 3 allergic reaction associated with infusion of the transduced cell product
- Grade 4 infection following infusion of the transduced cell product uncontrolled for >14 days

- Grade 4 neutropenia lasting >1 month following melphalan
- Grade 3 or 4 irreversible organ toxicity–neurologic, pulmonary, cardiac, GI, genitourinary, hepatic, or cutaneous–that is attributable to the study procedures
- Death due to study procedures
- Hematological malignancy due to vector insertion
- Hematologic malignancy related to investigational product or study medications/procedures

If a hematological cancer is reported in this study, the study will be temporarily suspended pending further analysis by the Sponsor and review by DSMB, to determine if the malignancy is attributable to the gene transfer procedure A detailed molecular analysis of the malignant clone will be performed in order to determine attribution to gene transfer. Insertional site analysis of the malignant clone and effect of the insertion on flanking cellular genes will be done to determine if the malignancy is caused by insertion of the vector.

If a subject should develop a hematopoietic malignancy while on the study, the subject will be referred to an oncologist. The oncologist will be contacted by the study investigator to review the subject's collected medical history and relay research-related information. Oncology records will be requested. Additional biological specimens may also be requested if needed to evaluate the causality of the malignancy. The study investigator will remain in contact with the subject's oncologist throughout the treatment period.

6.4.2.2 Feasibility Criteria

The feasibility criteria are defined as follows:

- Adequate harvest and recovery of CD34+ cells
- Transduced cell product meets release criteria
- Successful engraftment of ex-vivo manipulated gene-modified cells determined in BM aspirates at 1-year post-infusion with ≥1% gene-marked cells.

Failure to meet any one of the above 3 criteria will be considered as failure to meet the feasibility criteria.

6.4.3 Early Study Stopping Rules

The adult SCD population is a high-risk population where treatment-related mortality rates from HSCTs are nearly 25% following myeloablative conditioning for HSCT. A recent analysis of compiled HSCT data shows that even among children, adolescents do worse than younger children with a much lower overall and disease-free survival. Hence, an expected higher rate of toxicity and lack of feasibility is expected in the adult subject population in this study.

In the initial cohort of 6 subjects, the target is to obtain ≥3 subjects (out of 6) who meet both the safety and feasibility criteria. Accrual will be suspended pending DSMB review as specified in Section 6.4.2 if 1 or more of the first 3 subjects do not meet the safety criteria or 3 or more of 6 subjects do not meet either the safety or the feasibility criteria.

The study will be stopped if 2 of the first 3 subjects fail to meet the safety criteria or at least 4 subjects (of 6 subjects) fail to meet both the safety and feasibility criteria.

Up to 2 of 6 subjects are allowed to fail to meet the safety criteria (with the exception noted previously where study is stopped if 2 of first 3 subjects fail to meet safety criteria) or up to 3 of 6 subjects are allowed to fail to meet the feasibility criteria, since the safety and feasibility is expected to be low among adults due to the cumulative damage to organs and BM in subjects with severe SCD and the high risk from the chemotherapy conditioning regimen. Therefore, the target probability of the study drug being both safe and feasible is 48% to ~64%.

Table 6-3 presents the probability of early termination. The probability was calculated as: Prob (at or before 6 subjects) = Prob (safety criterion triggers the study to terminate early for the first 3 subjects) + Prob (safety criterion does not trigger the study to terminate early for the first 3 subjects, and the safety and feasibility criteria triggers the study to terminate for the first 6 subjects).

Table 6-3 Probability of Early Termination in the First Stage of the Initial Cohort (6 Subjects)

	Probability (being safe) / Feasibility Rate			
	Scenario A	**Scenario B**	**Scenario C**	**Scenario D**
	0.8 / 20%	**0.8 / 80%**	**0.6 / 20%**	**0.6 / 80%**
Probability (both safe and feasible)	0.16	0.64	0.12	0.48
At or before 3 subjects	10.4%	10.4%	35.2%	35.2%
At or before 6 subjects	33.2%	12.2%	64.6%	41.3%

Scenario B and D present cases close to the assumed target rate. When the probability of being both safe and feasible is 0.64 (64%), the study will be terminated at or before 3 subjects with 10.4% probability, whereas at or before 6 subjects with 12.2% probability (Scenario B). Scenario D shows that at much higher rates when the intervention is only moderately safe, although the overall probability of being both safe and feasible is close to the target.

In the cohort of 4 subjects, the target is to obtain ≥2 subjects who will meet both the safety and feasibility criteria. The study would suspend accrual pending DSMB review if the safety criteria are not met at any point.

6.5 Feasibility and Efficacy Assessments

The feasibility and efficacy endpoints in this study will be measured by analysis of BM and peripheral blood samples.

6.5.1 Feasibility Assessments

Feasibility of gene transfer will be measured by peripheral blood and/or BM sample analysis as indicated in Table 13-1 and Table 13-2. For subjects who have not yet transitioned to the LTFU study, analysis after Year 2 will also be performed as indicated in Table 13-3. Feasibility assessments include the following:

- Total number of CD34+ cells collected in peripheral blood and BM
- Amount of transduced cells (CD34+ cells/kg body weight) from all collections combined
- Percentage of gene-marked cells in BM aspirates

6.5.2 Efficacy Assessments

The efficacy of gene transfer will be measured by analysis of HbF in peripheral blood by 1) quantification of HbF and other variants; 2) vector copies in the WBC fraction; 3) the proportion of antisickling/sickling hemoglobin, (HbF+Hb^{G16D}+HbA2)/HbS; and 4) RBC testing.

Efficacy will be measured from BM samples for CFU-c analysis for gene-marked CFU-c. After the first year of BM sample collection, if no vector marking in peripheral blood WBC is observed, BM aspirates will be discontinued.

6.5.2.1 Hemoglobin Electrophoresis

Hemoglobin will be evaluated using peripheral blood as follows:

- Hemoglobin electrophoresis analysis (CZE) for HbF and other variants: Performed before ARU-1801 infusion and as indicated in Table 13-1 and Table 13-2. For subjects who have not yet transitioned to the LTFU study, analysis after Year 2 will also be performed as indicated in Table 13-3.

6.5.2.2 Peripheral Blood Gene Transfer Testing

Efficacy assessments of peripheral blood gene transfer will be performed as indicated in Table 13-1 and Table 13-2. For subjects who have not yet transitioned to the LTFU study, analysis after Year 2 will also be performed as indicated in Table 13-3. Testing will include the following:

- Vector copy number (VCN) analysis on DNA by quantitative polymerase chain reaction (qPCR) on DNA from WBC fraction
- VCN analysis (qPCR) on DNA from sorted cell subpopulations of erythroid, myeloid and B and T cells
- F-RBC and F-retics by flow cytometry
- RBC testing (P50 [partial pressure of oxygen in the blood at which hemoglobin is 50% saturated] and ektacytometry with Oxygenscan); performed at institutions with the capability to run the tests, or samples will be shipped within the stability window to an institution or laboratory with the capability to perform the testing.

- Mononuclear cells and plasma for archiving

6.5.2.3 Bone Marrow Gene Transfer Testing

Efficacy assessments of BM will be performed as indicated in Table 13-1 and Table 13-2. For subjects who have not yet transitioned to the LTFU study, if there is clinical or laboratory indication of a clonal dominance or oligoclonality, BM aspirates may be obtained as indicated in Table 13-3. Testing will include the following:

- VCN analysis by qPCR on DNA isolated from BM mononuclear cells and sorted myeloid, T, B and precursor erythroid hematopoietic lineages
- CFU-c assay for vector copies by qPCR on individual CFU-c

6.5.3 Incidence of SCD-Related Events and Parental Opioid Use

Subjects should be queried throughout the study as indicated in the schedules of events (Appendix 1) for any new occurrences of SCD-related events and parental opioid use. The incidence of SCD-related events and parental opioid use should also be completed within 24 hours of the start of each stem cell collection cycle.

For subjects enrolled under previous versions of the protocol (before Version 8.0), which did not stipulate recording of all VOEs in the study, the investigators or designee will query existing study source documents and medical records in a retrospective manner. The investigator or designee will record VOE information in the study database for VOEs previously collected in source documents or medical records but not entered into the study database.

Vaso-occlusive episodes include vaso-occlusive crises and other VOEs, including but not limited to ACS, stroke, priapism, dactylitis, and acute hepatic or splenic sequestration.

The following endpoint definitions are listed below to aid in the characterization of SCD-related events:

- Vaso-occlusive crisis are acute episodes of pain, with no medically determined cause other than a VOE, that resulted in a medical facility visit (emergency department, clinic, or hospital) and treatment with narcotic agents or with nonsteroidal anti-inflammatory drugs.
- Acute chest syndrome is an acute illness characterized by fever and/or respiratory symptoms, accompanied by a new pulmonary infiltrate on x-ray.
- Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

6.5.4 Performance Status

A modified Karnofsky Performance Status Scale will be administered at the time points indicated in the schedules of events (Appendix 1). The modified Karnofsky Scale is designed for recipients aged ≥10 years (Appendix 2). The scale is used to determine the score (10-100) that best represents the subject's activity status at the requested time point.

6.5.5 Patient-Reported Outcomes: ASCQ-Me System

As part of the secondary objectives of this study, changes from baseline in PROs will be assessed using the ASCQ-Me (Appendix 4). This PRO assessment should be administered in the subject's local language at the time points indicated in the schedules of events (Appendix 1). The ASCQ-Me should be administered before any other tests, treatments, or receipt of any test result to avoid biasing the subject's perspective.

The ASCQ-Me is a PRO measurement system that assesses the physical, social, and emotional impact of SCD on adults. It includes questions enabling adults to describe their functioning and well-being according to 7 topics (emotional impact, pain episodes, pain impact, SCD medical history checklist, sleep impact, social functioning impact, and stiffness impact; Appendix 4). It is a copyrighted instrument and is relatively new, with development initiated in 2005. Substantial qualitative and quantitative evidence has been gathered that supports the validity of ASCQ-Me scores in discriminating levels of symptoms and outcomes important to individuals with SCD (Keller et al 2014).

Subjects should be given sufficient space and time to complete the ASCQ-Me, and all administered questionnaires should be reviewed for completeness. If missing responses are noted, subjects should be encouraged to complete and missing responses. Attempts should be made to collect responses to all questionnaires for all subjects; however, if a subject refuses to complete the questionnaire, this should be documented in the study source records. A subject's refusal to complete study questionnaires will not be a protocol deviation.

Completed questionnaires, including both response to the questions and any unsolicited comments written by the subject, must be reviewed and assessed by the investigator or designee for responses that may indicate potential AEs or SAEs. This review should be documented in the study source records.

If an AE or SAE is confirmed, the investigator should record the event as instructed in Section 6.3.1.4 or Section 6.3.1.5, respectively. Investigators should not encourage the subjects to change responses reported in questionnaires.

6.6 Pharmacokinetic Assessments

Blood samples will be collected for analysis of melphalan PK for all subjects.

The conditioning dose will be administered and associated PK blood sampling will occur at the Chemotherapy Visit (Section 5.5.3), approximately 7 days after the Chemotherapy Clearance Visit.

Blood samples for quantification of melphalan concentration in plasma will be collected at pre-dose (within 60 minutes before the start of the melphalan infusion), immediately at the end of the melphalan infusion, and approximately 5 minutes, 15 minutes, 1 hour, 2 hours, 4 hours, and 6 hours after the end of the infusion. The actual date and time of the start and stop of the melphalan infusion and the actual date and time of each melphalan PK sample collection will be recorded.

Plasma melphalan concentrations will be determined using a validated analytical method under the direction of the Sponsor. Details of the PK sample collection, processing, storage, and shipping will be provided in the Laboratory Manual.

6.7 Pregnancy

Female subjects should refrain from getting pregnant or planning to become pregnant for 1 year after the ARU-1801 infusion. Male subjects should also refrain from a pregnancy with their prospective partner or planning to become pregnant for 1 year after the ARU-1801 infusion, due to the teratogenic side effects of melphalan conditioning.

Female subjects who become pregnant before the gene transfer procedure will be withdrawn from the study. They may be rescreened for potential inclusion once postpartum and no longer breastfeeding. Subjects who become pregnant after gene transfer may have selected study procedures deferred per investigator's discretion (eg, BM procedures).

All pregnancies, for both enrolled subjects and their prospective partners, from the time of informed consent until the end of the study will be reported on the appropriate pregnancy eCRF. For subjects who have not yet transitioned to the LTFU study, pregnancies will be reported on the appropriate pregnancy eCRF until the Year 15 follow-up visit.

Pregnancy is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication. Any pregnancy that occurs within 2 years (≤2 years) of the ARU-1801 infusion must be reported using the same procedures as for reporting an SAE (Section 6.3.1.5). To ensure subject safety, each pregnancy must be reported to Aruvant or designee within 2 weeks of learning of its occurrence. The pregnancy must be followed up to determine outcome (including spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) and status of mother and child, even if the subject was discontinued from the study. The child will be followed for 2 months after birth. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous miscarriages must be reported as an SAE.

6.8 Future Testing

Baseline and follow-up samples (plasma, blood cells and serum) will be collected for potential future biomedical research or biomarker analyses related to gene therapy and/or SCD for those subjects who consent to participate in this component of the study. Participation is optional. Blood samples will be collected at the time points indicated in

Appendix 1.If subjects provide consent, any unused intermediates from the ARU-1801 manufacturing process will also be utilized for research purposes.

A small portion of the final transduced product may be retained for research purposes for those subjects who consent to participate in this component of the study. Participation is optional.

The Sponsor will store the future testing samples in a secure storage space with adequate measures to protect confidentiality. The samples will be stored for no longer than the planned duration of the subject's follow-up, including the intended LTFU study (approximately 15 years).

7 Statistical and Analytical Plan

This is a clinical study that primarily concerns the safety and feasibility of the proposed treatment procedures in adult SCD subjects aged 18 to 45 years. The study uses a 2-stage design: in the first stage a cohort of 6 subjects will be accrued. Upon the establishment of the safety and feasibility of the procedures in this cohort, a cohort of up to 4 additional subjects will be enrolled in the second stage to further establish the safety, feasibility, and efficacy, for a total of 10 subjects treated in the study.

The study endpoints are presented in Section 2.2. The primary endpoints of the study are the safety and feasibility of the proposed treatment procedures. Safety endpoints include the number of subjects that experience significant toxicity events, the incidences of significant toxicity events, and time to neutrophil and platelet recovery. Feasibility endpoints include the number of subjects that meet each feasibility criteria and the recovery rate of CD34+ cells for gene transfer. The secondary endpoints are for efficacy. Baseline for the secondary efficacy endpoint for disease severity is defined in Section 7.6.2. Baseline for other study variables will be defined in the statistical analysis plan (SAP), which will be finalized before the study database lock.

7.1 Primary Safety and Feasibility Endpoints

The primary safety endpoints are presented in Section 2.2.1. Of particular interest is whether a subject fails to meet any one of the safety criteria, as listed in Section 6.4.2.1.

The primary feasibility endpoints are presented in Section 2.2.1. If a subject fails to meet any one of the feasibility endpoints, the subject will be considered as having failed the feasibility criteria.

7.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are presented in Section 2.2.2. The efficacy of gene transfer will be measured by the following:

- Quantification of HbF^{G16D} and other Hb subtypes, including HbF (endogenous), HbS, adult Hb (HbA), HbA2 and, if applicable, HbC and HbE

- Change in the proportion of antisickling/sickling hemoglobin ([HbF+HbF^{G16D}+HbA2]/HbS) in Months 6-24 post-transplantation compared with baseline
- Flow cytometry for percentage of F-RBC and F-retics in peripheral blood
- Determining vector copies in the WBC fraction
- Determining gene transfer in BM based on the following:
  - Vector copies
  - CFU-c analysis for gene-marked CFU-c

Change in disease severity from baseline will be measured by annualized VOEs pre-transplant versus post-transplant. Quality of life will be assessed using the ASCQ-Me.

7.3 Exploratory Endpoints

The exploratory endpoint for the PK of melphalan is presented in Section 2.2.3. Plasma PK parameters of melphalan will include AUC, Cmax, clearance, and volume of distribution.

Change in VOEs requiring parenteral opioids will also be reported.

7.4 Sample Size Calculations

The primary objective of this proof-of-concept study is to demonstrate the safety and feasibility of the proposed treatment procedures in SCD subjects. A sample size of 10 subjects is planned to obtain initial data on the safety and feasibility of γ-globin gene transfer in subjects with SCD, with enrollment in 2 stages, as described in Section 7.

As specified in Section 6.4.3, early stopping rules are in place. Accrual of subjects into the study will be suspended pending DSMB review (see Section 6.4.2) if 1 or more of the first 3 subjects do not meet the safety criteria or 3 or more of 6 subjects do not meet either the safety or the feasibility criteria. The study will be stopped if 2 of the first 3 subjects fail to meet the safety criteria or at least 4 subjects (of 6 subjects) fail to meet both the safety and feasibility criteria.

According to the study stopping criteria and assuming the probability of being both safe and feasible to be 0.64 (64%), the study will terminate at or before 3 subjects with a probability

of 0.104, and at or before 6 subjects with a probability of 0.122. For details see Scenario B in Table 6-3.

7.5 Analysis Sets

The following analysis sets will be used in the statistical analyses.

Safety analysis set: The safety analysis set will consist of all subjects who received the proposed treatment procedure.

Full-analysis set (FAS): The FAS will consist of all subjects who received the proposed treatment procedure and had a least 1 post-infusion efficacy measurement.

PK set (PKS): The PKS will consist of FAS subjects who receive melphalan and have at least 1 measurable plasma concentration of melphalan.

7.6 Statistical Analysis Methodology

Statistical analysis will be performed using SAS software (SAS Institute, Inc, Cary, North Carolina) Version 9.3 or later. Continuous variables (absolute values and change from baseline values) will be summarized using the mean, standard deviation, median, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages. Shift tables from baseline to post-infusion visits may be provided. Data will be listed in data listings. In general, the change from baseline for continuous variables will be analyzed using the 1-sample paired *t*-test, and the categorical variables will be analyzed by chi-square or Fisher exact test on the shift table.

Given the small sample size and single-arm design of the study, the focus of the statistical analysis will be on the provision of point estimates with 2-sided 95% confidence intervals. In addition, some exploratory informal testing analyses will be performed as outlined in Section 7.6.4, using a 2-sided significance level of 0.15 (15%) for informational purposes only.

Details of the statistical analyses, methods, and data conventions will be described in the SAP, which will be finalized before the study database lock.

7.6.1 Analysis of Primary Safety and Feasibility Endpoints

Summary statistics of the number of subjects that failed the safety, feasibility, or both safety and feasibility criteria will be provided. For each of the safety and feasibility endpoints, summary statistics will be provided. The incidence of AEs and SAEs will also be provided.

Moreover, the summary of the safety endpoints will be broken down into the following 3 distinct parts:

- Part 1 will include those events from study entry to the recovery from the stem cell collections.
- Part 2 will include those events from immediately before administration (baseline) through 30 days after gene transfer infusion.
- Part 3 will include capturing subject status at Day +30 post-infusion, defined as baseline through the remainder of the study.

7.6.2 Analysis of Secondary Efficacy Endpoints

For each of the secondary gene transfer efficacy endpoints, summary statistics will be provided.

Summary statistics will be presented for change from baseline in disease severity (eg, annualized VOE pre-transplant versus post-transplant). Baseline disease severity will be determined from a retrospective review of the subject's medical records for the last 2 years before the screening visit.

Summary statistics will be presented for ASCQ-Me scores at baseline and by post-baseline visits.

7.6.3 Analysis of Exploratory Pharmacokinetic Endpoint

Plasma PK parameters for melphalan will be estimated from the plasma concentration versus time data for all evaluable subjects by noncompartmental methods using Phoenix® WinNonlin® (Certara LP, Princeton, New Jersey) Version 8.0 or higher.

Individual plasma concentration of melphalan will be listed for each sampling time point and summarized using descriptive statistics (number of subjects, mean, standard deviation,

coefficient of variation, minimum, median, and maximum). Plasma PK parameters for melphalan including, but not limited to AUC from time zero (pre-dose) to time of last quantifiable concentration (AUC[0-t]), AUC from time zero (pre-dose) extrapolated to infinite time (AUC[0-∞]), C_{max}, clearance, and volume of distribution will be listed individually for each subject and summarized using descriptive statistics (number of subjects, mean, standard deviation, coefficient of variation, geometric mean, geometric coefficient of variation, minimum, median, and maximum). Dose-normalized parameters also will be calculated. Individual and mean plasma concentration versus time profiles for melphalan will be plotted in both linear and semilogarithmic scales.

The relationship between melphalan AUC and safety and efficacy endpoints will be explored using descriptive statistics. Correlation coefficients will be generated between PK versus pharmacodynamic parameters.

Detailed PK analysis methods will be provided in the SAP.

7.6.4 Exploratory and Other Analyses

Summary statistical analyses will be provided for demographics, medical history, physical examination, and risk factor variables at baseline. The Karnofsky Performance Status Scale will be summarized by visit.

Summary statistics will be presented for change from baseline VOEs requiring parenteral opioids. Baseline parenteral opioid use will be determined from a retrospective review of the subject’s medical records for the last 2 years before the screening visit.

The following exploratory informal testing analyses will be performed, using a 2-sided significance level of 0.15 (15%) for informational purposes only:

- Chi-Square test for a single proportion will be used to test the following feasibility and safety endpoints against a predefined threshold (which will be defined in the SAP):
  - Proportion of subjects with a total number of CD34+ cells recovered from all collections combined (mobilized peripheral blood and BM) of at least 8×10^6/kg viable CD34+ cells
  - Proportion of subjects for which a minimum of 4×10^6 CD34+ cells/kg body weight from all collections combined have been successfully transduced
  - Mortality rate
- Paired *t*-test will be used to assess the following changes from baseline:
  - Change of adult HbS measurements from baseline
  - Change in the proportion of antisickling/sickling hemoglobin ([HbF+HbF^{G16D}+HbA2]/HbS) from baseline

Detailed descriptions of these exploratory analyses will be documented in the SAP, which will be finalized before the study database lock.

7.6.5 Interim Analyses

No formal interim analysis is planned. However, the primary objective of this unblinded, proof-of-concept study is to show the safety and feasibility of the proposed treatment procedure in SCD subjects. An independent group of experts, therefore, will advise the Sponsor regarding the future of the study by periodically reviewing and evaluating the accumulated study data for subject safety, study conduct, and progress, as specified in Section 6.4.1.

Analyses may be performed for planning and regulatory purposes, such as in support of meetings or submission discussions, or to inform planning for future development.

8 Data Quality Assurance

This study will be conducted according to the International Council for Harmonisation (ICH) E6 risk and quality processes described in the applicable procedural documents. The quality management approach to be implemented in this study will be documented and will comply with the current ICH guidance on quality and risk management.

8.1 Data Management

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include laboratory reports, radiology reports, etc.

Investigative site personnel will enter subject data into Medidata Rave. The analysis data sets will be a combination of these data and data from other sources (eg, laboratory data).

Clinical data management will be performed in accordance with applicable Aruvant standards and data cleaning procedures to ensure the integrity of the data, eg, removing errors and inconsistencies in the data. Adverse event terms will be coded using the MedDRA, an internal validated medical dictionary, and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug).

All serious breaches must be reported to the Sponsor or designee, where a serious breach is defined as a breach likely to affect, to a significant degree, the safety and rights of a participant or the reliability and robustness of the data generated in the clinical study. The Sponsor or designee is responsible for notifying the IRB/IEC/REB and, if required, regulatory authorities.

After database lock, each study site will receive a CDROM containing all of their site specific eCRF data as entered into Medidata Rave for the study, including full discrepancy and audit history. Additionally, a CDROM copy of all of the study site’s data from the study will be created and sent to the Sponsor for storage. PPD will maintain a duplicate CDROM copy for their records. In all cases, subject initials will not be collected or transmitted to the Sponsor.

9 Ethics

9.1 Institutional Review Board

Federal regulations and the ICH guidelines require that approval be obtained from an IRB/IEC/REB before participation of human subjects in research studies. Before study onset, the protocol, informed consent, advertisements to be used for the recruitment of study subjects, and any other written information regarding this study to be provided to the subject must be approved by the IRB/IEC/REB. Documentation of all IRB/IEC/REB approvals and of the IRB/IEC/REB compliance with ICH harmonised tripartite guideline E6: Good Clinical Practice (GCP) will be maintained by the site and will be available for review by the Sponsor or its designee.

All IRB/IEC/REB approvals should be signed by the IRB/IEC/REB chairman or designee and must identify the IRB/IEC/REB name and address, the clinical protocol by title or protocol number or both, and the date approval or a favorable opinion was granted.

The investigator is responsible for providing written summaries of the progress and status of the study at intervals not exceeding 1 year or otherwise specified by the IRB/IEC/REB. The investigator must promptly supply the Sponsor or its designee, the IRB/IEC/REB, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to subjects.

9.2 Ethical Conduct of the Study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki, ICH GCP, the protocol, and all applicable regulations.

9.3 Subject Information and Consent

A written informed consent in compliance with US Title 21 Code of Federal Regulations (CFR) Part 50 shall be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An informed consent template may be provided by the Sponsor to investigative sites. If any institution-specific modifications to study-related procedures are proposed or made by the site, the consent should be reviewed by the Sponsor or its designee or both before IRB/IEC/REB submission. Once reviewed, the consent will be submitted by the investigator to his or her IRB/IEC/REB

for review and approval before the start of the study. If the ICF is revised during the course of the study, all active participating subjects must sign the revised form.

Subjects will be provided an IRB/IEC/REB-approved informed consent document to review. The study investigator or designee will review the content of this document with the subject.

Subjects will be allowed sufficient time to ask questions and consider participation in the study. Subjects will be explicitly informed that choosing not to participate will in no way affect the quality of the medical care that they will receive.

In addition, the consenting process will be witnessed by a person/advocate not directly involved in the study. The advocate should have knowledge of the informed consent process; however, the advocate does not need to be familiar with the study protocol. The advocate should document that sufficient time was allowed to review the consent, to ask questions, and to allow the subject to consider enrolling in the study. The advocate should document that the subject appears to understand what the study involves, understand the importance of coming to all study visits, and understand the potential risks. This will be documented by the advocate's signature on the consent form.

Prior to commencement of study procedures, the subject will be questioned again, in the presence of a witness, regarding his/her willingness to proceed.

The investigator shall retain the signed original ICF(s) and give a copy of the signed original form to the subject. If subjects are seen at multiple sites, the responsibilities of the principal investigators with regard to consent should be followed as detailed in the Subject Management Plan (see Section 10).

10 Investigator's Obligations

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes will be reported to the IRB/IEC/REB but will not result in protocol amendments.

In this study, the investigator will be the person responsible for the conduct of the study at a site. At sites where a team of individuals are involved in conducting the study, the investigator will be the responsible leader of the team and will be referred to as the principal investigator. Subinvestigators are any individual members of the clinical trial team designated and supervised by the investigator at a trial site to perform critical study-related procedures and/or to make important study-related decisions. Each principal investigator will maintain a list of appropriately qualified persons to whom the principal investigator has delegated significant study-related duties and ensure that all persons assisting with the study are adequately informed about the protocol, the investigational product, and their study-related duties and functions. For subjects who are seen at multiple sites, principal investigator responsibilities are detailed in the Subject Management Plan, which will outline issues related to the informed consent process, safety monitoring activities (including AE reporting), and, if applicable, transfer of responsibilities.

10.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject, except as necessary for monitoring and auditing by the Sponsor, its designee, the FDA, or the IRB/IEC/REB.

The investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

10.2 Financial Disclosure and Obligations

Investigators are required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigator must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Neither the Sponsor nor PPD is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, neither the Sponsor nor PPD is financially responsible for further treatment of the subject's disease.

10.3 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ICH E6 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- IRB/IEC/REB approval.
- Original investigator-signed investigator agreement page of the protocol.
- Form FDA 1572, fully executed, and all updates on a new fully executed Form FDA 1572 (or equivalent forms, as applicable, for non-US sites).
- Curriculum vitae for the investigator and each subinvestigator listed on Form FDA 1572 (or equivalent forms, as applicable, for non-US sites).
- Financial disclosure information to allow the Sponsor to submit complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigators must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.

- IRB/IEC/REB-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject.
- Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with 42 CFR 493.

10.4 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH E6. The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. Study information from this protocol will be posted on publicly available clinical trial registers before enrollment of subjects begins.

10.5 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6 and all applicable guidelines and regulations.

10.6 Adverse Events and Study Report Requirements

By participating in this study, the investigator agrees to submit reports of SAEs to the Sponsor and/or IRB/IEC/REB according to the time line and method outlined in the protocol. In addition, the investigator agrees to submit annual reports to the study site IRB/IEC/REB as appropriate.

10.7 Investigator's Final Report

Upon completion of the study, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC/REB with a summary of the study's outcome and the Sponsor and regulatory authority(ies) with any reports required.

10.8 Records Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory

requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10.9 Publications

After completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the Sponsor will be responsible for these activities and will work with the investigators to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues. The Sponsor has final approval authority over all such issues.

Data are the property of the Sponsor and cannot be published without prior authorization from the Sponsor, but data and publication thereof will not be unduly withheld.

11 Study Management

The administrative structure will include an DSMB. Details on the composition and the activities of the DSMB are outlined in the DSMB charter.

11.1 Monitoring

11.1.1 External Data Monitoring Committee

An external DSMB will regularly monitor safety in this study; details are provided in Section 6.4.1.

11.1.2 Monitoring of the Study

The clinical monitor, as a representative of the Sponsor, has the obligation to follow the study closely. In doing so, the monitor will visit the investigator and study site at periodic intervals, in addition to maintaining necessary telephone and letter contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and personnel.

All aspects of the study will be carefully monitored, by the Sponsor or its designee, for compliance with applicable government regulation with respect to current GCP and current standard operating procedures.

11.1.3 Inspection of Records

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB/IEC/REB review, and regulatory inspections by providing direct access to all study records. In the event of an audit, the investigator agrees to allow the Sponsor, representatives of the Sponsor, or a regulatory agency access to all study records.

The investigator should promptly notify the Sponsor and PPD of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the Sponsor.

11.2 Management of Protocol Amendments and Deviations

11.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the subject, must be reviewed and approved by the Sponsor or its designee. Amendments to the protocol must be submitted in writing to the investigator's IRB/IEC/REB for approval before subjects can be enrolled into an amended protocol.

11.2.2 Protocol Deviations

The investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study subjects without prior IRB/IEC/REB approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB/IEC/REB for review and approval, to the Sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the Sponsor and the IRB/IEC/REB and agreed to by the investigator. A significant deviation occurs when there is nonadherence to the protocol by the subject or investigator that results in a significant, additional risk to the subject or has a significant impact on data quality or the scientific integrity of the study. Significant deviations can include nonadherence to inclusion or exclusion criteria, or nonadherence to FDA regulations or ICH GCP guidelines, and will lead to the subject being withdrawn from the study (Section 4.2).

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Principal investigators will be notified in writing by the monitor of deviations. The IRB/IEC/REB should be notified of all protocol deviations in a timely manner.

11.3 Study Termination

Although Aruvant has every intention of completing the study, Aruvant reserves the right to discontinue the study at any time for clinical or administrative reasons. Predefined early

study stopping rules are detailed in Section 6.4.3. Should the Sponsor decide to terminate the study subjects who received ARU-1801 will be followed for safety for 15 years.

The end of the study is defined as the date on which the last subject completes the last visit (includes follow-up visits).

11.4 Final Report

Whether the study is completed or prematurely terminated, the Sponsor will ensure that the clinical study reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The Sponsor will also ensure that the clinical study reports in marketing applications meet the standards of the ICH harmonised tripartite guideline E3: Structure and content of clinical study reports.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results.

Upon completion of the clinical study report, the Sponsor will provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate. The study results will be posted on publicly available clinical trial registers.

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13 Appendices

13.1 Appendix 1: Schedules of Events (Screening and Study Treatment Visits, Post-infusion Visits, and Long-term Follow-up Visits)

Table 13-1 Schedule of Events: Screening and Study Treatment Visits

Assessment	Screening Visit	HbS Reductive Visit[a]	Stem Cell Collection Clearance Visit[b] (HbS ≤20%)	Stem Cell Collection Visit[c]	Chemo-therapy Clearance Visit	Chemo-therapy Visit[d]	Infusion Visit (Day 0)
Visit Window	**NA**	**Before stem cell collection[a]**	**≤7 days before stem cell collection**	**≥2 weeks after CD34+ kinetics**	**≤7 days before chemo-therapy**	**≥36 hours before infusion**	**NA**
Screening and Other Clinical Assessments							
Informed consent	X						
Inclusion/exclusion criteria	X						
Demographics	X						
Medical history, including reproductive health, VOE, opioid use, and other sickle events[e]	X						
Physical examination (complete)[f]	X	X[g]	X				
Physical examination (targeted)[h]				X[i]	X	X	X
Vital sign measurements	X	X	X	X	X	X	X
SCD-related events and parental opioid use		X	X	X[i]	X	X	X
Iron overload status[j]	X						

Assessment	Screening Visit	HbS Reductive Visit[a]	Stem Cell Collection Clearance Visit[b] (HbS ≤20%)	Stem Cell Collection Visit[c]	Chemo-therapy Clearance Visit	Chemo-therapy Visit[d]	Infusion Visit (Day 0)
Visit Window	**NA**	**Before stem cell collection[a]**	**≤7 days before stem cell collection**	**≥2 weeks after CD34+ kinetics**	**≤7 days before chemo-therapy**	**≥36 hours before infusion**	**NA**
Abdominal (spleen) ultrasound			X[k]				
Echocardiogram	X						
Electrocardiogram	X						
Brain MRI and MRA[l]	X						
Review concomitant medications	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X
Karnofsky performance status	X		X		X		
Patient-reported outcomes (ASCQ-Me)		X			X		
Pulmonary function tests (oxygen saturation, spirometry, and diffusion)	X						
Nuclear medicine GFR					X[y]		
Clinical Laboratory Testing							

Assessment	Screening Visit	HbS Reductive Visit[a]	Stem Cell Collection Clearance Visit[b] (HbS ≤20%)	Stem Cell Collection Visit[c]	Chemo-therapy Clearance Visit	Chemo-therapy Visit[d]	Infusion Visit (Day 0)
Visit Window	**NA**	**Before stem cell collection[a]**	**≤7 days before stem cell collection**	**≥2 weeks after CD34+ kinetics**	**≤7 days before chemo-therapy**	**≥36 hours before infusion**	**NA**
Clinical chemistry	X		X		X		X
Urinalysis	X		X		X		
Urine microalbumin	X						
CBC with reticulocyte and differential (automated)[m]	X	X	X	X	X	X[m]	X
Serum ferritin	X						
Reproductive testing[n]	X						
Globin gene mapping (and globin deletional analysis if not done previously)	X						
Enzyme genetic analysis	X						
Serum pregnancy test	X						
Urine pregnancy test		X[o]	X[o]	X[o]	X[o]	X	
Hepatitis and HIV testing	X						
Cystatin C eGFR	X		X		X		

Assessment	Screening Visit	HbS Reductive Visit[a]	Stem Cell Collection Clearance Visit[b] (HbS ≤20%)	Stem Cell Collection Visit[c]	Chemo-therapy Clearance Visit	Chemo-therapy Visit[d]	Infusion Visit (Day 0)
Visit Window	**NA**	**Before stem cell collection[a]**	**≤7 days before stem cell collection**	**≥2 weeks after CD34+ kinetics**	**≤7 days before chemo-therapy**	**≥36 hours before infusion**	**NA**
Infectious disease titer			X				
Hemoglobin electrophoresis	X		X				
Quantitative HbS only		X	X	X[p]	X		
Stem Cell Preparation and Collection							
Plerixafor administration (240 µg/kg)				X			
HSC collection via P-MPB				X			
CD34+ cell count[q]				X[q]			
Gene Transfer Testing							
Blood: RCL assay[r]			X				
Blood: mononuclear cells and plasma for archiving[s]			X				
Blood: F-RBC and F-retics by flow cytometry[r]			X				

Assessment	Screening Visit	HbS Reductive Visit[a]	Stem Cell Collection Clearance Visit[b] (HbS ≤20%)	Stem Cell Collection Visit[c]	Chemo-therapy Clearance Visit	Chemo-therapy Visit[d]	Infusion Visit (Day 0)
Visit Window	**NA**	**Before stem cell collection[a]**	**≤7 days before stem cell collection**	**≥2 weeks after CD34+ kinetics**	**≤7 days before chemo-therapy**	**≥36 hours before infusion**	**NA**
RBC testing[s]		X[s]					
Blood sample for future testing (optional)[t]		X	X				
Bone marrow: standard morphology assessment[u]		X[u]					
Bone marrow: flow cytometry for leukemia panel[u]		X[u]					
Bone marrow: Genomic evaluation for myeloid malignancy[u]		X[u]					
Bone marrow sample for gene transfer testing[u, v]		X[u]					
Study Treatment Administration and Pharmacokinetic Assessments							
Melphalan Dose Modeling					X		
Administration of melphalan						X	

Assessment	Screening Visit	HbS Reductive Visit[a]	Stem Cell Collection Clearance Visit[b] (HbS ≤20%)	Stem Cell Collection Visit[c]	Chemo-therapy Clearance Visit	Chemo-therapy Visit[d]	Infusion Visit (Day 0)
Visit Window	**NA**	**Before stem cell collection[a]**	**≤7 days before stem cell collection**	**≥2 weeks after CD34+ kinetics**	**≤7 days before chemo-therapy**	**≥36 hours before infusion**	**NA**
Melphalan PK sample collection[w]						X	
ARU-1801 infusion[x]							X

Abbreviations: ASCQ-Me, adult sickle cell quality-of-life measurement; CBC, complete blood count; CFU-c, colony-forming unit cells; eGFR, estimated glomerular filtration rate; F-RBC, fetal hemoglobin content in red blood cells; F-retics, fetal hemoglobin content in reticulocytes; HbF, fetal hemoglobin; HbS, sickle hemoglobin; HIV, human immunodeficiency virus; HSC, hematopoietic stem cell; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; NA, not applicable; P50, partial pressure of oxygen in the blood at which hemoglobin is 50% saturated; PK, pharmacokinetic; P-MPB, plerixafor-mobilized peripheral blood; qPCR, quantitative polymerase chain reaction; RBC, red blood cell; RCL, replication competent lentivirus; SCD, sickle cell disease; VOE, vaso-occlusive episode.

a. Subjects enrolled in this study will begin HbS reduction to ≤30% via erythrocytapheresis or transfusions over a period of 2 to 3 months. Multiple visits may be needed and will be permitted in order to achieve the target HbS at least 2 months before HSC collection; frequency of visits is at the investigator's discretion (Section 5.5.1).

b. Within 7 days prior to stem cell collection, the subject should have documented HbS <20% or have completed erythrocytapheresis to the goal HbS of ≤20%. The Stem Cell Collection Clearance Visit may be repeated, as required, if subjects require multiple cycles for stem cell collection.

c. Subjects may undergo multiple procedures over multiple visits, if required, in order to obtain sufficient CD34+ cell content (Section 5.5.1.1).

d. Subjects should be hospitalized before the infusion of the melphalan dose for conditioning and will remain hospitalized until the investigator determines they are fit for discharge but not before the last melphalan PK sample has been collected (Section 6.6). At the investigator's discretion and based on the subject's compliance, subjects may complete clinical monitoring assessments as an outpatient (Section 5.5.3). Testing performed for clinical monitoring up to 30 days post-infusion should be recorded in the study database (CBC with reticulocyte and differential, clinical chemistry, and hemoglobin electrophoresis).

e. Available medical records pertaining to SCD status (VOEs, organ function parameters, laboratory tests, medications, transfusions, etc) may be used to obtain history from the 2-year period before enrollment. Medical history will also include prior procedures performed up to 2 years before the screening visit and significant surgeries and medical events that occurred >2 years before screening. Reproductive health history will also be

recorded. For subjects enrolled under previous versions of the protocol, baseline disease severity will also be determined from a retrospective review of medical records for the last 2 years before the screening visit.

f. A complete physical examination will include height (screening visit only), weight, and evaluation of body systems, including but not limited to the following: skin; head, eyes, ears, nose, and throat; respiratory system; cardiovascular system; abdomen (liver, spleen); reproductive systems; and lymph nodes.

g. Physical examination required only if >30 days have elapsed since last assessment.

h. A targeted physical examination will include evaluation of the subject's general appearance and other components as indicated by the subject's medical history or symptoms.

i. Physical examination (targeted) and incidence of SCD-related events and parental opioid use should be completed within 24 hours of the CD34+ kinetics assessment and the start of each stem cell collection cycle.

j. At screening, subjects will be evaluated for chronic transfusions >200 mL/kg, evidence of iron overload from FerriScan imaging performed as part of standard of care, and hepatic fibrosis by noninvasive liver imaging obtained per the subject's standard of care.

k. An abdominal ultrasound for examination of the spleen will be performed once, within 7 days before the first plerixafor dose.

l. Brain MRI and MRA will be performed at screening only, to assess risk of stroke (per exclusion #7) and to establish the subject's baseline status. If such imaging has been performed within 6 months before the date of informed consent, and the report and images are available within the medical record, there is no need to repeat the procedure at screening.

m. After chemotherapy (melphalan) administration, CBC monitoring performed at local laboratory is required at a minimum of twice weekly until absolute neutrophil count $>1\times10^9$/L and weekly thereafter until the investigator considers the subject recovered (Section 5.6.2).

n. Reproductive laboratory tests for female and male subjects are listed in Section 6.3.2. Testing will be performed before fertility preservation (Section 5.6.4) for males and females; at a time point after fertility preservation for females; and at Month 12, Year 2, and Year 5 for males and females. The date of the last menstrual period should be obtained from females at the time of testing.

o. Perform urine pregnancy test only if last test performed at study site was >48 hours before visit.

p. Quantitative HbS should be performed within 24 hours of the CD34+ kinetics assessment and Stem Cell Collection. The HbS level only needs to be performed within 24 hours of the first HSC collection per cycle (Section 5.5.1).

q. CD34+ cell count will be analyzed locally before administration of plerixafor (baseline) and at approximately 1, 2, 4 and 8 hours after plerixafor dosing to evaluate the stem cell mobilization kinetics of each subject. After consultation with the Sponsor, the collection time points may be changed and the number of blood samples collected for peripheral blood CD34+ measurements may be reduced.

r. A baseline blood sample should be collected at the first Stem Cell Collection Clearance Visit, and sample collection does not need to be repeated.

s. Testing (P50 and ektacytometry with Oxyscan) will be performed at institutions with the capability to run the tests, or samples will be shipped within the stability window to an institution or laboratory with the capability to perform the testing. The testing is performed at the HbS Reductive Visit before the first blood transfusion, then at the Month 6, Month 12 (Year 1), and Year 2 post-infusion visits. Efforts should be made to perform baseline testing before initiation of transfusions.

t. Baseline and follow-up blood samples (1 tube each for blood cells, plasma and serum) will be collected for potential future biomedical research or biomarker analyses for subjects who consent to participate in this component of the study (Section 6.8). Participation is optional.
u. The baseline bone marrow testing must be completed after enrollment and prior to initiation of additional research procedures. Specifically, the testing should be completed before a central line is placed or the subject is initiated on RBC transfusions. Results from the baseline bone marrow evaluation must be complete and reviewed by the investigator before the initiation of the first HbS Reduction visit.
v. Bone marrow samples for gene transfer testing will be performed before ARU-1801 infusion and every 6 months through Year 2 (Months 6, 12, 18, and 24), and at Year 3 (Month 36) and comprises the following (see Section 6.5.2.3): qPCR and DNA for vector copies and hematopoietic lineages, CFU-c assay for vector copies by qPCR on individual CFU-c and vector insertion site analysis.
w. Blood samples for quantification of melphalan concentration in plasma will be collected at pre-dose (within 60 minutes before start of melphalan infusion), immediately at the end of the melphalan intravenous infusion, and approximately 5 minutes, 15 minutes, 1 hour, 2 hours, 4 hours, and 6 hours after the end of infusion (Section 6.6).
x. Before ARU-1801 infusion, subjects will be premedicated with acetaminophen, diphenhydramine, and hydrocortisone (Section 5.5.5).
y. May be performed up to 30 days before the chemotherapy infusion.

Table 13-2 Schedule of Events: Post-infusion Visits (Day 7 Through Year 2)

Assessments	D7, 14, and 21	D30	D60	D90	M4 and 5	M6	M7	M8 and 9	M10	M11	M12	M15	M18	M21	Y2
Visit Window (days)	**±2**	**±5**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±14**
SCD-related events and parental opioid use	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination (complete)[a]					X	X	X	X	X	X	X	X	X	X	X
Physical examination (targeted)[b]	X	X	X	X											
Vital sign measurements	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Echocardiogram											X				X
Karnofsky performance status					X	X	X	X	X	X	X	X	X	X	X
Patient-reported outcomes (ASCQ-Me)						X					X				X

Assessments	D7, 14, and 21	D30	D60	D90	M4 and 5	M6	M7	M8 and 9	M10	M11	M12	M15	M18	M21	Y2
Visit Window (days)	**±2**	**±5**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±14**
Pulmonary function tests (oxygen saturation, spirometry, and diffusion)											X				X
Clinical Laboratory Testing															
Clinical chemistry	X[e]	X[e]	X	X	X	X	X	X	X	X	X		X		X
Urinalysis		X									X				X
Urine microalbumin		X									X				X
Urine pregnancy test		X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC with reticulocyte and differential (automated)	X[e]	X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X
Reproductive testing[f]											X				X
Cystatin C eGFR		X	X	X	X	X	X	X	X	X	X				X
Hemoglobin electrophoresis		X[e]	X	X	X	X	X	X	X	X	X	X	X	X	X
Gene Transfer Testing															

Assessments	**D7, 14, and 21**	**D30**	**D60**	**D90**	**M4 and 5**	**M6**	**M7**	**M8 and 9**	**M10**	**M11**	**M12**	**M15**	**M18**	**M21**	**Y2**
Visit Window (days)	**±2**	**±5**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±14**
Blood: bulk vector DNA qPCR		X	X	X	X	X		X[g]			X		X	X	X
Blood: sorted cell subpopulations and vector DNA qPCR						X					X		X		X
Blood: RCL assay[h]				X		X					X				
Blood: vector insertion site analysis				X		X		X[g]			X		X		X
Blood: F-RBC and F-retics by flow cytometry				X	X	X	X	X			X	X	X	X	X
Blood sample for future testing (optional)[i]						X					X				
RBC testing[j]						X[j]					X				X
Bone marrow: standard morphology assessment						X					X		X		X
Bone marrow: flow cytometry for leukemia panel						X					X		X		X

Assessments	D7, 14, and 21	D30	D60	D90	M4 and 5	M6	M7	M8 and 9	M10	M11	M12	M15	M18	M21	Y2
Visit Window (days)	**±2**	**±5**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±14**
Bone marrow: Genomic evaluation for myeloid malignancy						X					X		X		X
Bone marrow samples for gene transfer testing[k]						X					X		X[l]		X[l]

Abbreviations: AE, adverse event; ASCQ-Me, adult sickle cell quality-of-life measurement; CBC, complete blood count; CFU-c, colony-forming unit cells; D, day; eGFR, estimated glomerular filtration rate; F-RBC, fetal hemoglobin content in red blood cells; F-retics, fetal hemoglobin content in reticulocytes; M, month; P50, partial pressure of oxygen in the blood at which hemoglobin is 50% saturated; qPCR, quantitative polymerase chain reaction; RCL, replication competent lentivirus; SAE, serious adverse event; SCD, sickle cell disease; VOE, vaso-occlusive episodes; Y, year.

a. A complete physical examination will include height (screening visit only), weight, and evaluation of body systems, including but not limited to the following: skin; head, eyes, ears, nose, and throat; respiratory system; cardiovascular system; abdomen (liver, spleen); reproductive systems; and lymph nodes.

b. A targeted physical examination will include evaluation of the subject's general appearance and other components as indicated by the subject's medical history or symptoms.

c. Adverse events will be assessed from the time the subject signs the informed consent form until 2 years after the ARU-1801 infusion. After the Year 2 follow-up visit in this study, AE collection and reporting will be limited to all SAEs, nonserious AEs leading to study withdrawal or that are considered at least possibly related to the investigation product or study medications/procedures, AEs of special interest, and SCD VOEs (Section 6.3.1.3).

d. Collect at Month 6 only.

e. Testing performed for clinical monitoring up to 30 days post-infusion should be recorded in the study database (CBC with reticulocyte and differential, clinical chemistry, and hemoglobin electrophoresis).

f. Reproductive laboratory tests for female and male subjects are listed in Section 6.3.2. Testing will be performed before fertility preservation (Section 5.6.4) for males and females; at a time point after fertility preservation for females; and at Month 12, Year 2, and Year 5 for males and females. The date of last menstrual period should be obtained from females at the time of testing.

g. Collect at Month 9 only.
h. If all post-infusion RCL assay results are negative in the first year, yearly follow-up samples may be discontinued. If any post-infusion results are positive, assay should be performed annually thereafter until Year 15.
i. Baseline and follow-up blood samples (1 tube each of blood cells, plasma and serum) will be collected for potential future biomedical research or biomarker analyses for subjects who consent to participate in this component of the study (Section 6.8). Participation is optional.
j. Testing (P50 and ektacytometry with Oxyscan) will be performed at institutions with the capability to run the tests, or samples will be shipped within the stability window to an institution or laboratory with the capability to perform the testing. The testing is performed at the Month 6, Month 12 (Year 1), and Year 2 post-infusion visits.
k. Bone marrow samples for gene transfer testing will be performed before ARU-1801 infusion, every 6 months through Year 2 (Months 6, 12, 18, and 24), and at Year 3 (Month 36) and comprises the following (see Section 6.5.2.3): qPCR and DNA for vector copies and hematopoietic lineages, CFU-c assay for vector copies by qPCR on individual CFU-c, and vector insertion site analysis.
l. If there is no vector marking in peripheral blood WBC at 2 previous time points, bone marrow aspirates will be discontinued.

Table 13-3 Schedule of Events: Long-term Follow-up Visits (Month 30 Through Year 15)

Assessment	M30	Year 3	M42	Year 4	M54	Year 5	Year 6, 7, 8, and 9	Year 10	Year 11 and 12	Year 13 and 14	Year 15
Visit Window (days)	**±14**	**±30**	**±14**	**±30**	**±14**	**±30**	**±30**	**±30**	**±30**	**±30**	**±30**
SCD-related events and parental opioiduse	X	X	X	X	X	X	X	X	X	X	X
Physical examination (complete)[a]	X	X	X	X	X	X	X	X	X	X	X
Vital sign measurements	X	X	X	X	X	X	X	X	X	X	X
Review concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Adverse events[b]	X	X	X	X	X	X	X	X	X	X	X
Karnofsky performance status		X		X		X	X	X	X	X	X
Patient-reported outcomes (ASCQ-Me)		X		X		X					
Clinical Laboratory Testing											
Clinical Chemistry	X	X	X	X	X	X	X	X	X	X	X
Urinalysis		X		X		X	X	X	X	X	X
Urine microalbumin		X		X		X	X	X	X	X	X
Urine pregnancy test	X	X									
CBC with reticulocyte and differential	X	X	X	X	X	X	X	X	X	X	X
Reproductive testing[c]						X					
Cystatin C eGFR		X		X		X	X	X	X	X	X
Hemoglobin electrophoresis	X	X[d]	X	X[d]	X	X[d]	X	X	X	X	X

Assessment	M30	Year 3	M42	Year 4	M54	Year 5	Year 6, 7, 8, and 9	Year 10	Year 11 and 12	Year 13 and 14	Year 15
Visit Window (days)	**±14**	**±30**	**±14**	**±30**	**±14**	**±30**	**±30**	**±30**	**±30**	**±30**	**±30**
Gene Transfer Testing											
Blood: bulk vector DNA qPCR	X	X	X	X	X	X	X	X	X	X	X
Blood: sorted cell subpopulations and vector DNA qPCR		X		X		X					
Blood: RCL assay[d]		(X)		(X)		(X)	(X)	(X)	(X)	(X)	(X)
Blood: vector insertion site analysis	X	X	X	X	X	X	X	X	X	X	X
Blood: F-RBC and F-retics by flow cytometry		X									
Bone marrow: standard morphology assessment		X									
Bone marrow: flow cytometry for leukemia panel		X									
Bone marrow: Genomic evaluation for myeloid malignancy		X									
Bone marrow sample for gene transfer testing[e, f, g]		X									

Abbreviations: AE, adverse event; ASCQ-Me, adult sickle cell quality-of-life measurement; CBC, complete blood count; CFU, colony-forming unit cells; eGFR, estimated glomerular filtration rate; F-RBC, fetal hemoglobin content in red blood cells; F-retics, fetal hemoglobin content in reticulocytes; LTFU, long-term follow-up; M, month; qPCR, quantitative polymerase chain reaction; RCL, replication competent lentivirus; SAE, serious adverse event; SCD, sickle cell disease; VOE, vaso-occlusive episode; WBC, white blood cell.

Note: It is anticipated that a separate LTFU clinical study will be initiated. Once the LTFU study is approved at the site, all subjects who complete the Year 2 study visit in this study will be asked to consent and enroll into the LTFU study and will be followed for a total of 15 years after the ARU-1801 infusion. Subjects who have not yet transitioned to the LTFU study should follow the assessments detailed in this table.

a. A complete physical examination will include height (screening visit only), weight, and evaluation of body systems, including but not limited to the following: skin; head, eyes, ears, nose, and throat; respiratory system; cardiovascular system; abdomen (liver, spleen); reproductive systems; and lymph nodes.

b. After the Year 2 follow-up visit in this study, AE collection and reporting will be limited to all SAEs, nonserious AEs leading to study withdrawal or that are considered at least possibly related to the investigation product or study medications/procedures, AEs of special interest, and SCD VOEs (Section 6.3.1.3).

c. Reproductive laboratory tests for female and male subjects are listed in Section 6.3.2. Testing will be performed before fertility preservation (Section 5.6.4) for males and females, at a time point after fertility preservation for females, and at Month 12, Year 2, and Year 5 for males and females. The date of last menstrual period should be obtained from females at the time of testing.

d. If any post-infusion results are positive during the first year, the assay should be performed annually thereafter until Year 15.

e. Bone marrow samples for gene transfer testing will be performed before ARU-1801 infusion, every 6 months through Year 2 (Months 6, 12, 18, and 24), and at Year 3 (Month 36) and comprises the following (see Section 6.5.2.3): qPCR and DNA for vector copies and hematopoietic lineages, CFU-c assay for vector copies by qPCR on individual CFU-c, burst-forming unit-erythroid (pooled) for RNA, and vector insertion site analysis.

f. If there is no vector marking in peripheral blood WBC at 2 previous time points, bone marrow aspirates will be discontinued.

g. For LTFU from Year 4 through Year 15, bone marrow will be collected for gene transfer testing only if warranted.

13.2 Appendix 2: Karnofsky Scores

Table 13-4 Karnofsky Scores (Age 10 and Older)

Performance Status	Karnofsky Score
Asymptomatic and fully active	100%
Symptomatic; fully ambulatory; restricted in physically strenuous activity	80%-90%
Symptomatic; ambulatory; capable of self-care; more than 50% of waking hours are spent out of bed	60%-70%
Symptomatic; limited self-care; spends more than 50% of time in bed, but not bedridden	40%-50%
Completely disabled; no self-care; bedridden	20%-30%

13.3 Appendix 3: Side Effects Related to Melphalan Chemotherapy

Table 13-5 Risks and Side Effects Related to Melphalan

	Common Occurs for 21 to 100 of every 100 persons	**Occasional** Occurs for 5 to 20 of every 100 persons	**Rare** Occurs for <5 of every 100 persons
Immediate: Within 1 to 2 days of receiving drug	Anorexia Nausea Vomiting Hyponatremia		Anaphylaxis Hypotension Diaphoresis Pruritus Atrial fibrillation SIADH Seizures
Prompt: Within 2-3 weeks	Myelosuppression Mucositis Diarrhea Alopecia		Abnormal liver function tests Jaundice Hepatitis
Delayed: Any time later during therapy, excluding the above conditions		Amenorrhea Testicular suppression	Bone marrow failure Hemolytic anemia Pulmonary fibrosis Interstitial pneumonitis Rash
Late: Any time after completion of treatment		Sterility Primary ovarian failure	Secondary malignancy

Abbreviations: SIADH, syndrome of inappropriate antidiuretic hormone secretion.

13.4 Appendix 4: Adult Sickle Cell Quality-of-Life Measurement (ASCQ-Me)

ASCQ-Me® v2.0 Emotional Impact - Short Form

Emotional Impact - Short Form

Please respond to each question or statement by marking one box per row.

		Never	Rarely	Sometimes	Often	Always
EmotionalImpactQ11	In the past 7 days, how often did you feel completely hopeless because of your health?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
		Not at all	**A little**	**Somewhat**	**Quite**	**Very**
EmotionalImpactQ12	In the past 7 days, how lonely did you feel because of your health problems? ..	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
EmotionalImpactQ15	In the past 7 days, how depressed were you about your health problems?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
		Not at all	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
EmotionalImpactQ9	In the past 7 days, how much did you worry about getting sick?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
		Never	**Rarely**	**Sometimes**	**Often**	**Always**
EmotionalImpactQ17	In the past 7 days, how often were you very worried about needing to go to the hospital?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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ASCQ-Me® v2.0 Pain Episode Frequency and Severity Measure

Pain Episode Frequency and Severity Measure

Please respond to each question or statement by marking one box.

PainEpisodeQ1	In the past 12 months, how many sickle cell pain attacks (crises) did you have? .....

- ☐ X I did not have a pain attack (crises) in the past 12 months
- ☐ 1 1
- ☐ 2 2
- ☐ 3 3
- ☐ 4 4 or more

PainEpisodeQ2	When was your last pain attack (crisis)?.....

- ☐ X I've never had a pain attack (crises)
- ☐ 1 More than 5 years ago
- ☐ 2 1-5 years ago
- ☐ 3 7-11 months ago
- ☐ 4 1-6 months ago
- ☐ 5 1-3 weeks ago
- ☐ 6 Less than a week ago
- ☐ 7 I have one right now

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ASCQ-Me® v2.0 Pain Episode Frequency and Severity Measure

PainEpisodeQ3	Using any number from 0 to 10, where 0 is no pain and 10 is the worst pain imaginable, how severe was your pain during your last pain attack (crisis)?

- ☐ 0 No pain
- ☐ 1 1
- ☐ 2 2
- ☐ 3 3
- ☐ 4 4
- ☐ 5 5
- ☐ 6 6
- ☐ 7 7
- ☐ 8 8
- ☐ 9 9
- ☐ 10 Worst pain imaginable
- ☐ X I've never had a pain attack (crisis)

PainEpisodeQ4	How much did your last pain attack (crisis) interfere with your life?

- ☐ X I've never had a pain attack (crisis)
- ☐ 1 Not at all, I did everything I usually do
- ☐ 2 I had to cut down on some things I usually do
- ☐ 3 I could not do most things I usually do
- ☐ 4 I could not take care of myself and needed some help from family or friends
- ☐ 5 I could not take care of myself and needed constant care from family, friends, doctors, or nurses

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ASCQ-Me® v2.0 Pain Episode Frequency and Severity Measure

PainEpisodeQ5	About how long did your most recent pain attack (crisis) last?

- ☐ X I've never had a pain attack (crisis)
- ☐ 1 Less than 1 hour
- ☐ 2 1-12 hours
- ☐ 3 13-23 hours
- ☐ 4 1-3 days
- ☐ 5 4-6 days
- ☐ 6 1-2 weeks
- ☐ 7 More than 2 weeks

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ASCQ-Me® v2.0 Pain Impact - Short Form

Pain Impact - Short Form

Please respond to each question or statement by marking one box per row.

		Never	Rarely	Sometimes	Often	Always
PainImpact02	In the past 7 days, how often did you have pain so bad that you could not do anything for a whole day?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpact07	In the past 7 days, how often did you have pain so bad that you could not get out of bed?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpact08	In the past 7 days, how often did you have very severe pain?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpact010	In the past 7 days, how often did you have pain so bad that you had to stop what you were doing?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpact012	In the past 7 days, how often did you have pain so bad that it was hard to finish what you were doing?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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ASCQ-Me® Sickle v2.0 Cell Disease – Medical History Checklist

Sickle Cell Disease – Medical History Checklist

Please respond to each question or statement by marking one box per row.

		Yes	No
SCDMHCQ1	Have you ever had open sores on your legs or feet (leg ulcers)?	☐ 1	☐ 0
SCDMHCQ2	Has a doctor or nurse ever told you that you have lung damage?	☐ 1	☐ 0
SCDMHCQ3	Has a doctor or nurse ever told you that you have kidney damage?	☐ 1	☐ 0
SCDMHCQ4	Has a doctor or nurse ever told you that you have eye damage called retinopathy?	☐ 1	☐ 0
SCDMHCQ5	Has a doctor or nurse ever told you that you have damage to your hip or shoulder due to sickle cell disease?	☐ 1	☐ 0
SCDMHCQ6	Has a doctor or nurse ever told you that you have had a stroke?	☐ 1	☐ 0
SCDMHCQ7	Has your spleen either been removed or seriously damaged due to sickle cell disease?	☐ 1	☐ 0
SCDMHCQ8	Do you get regular blood transfusions for your sickle cell disease?	☐ 1	☐ 0
SCDMHCQ9	Do you take pain medicine every day for your sickle cell disease?	☐ 1	☐ 0

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ASCQ-Me® v2.0 Sleep Impact - Short Form

Sleep Impact Short Form

Please respond to each question or statement by marking one box per row.

		Never	Rarely	Sometimes	Often	Always
SleepImpactQ2	In the past 7 days, how often did you stay up most of the night because you could not fall asleep?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SleepImpactQ5	In the past 7 days, how often was it very easy for you to fall asleep?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
SleepImpactQ8	In the past 7 days, how often did you have a lot of trouble falling asleep	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SleepImpactQ10	In the past 7 days, how often did you stay up all night because you could not fall asleep?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SleepImpactQ11	In the past 7 days, how often did you stay up half of the night because you could not fall asleep?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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ASCQ-Me® v2.0 Social Functioning – Short Form

Social Functioning – Short Form

Please respond to each question or statement by marking one box per row.

		Not at all	A little bit	Somewhat	Quite a bit	Very much
SocialFunctioningQ7	In the past 30 days, how much did you rely on others to take care of you because of your health?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
		Never	**Rarely**	**Sometimes**	**Often**	**Always**
SocialFunctioningQ10	In the past 30 days, how often did your health slow you down?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SocialFunctioningQ15	In the past 30 days, how often did your health make it hard for you to do things?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
SocialFunctioningQ9	In the past 30 days, how often did your health keep you from going out?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
		Not at all	**A little bit**	**Somewhat**	**Quite a bit**	**Very much**
SocialFunctioningQ14	In the past 30 days, how much did your health make it hard for you to do things with your friends?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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ASCQ-Me® v2.0 Stiffness Impact - Short Form

Stiffness Impact - Short Form

Please respond to each question or statement by marking one box per row.

		Never	Rarely	Sometimes	Often	Always
Stiffimpact03	In the past 7 days, how often were your joints very stiff when you woke up?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Stiffimpact07	In the past 7 days, how often were your joints very stiff during the day?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Stiffimpact09	In the past 7 days, how often were your joints so stiff during the day that you could not move?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Stiffimpact010	In the past 7 days, how often did you wake up so stiff that you could not move?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
Stiffimpact015	In the past 7 days, how often did it take you a very long time to get out of bed because of stiffness?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

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13.5 Appendix 5: Formulas and Methods for Melphalan Dosing

Body surface area (BSA) in m^2 by Dubois formula (Dubois, 1916)

- Where WT is total body weight in kg, HT is height in cm, and

$$BSA = WT^{0.425} * HT^{0.725} * 0.007184 \quad (1)$$

Ideal body weight (IBW) in kg by Devine formula (Devine, 1974)

- Where HT is height in inches

$$IBW\ (male) = 50 + 2.3(HT - 60)$$
$$IBW\ (female) = 45.5 + 2.3(HT - 60) \quad (2)$$

Fat free mass (FFM) in kg from Janmahasatian et al, 2005

- Where WT is total body weight in kg, HT is height in m

$$FFM\ (male) = \frac{9270 \cdot WT}{\left(6680 + 216 \cdot \frac{WT}{HT^2}\right)} \quad (3)$$

$$FFM\ (female) = \frac{9270 \cdot WT}{\left(8780 + 244 \cdot \frac{WT}{HT^2}\right)}$$

Cystatin C eGFR ($eGFR_{CysC,input}$) provided in units of mL/min/1.73m^2 by Aruvant. Converted to units of L/h for use in Nath model ($eGFR_{CysC}$) as follows:

$$eGFR_{CystC} = eGFR_{CystC,input} \cdot \frac{BSA}{1.73m^2} \cdot \frac{1L}{1000mL} \cdot \frac{60\ min}{1h} \quad (4)$$

Cystatin C eGFR normalized by weight in kg ($eGFR_{CysC,normalized}$; in L/h):

$$eGFR_{CystC,normalized} = eGFR_{CystC} \cdot \frac{70kg}{WT} \quad (5)$$

Predicted melphalan clearance ($CL_{predicted}$) in units of L/h, calculated using subject covariates:

- Where hematocrit (HCT) is provided in %, FFM in kg, $eGFR_{CystC,normalized}$ in L/h
- Modifications from the paper:

- In the paper, the normalization for FFM is 50 kg. The Sponsor implemented the Nath model based on a NONMEM file provided by Nath, where 53 kg is used as the normalization.
- In the paper, normalization for eGFR is given as 88 mL/min. Since CL is used in units of L/h, the Sponsor converted 88 mL/min to 5.28 L/h here.

$$CL_{predicted} = 17 \cdot \left(\frac{HCT}{34}\right)^{0.462} \cdot \left(\frac{FFM}{53}\right)^{0.75} + 11.1 \cdot \frac{eGFR_{CystC,normalized}}{5.28} \quad (6)$$

Model-informed Individualized Dose Determination:

Predicted melphalan AUC ($AUC_{predicted}$) in subjects after conventional 140 mg/m^2 dose, units of ug.h/mL:

$$AUC_{predicted} = \frac{140 \cdot BSA_{capped}}{CL_{predicted}} \quad (7)$$

Where BSA_{capped} is BSA calculated with ideal body weight replacing total body weight in Equation 1 if the patient's total body weight (WT) is greater than 1.3 times their ideal body weight (IBW), and using total body weight when WT ≤ 1.3 x IBW.

Subjects with a $AUC_{predicted}$ ≥ 8.0 µg.h/mL, as determined using Equation 7 will receive a dose of 140 mg/m^2, normalized to BSA_{capped}.

If the subject's $AUC_{predicted}$ is < 8.0 µg.h/mL, the subject will receive a model-informed dose calculated to achieve a target AUC of 10.4 µg.h/mL, limited to a maximum of 200 mg/m^2, i.e. the lesser of Equations 8 and 9:.

$$Melphalan\ Dose_{AUC10.4} = 10.4 \cdot CL_{predicted} \quad (8)$$
$$Melphalan\ Dose_{200mg/m2} = 200 \cdot BSA_{capped} \quad (9)$$

13.6 Appendix 6: Flow Chart for Melphalan Dosing

