PROTOCOL AVRO-RD-02-201

THE GUARD1 TRIAL, AN OPEN-LABEL, MULTINATIONAL PHASE 1/2 STUDY OF THE SAFETY AND EFFICACY OF EX VIVO, LENTIVIRAL VECTOR-MEDIATED GENE THERAPY AVR-RD-02 FOR SUBJECTS WITH TYPE 1 GAUCHER DISEASE

Sponsor:	AVROBIO, Inc. One Kendall Square Building 300, Suite 201 Cambridge, MA 02139 USA
Sponsor Representative:	[redacted]
Protocol Version and Date:	Original protocol: 21 August 2018 Amendment 1: 18 January 2019 Amendment 1A: 12 March 2020 Amendment 2: 04 October 2019 Amendment 3: 12 December 2019 Amendment 4: 01 July 2020 Amendment 5: 01 July 2021 Amendment 6: 15 June 2022
IND#	19253

SPONSOR SIGNATURE PAGE

THE GUARD1 TRIAL, AN OPEN-LABEL, MULTINATIONAL PHASE 1/2 STUDY OF THE SAFETY AND EFFICACY OF EX VIVO, LENTIVIRAL VECTOR-MEDIATED GENE THERAPY AVR-RD-02 FOR SUBJECTS WITH TYPE 1 GAUCHER DISEASE

PROTOCOL NUMBER: AVRO-RD-02-201; Amendment 6

[redacted]

15-Jun-2022

Date

INVESTIGATOR'S AGREEMENT

I have read the AVRO-RD-02-201 study protocol and agree to conduct the study in accordance with this protocol, all applicable government regulations, the principles of the International Council for Harmonisation (ICH) E6 Guidelines for Good Clinical Practice (GCP), and the principles of the World Medical Association Declaration of Helsinki. I also agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

_____

Site Number

1 SYNOPSIS

Protocol Number: AVRO-RD-02-201

Name of Sponsor/Company: AVROBIO, Inc.

Name of Investigational Product: AVR-RD-02

Name of Active Ingredient: The active substance in AVR-RD-02 is an autologous cell product of CD34+ enriched hematopoietic stem cell (HSCs) that have been genetically modified *ex vivo* with a lentiviral vector (LV) to contain a ribonucleic acid (RNA) transcript that, after reverse transcription, results in codon-optimized, complementary deoxyribonucleic acid (cDNA) that, upon its integration into the human genome, encodes for functional human glucocerebrosidase (GCase).

Title of Study: The Guard1 Trial, an Open-Label, Multinational Phase 1/2 Study of the Safety and Efficacy of Ex Vivo, Lentiviral Vector-Mediated Gene Therapy AVR-RD-02 for Subjects with Type 1 Gaucher Disease

Study Phase: Phase 1/2

Study Center(s): This is a multicenter, multinational study.

Study Duration: Approximately 5 years (from first subject first visit [FSFV] to last subject last visit [LSLV])

Planned Duration of Treatment: The treatment is a one-time gene therapy infusion with AVR-RD-02. Other treatments will be given during other periods.

Objectives:

Primary Objectives:

The primary objectives of this study are to:

- Evaluate the safety and tolerability of AVR-RD-02 investigational product
- Assess measures of engraftment of gene-augmented HSCs by determining average vector copy number (VCN) in peripheral blood and bone marrow aspirates using quantitative polymerase chain reaction (qPCR) and/or droplet digital polymerase chain reaction (ddPCR) analysis
- Evaluate the effect of AVR-RD-02 investigational product on clinical and biomarker indices of Type 1 Gaucher disease, including:
  - Spleen volume assessed by abdominal magnetic resonance imaging (MRI)
  - Liver volume assessed by abdominal MRI
  - Hemoglobin concentration
  - Platelet count
  - Glucosylsphingosine (lyso-Gb1) plasma levels

Secondary Objectives:

The secondary objectives of this study are to:

- Evaluate GCase enzyme activity in peripheral blood
- Evaluate the need for enzyme replacement therapy (ERT) following treatment with AVR-RD-02 investigational product
- Assess the immunogenicity of AVR-RD-02 investigational product
- Evaluate the effect of AVR-RD-02 investigational product on clinical and biomarker indices of Type 1 Gaucher disease, including:
  - Chitotriosidase enzyme activity in plasma
  - Bone marrow burden (BMB) scoring by bone MRI
  - Bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry (DXA)

Exploratory Objectives:

The exploratory objectives of this study are to:

- Evaluate the effect of AVR-RD-02 investigational product on Quality of Life (QoL) assessed by the 36-Item Short Form Health Survey (SF-36)
- Evaluate the effect of AVR-RD-02 investigational product on pain assessed by the Brief Pain Inventory-Short Form (BPI-SF)
- Assess exploratory biomarkers for Gaucher disease in serum, plasma, PBLs, and urine
- Assess the impact of the conditioning regimen on reproductive potential

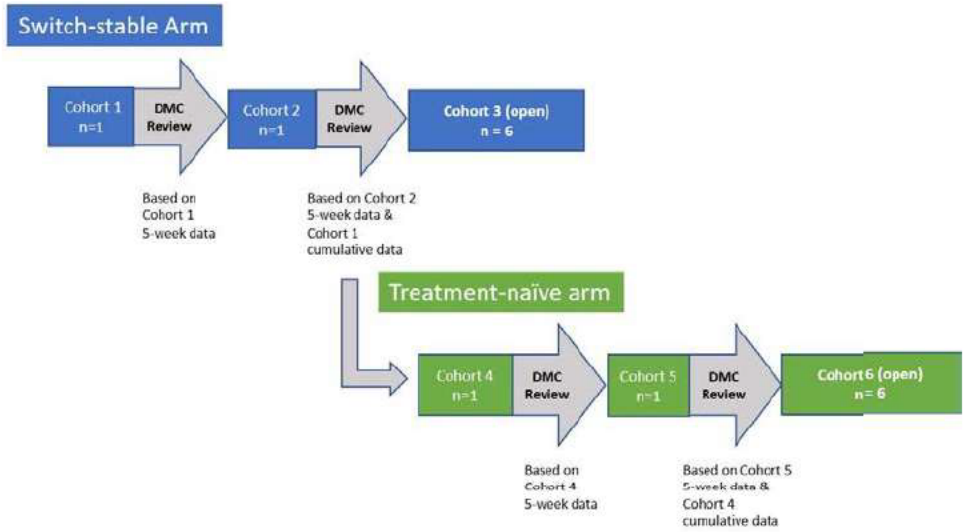
Study Design and Methodology:

This is a Phase 1/2 multinational, open-label study to assess the safety and efficacy of AVR-RD-02 in approximately 8 to 16 subjects (male or female) who are ≥ 18 years and ≤ 50 years of age and postpubertal at Screening with a confirmed diagnosis of Type 1 Gaucher disease (based on clinical phenotype and deficient GCase enzyme activity in peripheral blood). The planned study may consist of 2 arms that are to be enrolled sequentially:

- Switch-stable arm: Subjects who have undergone ERT ≥ 15 U/kg and ≤ 60 U/kg every other week (or equivalent; ie, any combination of infusions resulting in a total monthly ERT dose of > 30 U/kg and < 120 U/kg) for ≥ 24 consecutive months for Gaucher disease at the time of Screening. Switch-stable subjects must discontinue ERT at least 2 weeks before the scheduled gene therapy infusion day. Subjects must not have been on substrate reduction therapy (SRT) within 12 months of Screening.
- Treatment-naïve arm: Subjects with Type 1 Gaucher disease who have never received either ERT or SRT for Gaucher disease or have not received either ERT or SRT for Gaucher disease within 12 months of Screening (ie, treatment-naïve subjects). Enrollment will follow a similar scheme as for the switch-stable subjects.

Initially, 1 adult, switch-stable subject will undergo treatment with AVR-RD-02. Following Week 5 (Day 30) post-gene therapy infusion data review by an independent Data Monitoring Committee (DMC), if it is determined that study enrollment may proceed, a second safety cohort 1 adult switch-stable subject may undergo mobilization. If DMC data review of these initial 2 subjects indicates no significant safety concerns, enrollment may be expanded to include unrestricted enrollment of the switch-stable study arm. In addition, the treatment-naïve study arm may be open to enrollment and will follow the same gated enrollment scheme as outlined above for the switch-stable arm. Neither switch-stable subjects nor treatment-naïve subjects will receive ERT following gene therapy infusion, unless clinically warranted by prespecified criteria (see criteria for post-transplant ERT initiation below).

Below is an overview of the clinical study design:



Abbreviation: DMC = Data Monitoring Committee.

Five study periods (Screening, Baseline, Pre-gene Therapy Infusion, Gene Therapy Infusion, and Post-gene Therapy Infusion Follow-up) comprise the study. During the Screening Period (up to 60 days), written informed consent will be obtained and the subject will complete other Screening procedures to confirm study eligibility. Once study eligibility is confirmed, subjects will enter the Baseline Period (up to 7 days) during which time assessments will be performed to establish pre-gene therapy infusion baseline. Once baseline assessments are complete, the subject will enter the Pre-gene Therapy Infusion Period (approximately 8 weeks to 10 weeks [in some instances, the Pre-gene Therapy Infusion Period may be extended beyond 10 weeks due to subject and/or site availability to initiate conditioning]) during which time mobilization, apheresis, AVR-RD-02 investigational product preparation and testing for release, conditioning regimen administration, and conditioning washout period will take place. Enzyme replacement therapy must be discontinued at least 2 weeks before the scheduled gene therapy infusion day. Following completion of the Pre-gene Therapy Infusion Period, the subject will enter the Gene Therapy Infusion Period (1 day) during which AVR-RD-02 infusion will take place. After AVR-RD-02 gene therapy infusion, the subject will enter the Post-gene Therapy Infusion Follow-up Period (approximately 52 weeks) during which time periodic safety and efficacy assessments will be performed to assess measures of safety, engraftment, and clinical response post-gene therapy infusion. Post-gene therapy infusion follow-up will occur at the following time points: Week 1 (Days 1 through 7), Week 2 (Days 10 and 14), Week 5 (Day 30), Week 9 (Day 60), Week 13 (Day 90), Week 18 (Day 120), Week 22 (Day 150), Week 26 (Day 180), Week 39 (Day 270), and Week 52 (Day 365). Subjects that withdraw any time after gene therapy infusion or complete this study have the opportunity to participate in the long-term follow-up study. During the Post-gene Therapy Infusion Period, subjects will not receive ERT unless prespecified laboratory and clinical criteria, which suggest the need for ERT initiation, are met.

Visit delays due to the global pandemic (coronavirus disease 2019 [COVID-19]) or other unforeseen reasons may necessitate that some procedures/assessments be performed locally, performed out of window, or repeated if performed outside the acceptable timelines for this study. Under such circumstances and in consultation with the Sponsor, remote visits (eg, telemedicine, home nursing visits, etc.) may be considered and may replace some protocol procedures/assessments, when possible. If required, COVID-19 testing will be performed per the institutional guidelines prior to protocol procedures/assessments. To facilitate detection of AEs, other suitable healthcare providers of study subjects (eg, primary care physician, physician's assistant, nurse practitioner, or equivalent) may be engaged in notifying participating Investigators of AEs being collected for the study. Similarly, blood collection on dried blood spot (DBS) cards for GCase activity measurement may occur at the indicated study time points in mitigation of unforeseen logistic delays.

Individuals may be allowed to rescreen based on discussions with the Principal Investigator (PI) and the Sponsor's medical representative or designee's review and approval. Subjects who discontinue from the study prior to gene therapy infusion of AVR-RD-02 may be replaced.

An independent DMC will be established for the study to review safety and efficacy information during the Pre-gene Therapy Infusion, Gene Therapy Infusion, and Post-gene Therapy Infusion Follow-up Periods of the study to assess for signals that may preclude continued study enrollment and/or necessitate changes to the protocol.

Details on the DMC including meeting frequency will be outlined in a DMC Charter for the study.

The DMC will review data from the safety and efficacy assessments periodically throughout the study to ensure the ongoing safety of subjects.

Number of Subjects (planned): Approximately 8 to 16 subjects with a confirmed diagnosis of Type 1 Gaucher disease are planned for study enrollment.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Individuals may be allowed to rescreen based on discussions with the PI and the Sponsor's medical representative or designee's review and approval. Subjects who discontinue from the study prior to gene therapy infusion of AVR-RD-02 may be replaced. Prospective approval of protocol deviations, but not limited to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

Inclusion Criteria:

Common Inclusion Criteria for all Enrolled (Switch-stable and Treatment-naïve) Subjects:

Subjects must meet all of the following inclusion criteria for participation in this study:

1. Subject must be willing and able to provide written informed consent for the study in accordance with applicable regulations and guidelines and to comply with all study visits and procedures, including the use of any data collection device(s) that may be used to directly record subject data.
2. Subject is ≥ 18 years and ≤ 50 years old and postpubertal (Tanner Stage 5 genital development and pubic hair distribution in males and breast development in females). Guidelines for Tanner staging will be provided in the appropriate Study Manual.
3. Subject has a confirmed diagnosis of Type 1 Gaucher disease based on deficient GCase enzyme in blood (and/or documented historical deficiency of GCase activity, verified by the PI) and clinical phenotype (Gary 2018) at Screening. Deficient GCase activity results from DBS cards are acceptable as inclusion criterion at Screening (Miyamoto 2021).
    a. For switch-stable subjects, documentation of deficient GCase enzyme activity in peripheral blood prior to having been started on ERT is required. If GCase levels prior to ERT are not available, deficient trough GCase enzyme activity in peripheral blood at Screening is required.
4. Female subjects of reproductive potential will be counseled regarding the risks, benefits, limitations, and alternatives associated with female fertility preservation. Oocyte harvesting and cryopreservation will be offered.
5. Male subjects must be willing to refrain from donating sperm at any time after receiving conditioning therapy. For subjects planning on (or for whom there is a possibility of) fathering children in the future, cryopreservation before administration of the conditioning regimen will be recommended.
6. All subjects who have not undergone successful surgical sterilization (ie, vasectomy, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) must agree to remain sexually abstinent or use two effective methods of contraception while sexually active from the day of conditioning administration until 52 weeks post-gene therapy infusion (ie, the Week 52 study visit). Two methods of contraception are required even with documented medical assessment of surgical success of sterilization.
    a. For male subjects and for male spouses/partners of female subjects, condoms are an acceptable method of barrier contraception.
    b. For female subjects and for female spouses/partners of male subjects, acceptable methods of barrier contraception include diaphragm, cervical cap, or contraceptive sponge.
7. Male and female subjects must agree to refrain from donating sperm and eggs, respectively, after undergoing conditioning.
8. Subject must be willing to refrain from donating blood, organs, tissues, or cells for gene therapy infusion any time after AVR-RD-02 treatment.
9. Subject must be willing to receive blood or blood products transfusion to manage adverse events (AEs).

Additional Inclusion Criteria for Switch-stable Subjects:

In addition to inclusion criteria 1 through 9, switch-stable subjects must meet all of the following inclusion criteria for participation in this study:

10. Subject has undergone a stable dose (within 75% to 125% of the prescribed dose) of ERT ≥ 15 U/kg and ≤ 60 U/kg every other week (or equivalent; ie, any combination of infusions resulting in a total monthly ERT dose of > 30 U/kg and < 120 U/kg) for ≥ 24 consecutive months with no significant interruptions, in the opinion of the Investigator, in dosing over the last 6 months prior to Screening.
11. Subject has normal or near-normal hematologic values at Screening defined as one or more of the following:
    a. Hemoglobin concentration ≥ 10 g/dL
    b. Platelet count ≥ 80×10^9/L
12. Subject has stable Gaucher disease during the 6 months immediately preceding Screening defined by:
    a. Stable hemoglobin concentration (ie, within a range of ± 2 g/dL of the Screening value) based on documented historical clinical laboratory results

and

b. Stable platelet count (within ± 20% of the Screening value) based on documented historical clinical laboratory results

13. Subject has not received SRT for Gaucher disease during the 12 months immediately preceding Screening.

Additional Inclusion Criteria for Treatment-naïve Subjects:

In addition to inclusion criteria 1 through 9, treatment-naïve subjects must meet the following inclusion criteria for participation in this study:

14. Subject has never received either ERT or SRT for Gaucher disease or has not received either ERT or SRT for Gaucher disease within 12 months of Screening.
15. Subject has a hemoglobin level ≤ 2 g/dL below the lower limit of normal (LLN) for age and sex at Screening and at least one of the following at Screening:
    a. Platelet count < 120×10^9/L
    b. Enlarged liver by palpation, confirmed on abdominal MRI
    c. Moderate splenomegaly by palpation, confirmed on abdominal MRI
16. For any subject who is treatment-naïve, ERT peri-procedurally (from the Screening Period throughout 2 weeks prior to Gene Therapy infusion) will be considered in consultation with the PI and Sponsor Medical Monitor.

Exclusion Criteria:

Subjects will be excluded from participation in this study if they meet any of the following exclusion criteria:

1. Subject has Type 2 or 3 Gaucher disease, has severe neurological signs and symptoms, defined as complete ocular paralysis, overt myoclonus or history of seizures, characteristic of neuronopathic Gaucher disease, or has a tremor, peripheral neuropathy or symptoms of Parkinson's disease.
2. Subject has any one of the following:
    a. Hemoglobin value < 9.0 g/dL, or
    b. Platelet count < 70×10^9/L, or
    c. Spleen volume > 10 × normal, or
    d. Pulmonary hypertension
3. Subject has experienced a prior anaphylactic or anaphylactoid reaction (of any severity) to ERT
4. Treatment-naïve subject has history of clinically significant (CS) anti-GCase antibodies.
5. Subject has a contraindication to ERT, in the opinion of the Investigator.
6. Subject has a contraindication to HSC transplantation (HSCT), in the opinion of the Investigator.
7. Subject presents with iron, folic acid, and/or vitamin B12 deficiency sustained anemia during Screening.
8. Subject has idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), thrombocytopenia, anemia, hepatomegaly, splenomegaly, and/or osteoporosis, unrelated to Gaucher disease, in the opinion of the Investigator.
9. Subject has a clinical co-morbidity such as neurologic, cardiovascular, pulmonary, hepatic, gastrointestinal, renal, hematologic, endocrine, metabolic, genetic, immunologic, neoplastic, or psychiatric disease, other medical condition(s), or intercurrent illnesses that may confound the study results or, in the opinion of the Investigator, may preclude participation in the study.
10. Subject is a pregnant and/or lactating female.
11. Subject is unable to understand the nature, scope, and possible consequences of the study.
12. Subject has diabetes mellitus (Type 1 or Type 2).
13. Subject has active, progressive bone necrosis.
14. Subject has an active chronic infection during the Screening, Baseline, or Pre-gene Therapy Infusion Period of the study.
15. Subject has an active uncontrolled acute bacterial, viral, fungal, parasitic, or prion-associated infection during the Screening, Baseline, or Pre-gene Therapy Infusion Period of the study.
16. Subject has a history of (or current) tuberculosis.
17. Subject tests positive for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV, Type 1 or 2), human T-cell lymphotropic virus (HTLV)-1, HTLV-2, and/or syphilis on

Venereal Disease Research Laboratory (VDRL) test, chemiluminescent microplate immunoassay (CMIA), or enzyme immunosorbent assay (EIA) at Screening.

18. Subject has a prior history of (or current) cancer or precancerous lesion or has a known genetic predisposition to cancer. The one exception is a prior history of resected squamous cell carcinoma.
19. Subject has any other medical condition that predisposes him/her to (or conveys increased risk of) malignancy, in the opinion of the Investigator, including history of (or current) monoclonal gammopathy of undetermined significance (MGUS).
20. Subject has a history of alcohol or illicit drug use, according to the Investigator's judgment.
21. Subject has undergone, or is scheduled to undergo, bone marrow, HSC, and/or solid organ transplant. NOTE: Subjects who are otherwise eligible for the study but are scheduled for bone marrow or HSC transplant to treat Type 1 Gaucher disease may be enrolled in the study (instead of receiving an allogeneic transplant) and undergo gene therapy infusion with AVR-RD-02.
22. Subject has white blood cell count (WBC) $< 3.0 \times 10^9$/L and/or uncorrected bleeding disorder from enrollment (ie, signing of informed consent at Screening) through the Gene Therapy Infusion Period of the study (ie, the day of AVR-RD-02 gene therapy infusion).
23. Subject has CS immunosuppressive disease or condition, in the opinion of the Investigator, at Screening.
24. Subject is on (or requires treatment with) cytotoxic or immunosuppressive agents from 60 days prior to signing informed consent at Screening (ie, study enrollment) through the Week 52 study visit; the one exception is treatment with cytotoxic or immunosuppressive agents required per protocol for stem cell transplant.
25. Subject is on (or requires treatment with) red blood cell (RBC) growth factor (eg, erythropoietin) from 6 months prior to enrollment (ie, signing of informed consent at Screening) through the Week 52 study visit.
26. Subject has any condition that makes it impossible to perform MRI studies.
27. Subject has medical condition(s) and/or is receiving medication(s) that would contraindicate ability to undergo mobilization (including contraindication to granulocyte-colony stimulating factor [G-CSF] and/or plerixafor), apheresis, or conditioning.
28. Busulfan is contraindicated for the subject.
29. Subject has previously received treatment with AVR-RD-02 or any other gene therapy.
30. Subject is participating in (or plans to participate in) any other investigational drug trial, or plans to be exposed to any other investigational agent, device and/or procedure, from 30 days prior to enrollment (ie, signing of informed consent at Screening) through study completion.

Investigational Product, Dosage, and Mode of Administration:

Subjects will receive a single intravenous (IV) gene therapy infusion of between 3×10^6/kg and 10×10^6/kg body weight autologous CD34+ cell-enriched population that contains autologous CD34+ enriched HSCs that have been genetically modified *ex vivo* with an LV containing an RNA transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the human genome, encodes for functional human GCase.

Endpoints:

Primary

Primary endpoints include the following:

Safety endpoints:

- Incidence and severity of AEs, incidence of serious AEs (SAEs), and incidence and severity of AEs of interest, including drug-product related AEs, infections, and malignancy
- Change from Baseline in clinical laboratory values relevant to safety
- Change from Baseline in vital signs
- Change from Baseline in electrocardiogram (ECG) findings at Weeks 26 and 52

Engraftment endpoints:

- Average VCN assessed by qPCR and/or ddPCR in peripheral blood at Baseline and Weeks 13, 26, 39, and 52 and in bone marrow aspirate at Baseline and Weeks 26 and 52

Efficacy endpoints:

- Change from Baseline in spleen volume assessed by abdominal MRI at Weeks 26, 39, and 52
- Change from Baseline in liver volume assessed by abdominal MRI at Weeks 26, 39, and 52
- Change from Baseline in hemoglobin concentration at Weeks 13, 26, 39, and 52
- Change from Baseline in platelet count at Weeks 13, 26, 39, and 52
- Change from Baseline in lyso-Gb1 plasma levels at Weeks 13, 26, 39, and 52

Secondary

Secondary efficacy endpoints include the following:

- Change from Baseline in GCase enzyme activity level in peripheral blood at Weeks 13, 26, 39, and 52
- ERT frequency and dosing between Weeks 26 through 52, inclusive
- Change from Baseline in anti-GCase total antibodies, and subsequent titers and isotypes at Weeks 5, 13, 26, 39, and 52
- Change from Baseline in BMD assessed by DXA at Week 52
- Change from Baseline in clinical and biomarker indices of Type 1 Gaucher disease, including the following:
  - Chitotriosidase enzyme activity levels in plasma at Weeks 13, 26, 39, and 52
  - BMB score as assessed by bone MRI at Weeks 26, 39, and 52

Exploratory

Exploratory efficacy endpoints include the following:

- Change from Baseline in QoL as assessed by SF-36 at Week 52
- Change from Baseline in pain as assessed by BPI-SF at Weeks 13, 26, 39, and 52
- Change from Baseline in exploratory biomarkers for Gaucher disease in serum, plasma, urine, and whole blood derived leukocytes, or subpopulations at Weeks 13, 26, 39, and 52
- Evaluate exploratory genomic biomarkers for Gaucher disease in whole blood at Screening (ie, *CHIT1*, gene encoding chitotriosidase)
- Average VCN and ISA results in both whole blood and bone marrow cells at specified time points.

Exploratory safety endpoints include the following:

- Change from Baseline in ovarian reserve as assessed by anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (female subjects only) at Weeks 26 and 52
- Change from Baseline in menstrual cycle (female subjects only) monthly through Week 52
- Change from Baseline in sperm count, volume, sperm concentration, total motility, progressive motility, and morphology (male subjects only) at Week 52

Statistical Methods:

This first-in-human study is designed to assess both safety and early efficacy in a limited number of switch-stable subjects and treatment-naïve subjects. This is not a pivotal study. The results of this Phase 1/2 study will help inform the appropriateness and design of future clinical studies that evaluate AVR-RD-02.

Data collected will be presented in summary tabulations. All data, as well as any outcomes derived from the data, will be presented in detailed data listings. Graphical displays will also be provided, when appropriate. All analyses will be performed using validated statistical software. Continuous variables will be summarized using descriptive statistics, with the number of non-missing observations, mean, standard deviation, median, minimum, and maximum values provided. Categorical variables will be summarized by frequency counts and by percentages at each protocol-scheduled time point of subjects.

Subject disposition, demographic data, and Baseline data will be summarized.

Safety summaries will be provided for treatment-emergent adverse events (TEAEs), SAEs, AEs of interest (including investigational product-related AEs, infections, and malignancy), and AEs leading to discontinuation. Adverse events will be coded by Medical Dictionary for Regulatory Activities (MedDRA), and will be grouped by primary System Organ Class and Preferred Term. Concomitant medications will be coded using the most current World Health Organization (WHO) Drug Dictionary Enhanced. Changes from Baseline in vital signs and clinical laboratory assessments will be summarized at each protocol-scheduled time point. Abnormal ECG findings and results of immunogenicity testing for presence of antibodies against GCase will also be summarized.

All primary, secondary, and exploratory endpoints, as well as change from Baseline assessments and analyses will be summarized by study arm (ie, switch-stable or treatment-naïve). Change from Baseline assessments will include change from Baseline analysis by study arm. Change from Baseline analysis will also be performed by baseline values (ie, change from Baseline for normal baseline values and change from Baseline for abnormal baseline values) and by engraftment status.

Final statistical analysis for the study will be performed after all enrolled subjects complete the Week 52 assessments (or prematurely discontinue the study) and the clinical database has been locked. Prior to finalizing and locking the database, all decisions concerning the inclusion or exclusion of data from the analysis for each subject will be determined by appropriate medical and statistical personnel. Any and all exclusions will be documented in subject listings.

Full analytical details for the study, including definition of analysis populations and procedures for handling missing data, will be prospectively outlined in a statistical analysis plan (SAP) for the study, which will supersede the statistical sections of this protocol.

2 TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADA	Anti-drug antibodies
ADL	Activities of daily living
AE	Adverse event(s)
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMH	Anti-Müllerian hormone
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical (classification)
AUC	Area-under-the curve
BID	Twice a day
BMB	Bone marrow burden
BMD	Bone mineral density
BMI	Body mass index
BMT	Bone marrow transplant
BPI-SF	Brief Pain Inventory-Short Form
BUN	Blood urea nitrogen
CBER	Center for Biologics Evaluation and Research
cDNA	Complementary deoxyribonucleic acid
CIOMS	Council for International Organizations of Medical Sciences
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practice
CMIA	Chemiluminescent microplate immunoassay
CMP	Common myeloid progenitors
COVID-19	Coronavirus disease 2019
CS	Clinically significant
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
ddPCR	Droplet digital polymerase chain reaction
DBS	Dried blood spot
DHHS	Department of Health and Human Services
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic case report form

Abbreviation	Definition
EIA	Enzyme immunosorbent assay
EMA	European Medicines Agency
ERT	Enzyme replacement therapy
FDA	Food and Drug Administration
FSFV	First subject first visit
FSH	Follicle-stimulating hormone
GBA	Glucosylceramidase beta gene
GCase	Glucocerebrosidase
GCP	Good Clinical Practice
G-CSF	Granulocyte-colony stimulating factor
GGT	Gamma-glutamyl transferase
GLP	Good Laboratory Practice
GluCer	Glucosylceramide
GMP	Granulocytes/monocytes progenitors
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCT	Hematopoietic cell transplant
HIV	Human immunodeficiency virus
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HTLV	Human T-cell lymphotropic virus
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISA	Insertional site analysis
ITP	Idiopathic thrombocytopenic purpura
IV	Intravenous(ly)
LC-MS	Liquid chromatography-mass spectrometry
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
LLN	Lower limit of normal
LSLV	Last subject last visit
LSD	Lysosomal storage disorder
LV	Lentiviral vector
lyso-Gb1	Glucosylsphingosine
MDS	Myelodysplastic Syndrome
MedDRA	Medical Dictionary for Regulatory Activities
MEP	Megakaryocyte/erythroid progenitors

Abbreviation	Definition
MGUS	Monoclonal gammopathy of undetermined significance
MLD	Metachromatic leukodystrophy
MLP	Multilymphoid progenitors
MPP	Multipotent progenitors
MRI	Magnetic resonance imaging
NCS	Not clinically significant
NK	Natural killer
PBL	Peripheral blood leukocyte
PCR	Polymerase chain reaction
PI	Principal Investigator
PK	Pharmacokinetics
QD	Once daily
QoL	Quality of Life
qPCR	Quantitative polymerase chain reaction
RBC	Red blood cell
RCL	Replication-competent lentivirus
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SC	Subcutaneous
SCD	Sickle cell disease
SCID-Xl	X-linked severe combined immunodeficiency
SF-36	36-Item Short Form Health Survey
SRT	Substrate reduction therapy
SUSAR	Suspected unexpected serious adverse reaction(s)
TDM	Therapeutic drug monitoring
TDT	Transfusion-dependent beta-thalassemia
TEAE	Treatment-emergent adverse event
t-MDS	Therapy-related myelodysplastic syndrome
TTP	Thrombotic thrombocytopenic purpura
UK	United Kingdom
US	United States
VCN	Vector copy number
VDRL	Venereal Disease Research Laboratory
WBC	White blood cell
WHO	World Health Organization

4 INTRODUCTION

Gaucher disease is a rare, autosomal recessive lysosomal storage disorder (LSD) caused by mutations in the glucosylceramidase beta (*GBA*) gene, leading to deficiency of the lysosomal enzyme glucocerebrosidase (GCase). Diagnosis of Gaucher disease is made by *GBA* gene mutation analysis and documented GCase enzyme activity deficiency in whole blood, peripheral blood leukocytes (PBLs) or mononuclear cells, or in cultured fibroblasts (Pastores and Hughes 2020). Deficiency of GCase enzyme activity leads to accumulation of its major substrate, glucosylceramide (GluCer), primarily within the lysosomes of macrophages. These pathologic macrophages (called Gaucher cells) infiltrate various organs, including the liver, spleen, and bone marrow, causing disease-related manifestations and complications.

Gaucher disease is classified into three major subtypes (Types 1, 2, and 3). Type 1 disease is considered the most common form, representing 90% to 95% of all Gaucher disease in North America and Europe (Stirnemann 2017). In Type 1 Gaucher disease (non-neuronopathic), organ involvement is generally limited to the viscera and bone; Types 2 and 3 Gaucher disease (neuronopathic) are also associated with neurological involvement. Although there is significant heterogeneity in clinical presentation, common clinical features of Gaucher disease include splenomegaly (in more than 90% of patients), hepatomegaly (in 60% to 80% of patients), and cytopenias, including thrombocytopenia (in 60% to 90% of patients) and anemia (in 20% to 50% of patients). Spleno- and hepatomegaly may be associated with infarction and abdominal pain and/or distension. Bone manifestations of the disease include acute bone crises, primarily in the pelvis and lower extremities, which have clinical features consistent with osteomyelitis, bone infarcts, avascular necrosis, pathologic fractures, and chronic pain. Rarely, pulmonary involvement, manifested as pulmonary fibrosis, restrictive lung disease, and/or pulmonary arterial hypertension, may also be observed. Fatigue is a common complaint of Gaucher patients (50%) that can limit participation in academic, professional, and social activities (Stirnemann 2017). Taken together, these Gaucher disease-related manifestations and complications are associated with significant morbidity and have an adverse impact on Quality of Life (QoL).

Currently, five therapies are available for treatment of Gaucher disease: three intravenously (IV) administered enzyme replacement therapies (ERTs) (Cerezyme® [imiglucerase]; VPRIV® [velaglucerase alpha], most recently being offered as 10-minute home infusion (Becker-Cohen 2022); and ELELYSO® [taliglucerase alpha]) and two orally administered substrate reduction therapies (SRTs) intended to inhibit GluCer biosynthesis (Zavesca® [miglustat] and Cerdelga® [eliglustat]). Although these therapeutic options for patients with Gaucher disease are currently available, there are inherent limitations associated with each.

Despite availability of ERT and clinical improvements, some patients have achieved only a sub-optimal response to ERT after years on treatment (Biffi 2016; Charrow and Scott 2015; Bennett 2018; Weinreb 2021). This may be due, in part, to ERTs very short half-life and biweekly delivery by IV infusion, which provide a very high level of enzyme activity for less than 24 hours followed by a return to pre-treatment levels (CEREZYME PI; ELELYSO PI; VPRIV PI). In a long-term study on hematologic, visceral, bone pain, bone crisis, height, weight, and body mass index (BMI) outcomes in Gaucher disease Type 1 patients, with subset analysis based on pre-treatment severity, genotype, and age at treatment initiation, improvements seen during early treatment years are sustained by continuing treatment for 20 years, except for bone pain in non-splenectomized patients (Weinreb 2021). In addition, although ERTs are generally well tolerated, a small fraction of patients treated with ERT have also experienced hypersensitivity

reactions, anaphylactic reactions, and/or anaphylactoid reactions, and some have also developed anti-drug antibodies (ADA). The rates by which these immune-mediated reactions occur vary by product but appear less frequently among patients receiving the ERT formulation derived from the wild-type gene (ie, velaglucerase alfa).

Of the available SRTs, eliglustat is considered a first-line therapy for adults with Type 1 Gaucher disease; however, miglustat is only recommended as a second-line therapy for adult patients for whom ERT is not suitable. Furthermore, there are safety considerations with both eliglustat and miglustat administration. Eliglustat is contraindicated in patients with pre-existing cardiac disease, electrolyte disturbances (or conditions that lead to electrolyte disturbances), and/or who are receiving certain classes of anti-arrhythmic medications due to the propensity to cause concentration-related increases in electrocardiogram (ECG) intervals. Eliglustat is also contraindicated in patients with specific P450 metabolizer status (CERDELGA PI). Miglustat administration is associated with significant gastrointestinal symptoms, including diarrhea (in more than 80% of patients) and neurologic symptoms, including tremors and peripheral neuropathy, for which periodic neurologic assessment on an ongoing basis is recommended (ZAVESCA PI).

Considering the limitations of the currently approved ERTs and SRTs for Gaucher disease, AVROBIO is developing an *ex vivo* lentiviral vector (LV)-mediated, gene modified, autologous cell therapy for the treatment of Gaucher disease. This gene therapy aims to provide autologous gene therapy infusion of CD34+ enriched hematopoietic stem cells (HSCs) transduced with an LV that inserts codon-optimized, human *GBA* complementary deoxyribonucleic acid (cDNA) into the cells' genomes. Autologous CD34+ HSCs obtained from the subject's mobilized peripheral blood will be genetically modified *ex vivo* with an LV containing a ribonucleic acid (RNA) transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the human genome, encodes for functional human GCase. After the subject has undergone a conditioning regimen and washout period, the genetically-modified cells will be returned to the subject by IV infusion. Progeny of these genetically modified CD34+ enriched cells are expected to produce functional human, wild type GCase, thus, restoring the cell's missing enzymatic activity. This replacement GCase is expected to physiologically break down and prevent the accumulation of GluCer and its deacetylated form, glucosylsphingosine (lyso-Gb1), primarily in the lysosomes of cells of the reticuloendothelial system, especially monocytes and macrophages. In a mouse model of Gaucher disease, early or late *ex vivo*, LV-mediated gene therapy of hematopoietic stem and progenitor cells prevented or reversed Gaucher disease manifestations in bone marrow, liver, and spleen (Dahl 2015). Given the close similarities between the mammalian lysosomal systems, it is reasonable to expect a similar outcome in human Gaucher patients.

Unlike ERTs and SRTs, this LV gene therapy is intended for single administration, having the potential to provide sustained therapeutic levels of functional GCase over the patient's lifetime, thus eliminating the need for biweekly IV infusions and resulting in a beneficial effect on the disease manifestations, patient QoL, and healthcare burden associated with Gaucher disease. In support of this approach, a review of approximately 50 cases of allogeneic HSC transplantations (HSCTs) for the treatment of Types 2 and 3 Gaucher disease found that HSCT can completely correct visceral and bony changes (Ito and Barrett 2013).

Information about the known and expected benefits, risks, and reasonably anticipated adverse events (AEs) of AVR-RD-02 may be found in the current edition of the Investigator's Brochure.

5 STUDY OBJECTIVES AND ENDPOINTS

Study objectives and associated endpoints are presented in Sections 5.1 and 5.2, respectively. The frequency and timing of study measurements are provided in the Schedule of Assessments (Section 15). Information regarding sample and data collection are presented in Section 9.

5.1 Study Objectives

5.1.1 Primary Objectives

The primary objectives of this study are to:

- Evaluate the safety and tolerability of AVR-RD-02 investigational product
- Assess measures of engraftment of gene-augmented HSCs by determining average vector copy number (VCN) in peripheral blood and bone marrow aspirates using quantitative polymerase chain reaction (qPCR) and/or droplet digital polymerase chain reaction (ddPCR) analysis
- Evaluate the effect of AVR-RD-02 investigational product on clinical and biomarker indices of Type 1 Gaucher disease, including:
  - Spleen volume assessed by abdominal magnetic resonance imaging (MRI)
  - Liver volume assessed by abdominal MRI
  - Hemoglobin concentration
  - Platelet count
  - Lyso-Gb1 plasma levels

5.1.2 Secondary Objectives

The secondary objectives of the study are to:

- Evaluate GCase enzyme activity in peripheral blood
- Evaluate the need for ERT following treatment with AVR-RD-02 investigational product
- Assess the immunogenicity of AVR-RD-02 investigational product
- Evaluate the effect of AVR-RD-02 investigational product on clinical and biomarker indices of Type 1 Gaucher disease, including:
  - Chitotriosidase enzyme activity in plasma
  - Bone marrow burden (BMB) scoring by bone MRI
  - Bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry (DXA)

5.1.3 Exploratory Objectives

The exploratory objectives are to:

- Evaluate the effect of AVR-RD-02 investigational product on QoL assessed by the 36-Item Short Form Health Survey (SF-36)
- Evaluate the effect of AVR-RD-02 investigational product on pain assessed by the Brief Pain Inventory-Short Form (BPI-SF)
- Assess exploratory biomarkers for Gaucher disease in serum, plasma, PBLs, and urine
- Assess the impact of the conditioning regimen on reproductive potential

5.2 Endpoints

5.2.1 Primary Endpoints

Primary endpoints include the following:

Safety endpoints

- Incidence and severity of AEs, incidence of serious adverse events (SAEs), and incidence and severity of AEs of interest, including drug-product related AEs, infections, and malignancy
- Change from Baseline in clinical laboratory values relevant to safety
- Change from Baseline in vital signs
- Change from Baseline in ECG findings at Weeks 26 and 52

Engraftment endpoints

- Average VCN assessed by qPCR and/or ddPCR in peripheral blood at Baseline and Weeks 13, 26, 39, and 52 and in bone marrow aspirate at Baseline and Weeks 26 and 52

Efficacy endpoints

- Change from Baseline in spleen volume assessed by abdominal MRI at Weeks 26, 39, and 52
- Change from Baseline in liver volume assessed by abdominal MRI at Weeks 26, 39, and 52
- Change from Baseline in hemoglobin concentration at Weeks 13, 26, 39, and 52
- Change from Baseline in platelet count at Weeks 13, 26, 39, and 52
- Change from Baseline in lyso-Gb1 plasma levels at Weeks 13, 26, 39, and 52

5.2.2 Secondary Endpoints

Secondary efficacy endpoints include:

- Change from the Baseline in GCase enzyme activity level in peripheral blood at Weeks 13, 26, 39, and 52
- ERT frequency and dosing between Weeks 26 through 52, inclusive
- Change from Baseline in anti-GCase total antibodies, and subsequent titers and isotypes at Weeks 5, 13, 26, 39, and 52
- Change from Baseline in BMD assessed by DXA at Week 52
- Change from Baseline in clinical and biomarker indices of Type 1 Gaucher disease, including the following:
  - Chitotriosidase enzyme activity levels in plasma at Weeks 13, 26, 39, and 52
  - BMB score as assessed by bone MRI at Weeks 26, 39, and 52

5.2.3 Exploratory Endpoints

Exploratory efficacy endpoints include:

- Change from Baseline in QoL as assessed by SF-36 at Week 52
- Change from Baseline in pain as assessed by BPI-SF at Weeks 13, 26, 39, and 52
- Change from Baseline in exploratory biomarkers for Gaucher disease in serum, plasma, urine, and whole blood derived leukocytes or subpopulations at Weeks 13, 26, 39, and 52

- Evaluate exploratory genomic biomarkers for Gaucher disease in whole blood at Screening (ie, *CHIT1,* gene encoding chitotrioside)
- Average VCN and ISA results in whole blood and bone marrow cells at specified time points.

Exploratory safety endpoints include:

- Change from Baseline in ovarian reserve as assessed by anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (female subjects only) at Weeks 26 and 52
- Change from Baseline in menstrual cycle (female subjects only) monthly through Week 52
- Change from Baseline in sperm count, volume, sperm concentration, total motility, progressive motility, and morphology (male subjects only) at Week 52

6 INVESTIGATIONAL PLAN

6.1 Summary of Study Design

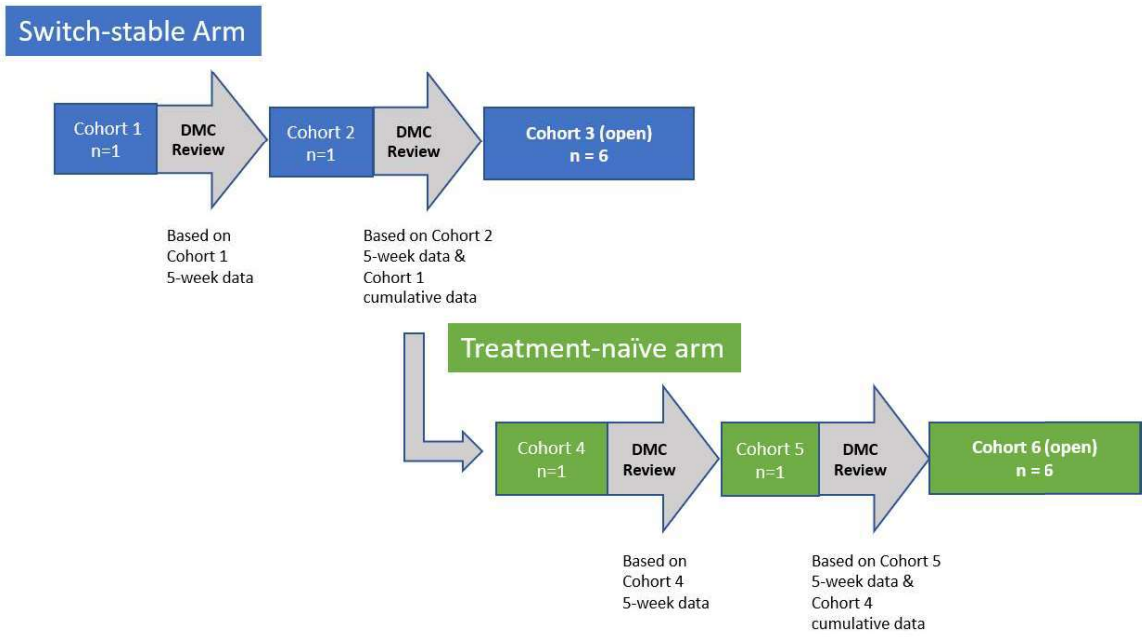
This is a Phase 1/2 multinational, open-label study to assess the safety and efficacy of AVR-RD-02 in approximately 8 to 16 subjects (male or female) who are ≥ 18 years and ≤ 50 years of age and postpubertal at Screening with a confirmed diagnosis of Type 1 Gaucher disease (based on clinical phenotype and deficient GCase enzyme activity in peripheral blood). The planned study may consist of 2 arms that are to be enrolled sequentially:

- Switch-stable arm: Subjects who have undergone ERT ≥ 15 U/kg and ≤ 60 U/kg every other week (or equivalent; ie, any combination of infusions resulting in a total monthly ERT dose of > 30 U/kg and < 120 U/kg) for ≥ 24 consecutive months for Gaucher disease at the time of Screening. Switch-stable subjects must discontinue ERT at least 2 weeks before the scheduled gene therapy infusion day. Subjects must not have been on SRT within 12 months of Screening.
- Treatment-naïve arm: Subjects with Type 1 Gaucher disease who have never received either ERT or SRT for Gaucher disease or have not received either ERT or SRT for Gaucher disease within 12 months of Screening (ie, treatment-naïve subjects). Enrollment will follow a similar scheme as for the switch-stable subjects. For any subject who is treatment-naïve, ERT peri-procedurally (from the Screening Period throughout 2 weeks prior to Gene Therapy Infusion Period) will be considered in consultation with the PI and Sponsor Medical Monitor.

Initially, 1 switch-stable subject will undergo treatment with AVR-RD-02. Following Week 5 (Day 30) post-gene therapy infusion data review by an independent Data Monitoring Committee (DMC), if it is determined that study enrollment may proceed, a second safety cohort of 1 adult switch-stable subject may undergo conditioning. If DMC data review of these initial 2 subjects indicates no significant safety concerns, enrollment may be expanded to enroll switch-stable subjects in an unrestricted manner. In addition, the treatment-naïve study arm may be open to enrollment and will follow the same gated enrollment scheme as outlined above for the switch-stable arm. Neither switch-stable subjects nor treatment-naïve subjects will receive ERT following gene therapy, unless clinically warranted by prespecified criteria (see Section 7.4.6 for post-transplant ERT initiation criteria).

An overview of the clinical study design can be found in Figure 1.

Figure 1: Clinical Study Design for AVRO-RD-02-201



Abbreviation: DMC = Data Monitoring Committee.

Five study periods (Screening, Baseline, Pre-gene Therapy Infusion, Gene Therapy Infusion, and Post-gene Therapy Infusion Follow-up) comprise the study. During the Screening Period (up to 60 days), written informed consent will be obtained and the subject will complete other Screening procedures to confirm study eligibility. Once study eligibility is confirmed, subjects will enter the Baseline Period (up to 7 days) during which time assessments will be performed to establish pre-gene therapy infusion baseline. Once Baseline assessments are complete, the subject will enter the Pre-gene Therapy Infusion Period (approximately 8 weeks to 10 weeks [in some instances, the Pre-gene Therapy Infusion Period may be extended beyond 10 weeks due to subject and/or site availability to initiate conditioning]) during which time mobilization, apheresis, AVR-RD-02 investigational product preparation and testing for release, conditioning regimen administration, and conditioning washout period will take place. Enzyme replacement therapy must be discontinued at least 2 weeks before the scheduled gene therapy infusion day. Following completion of the Pre-gene Therapy Infusion Period, the subject will enter the Gene Therapy Infusion Period (1 day) during which AVR-RD-02 gene therapy infusion will take place. After AVR-RD-02 gene therapy infusion, the subject will enter the Post-gene Therapy Infusion Follow-up Period (approximately 52 weeks) during which time periodic safety and efficacy assessments will be performed to assess measures of safety, engraftment, and clinical response post-gene therapy infusion. Post-gene therapy infusion follow-up will occur at the following time points: Week 1 (Days 1 through 7), Week 2 (Days 10 and 14), Week 5 (Day 30), Week 9 (Day 60), Week 13 (Day 90), Week 18 (Day 120), Week 22 (Day 150), Week 26 (Day 180), Week 39 (Day 270), and Week 52 (Day 365). Subjects that withdraw any time after gene therapy infusion or complete this study have the opportunity to participate in the long-term follow-up study. During the post-gene therapy infusion period, subjects will not receive ERT unless prespecified laboratory and clinical criteria, which suggest the need for ERT initiation, are met.

Visit delays due to the global pandemic (coronavirus disease 2019 [COVID-19]) or other unforeseen reasons may necessitate that some procedures/assessments be performed locally, performed out of the window, or repeated if performed outside the acceptable timelines for this study. Under such circumstances and in consultation with the Sponsor, remote visits (eg, telemedicine, home nursing visits, etc.) may be considered, and may replace some protocol procedures/assessments, when possible. If required, COVID-19 testing will be performed per the institutional guidelines prior to protocol procedures/assessments. To facilitate detection of AEs, other suitable healthcare providers of study subjects (eg, primary care physician, physician's assistant, nurse practitioner, or equivalent) may be engaged in notifying participating Investigators of AEs being collected for the study. Similarly, blood collection on dried blood spot (DBS) cards for GCase activity measurement may occur at the indicated study time points in mitigation of unforeseen logistic delays.

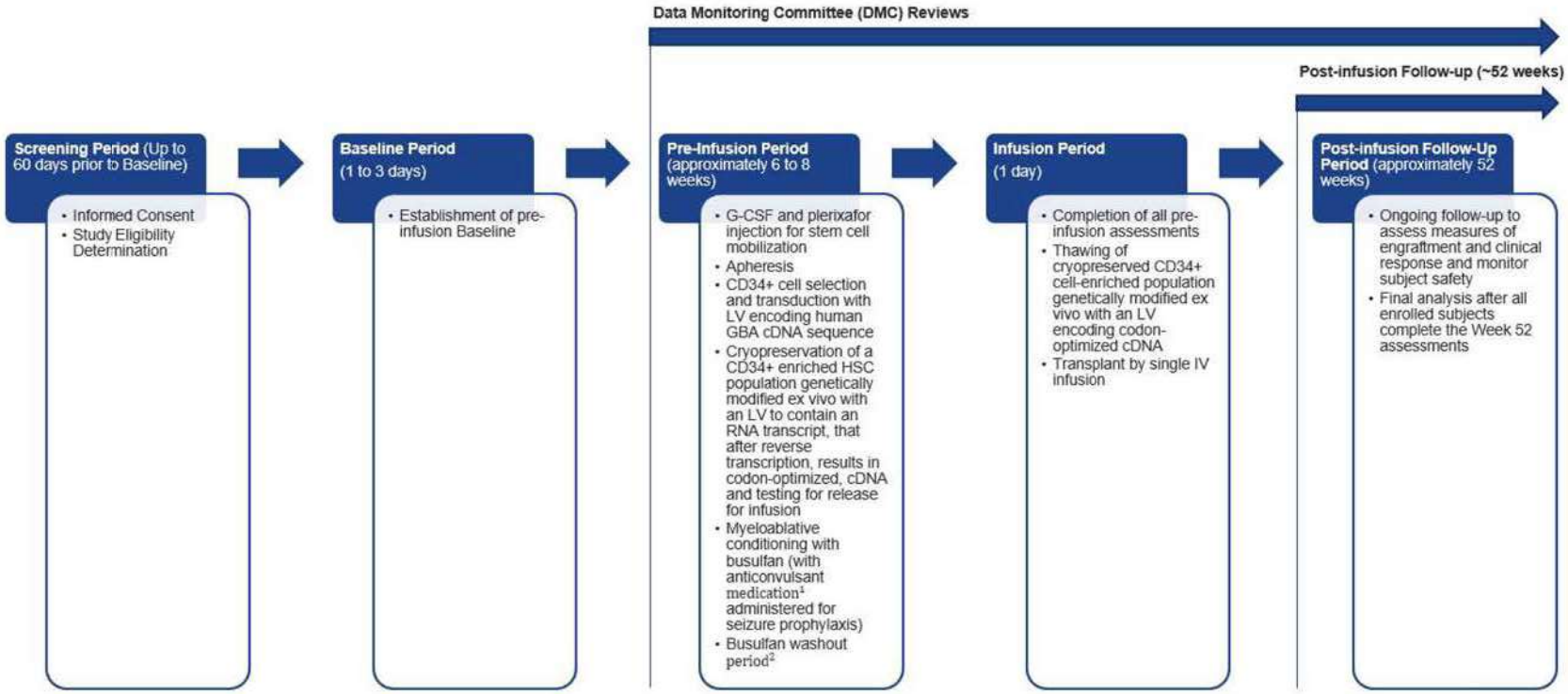
Individuals may be allowed to re-screen based on discussions with the Principal Investigator (PI) and the Sponsor's medical representative or designee's review and approval. Subjects who discontinue from the study prior to gene therapy infusion of AVR-RD-02 may be replaced.

An independent DMC will be established for the study to review safety and efficacy information during the Pre-gene Therapy Infusion, Gene Therapy Infusion, and Post-gene Therapy Infusion Follow-up Periods of the study to assess for signals that may preclude continued study enrollment and/or necessitate changes to the protocol.

Details on the DMC including meeting frequency will be outlined in a DMC Charter for the study, which outlines that the DMC will review data from the safety and efficacy assessments periodically throughout the study to ensure the ongoing safety of subjects.

An overview of the overall study design can be found in Figure 2. A detailed overview of the Pre-gene Therapy Infusion and Gene Therapy Infusion Periods is provided in Figure 3.

Figure 2: Overall Study Design for AVRO-RD-02-201

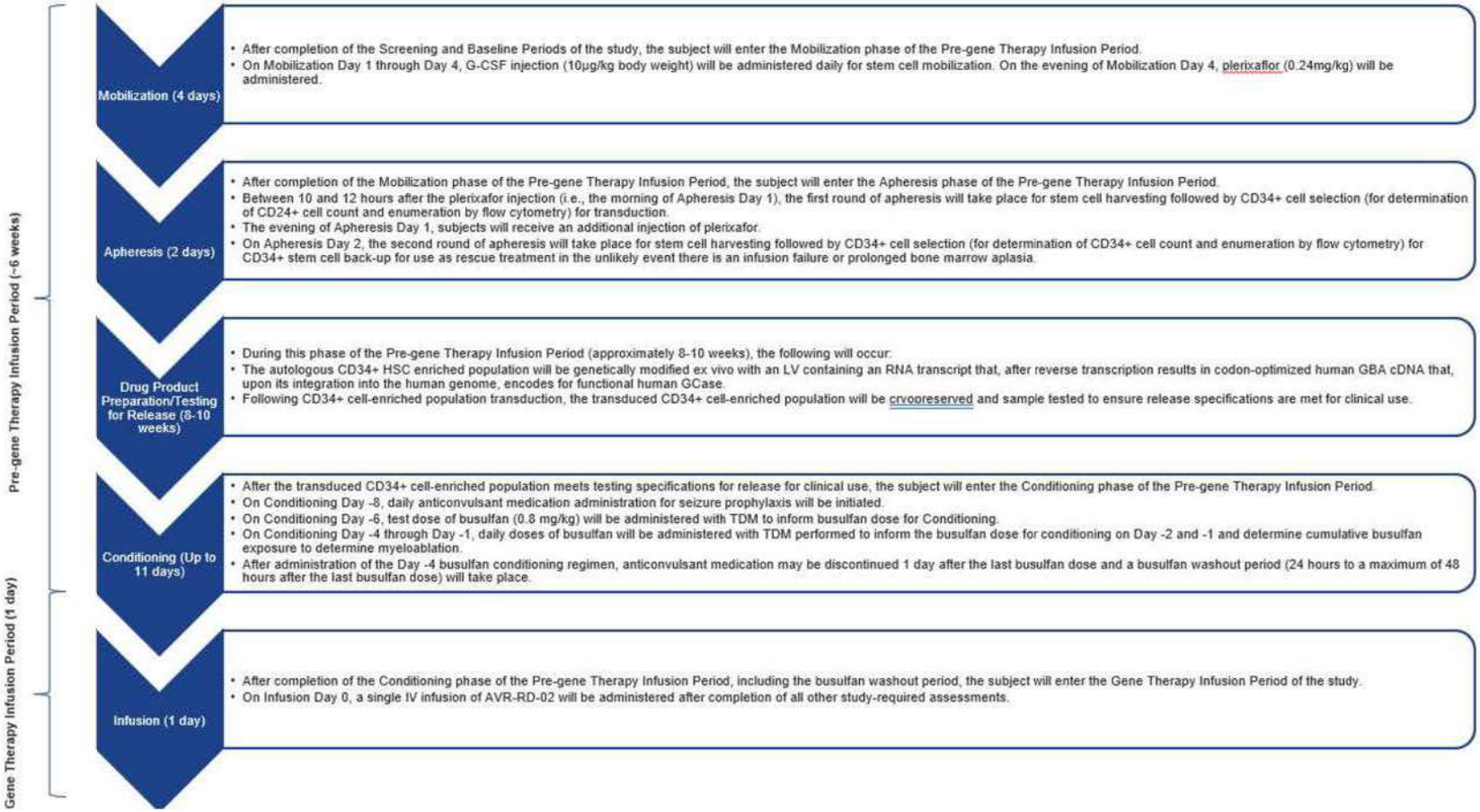


Abbreviations: cDNA = complementary deoxyribonucleic acid; DMC = Data Monitoring Committee; *GBA* = glucosylceramidase beta gene; G-CSF = granulocyte colony-stimulating factor; IV = intravenous; LV = lentiviral vector.

1 Levetiracetam will be administered in this study. If levetiracetam is contraindicated, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be administered.

2 The busulfan washout period prior to AVR-RD-02 gene therapy infusion must be a minimum of 24 hours up to a maximum of 48 hours. Enrollment in the study will be open to switch-stable subjects followed by treatment-naïve subjects. Subjects will initially be enrolled in cohorts of 1 (a total of 2 cohorts of 1 subject each are planned) to allow for safety review by the DMC prior to proceeding to further enrollment (see Table 7). The independent DMC will review safety information during the Pre-gene Therapy Infusion, Gene Therapy Infusion, and Post-gene Therapy Infusion Follow-up Periods of the study to assess for signals that may preclude continued study enrollment and/or necessitate changes to the protocol. Final statistical analysis for the study will be performed after all enrolled subjects complete the Week 52 assessments (or prematurely discontinue the study).

Figure 3: Overview of the Pre-gene Therapy Infusion and Gene Therapy Infusion Periods of Study AVRO-RD-02-201



Abbreviations: BID = twice a day; cDNA = complementary deoxyribonucleic acid; *GBA* = glucosylceramidase beta gene; GCase = glucocerebrosidase; G-CSF = granulocyte colony-stimulating factor; HSC = hematopoietic stem cell; IV = intravenous(ly); LV = lentiviral vector; SC = subcutaneously; RNA = ribonucleic acid; TDM = therapeutic drug monitoring.

Note: AVR-RD-02 gene therapy infusion will occur between a minimum of 24 hours and a maximum of 48 hours after the final busulfan dose.

For investigational sites with only prefilled syringes, a G-CSF dose from 10 µg/kg body weight up to 12 µg/kg body weight, administered in either a single or divided BID dose, may be administered. The G-CSF dose may be administered SC or IV.

Levetiracetam will be administered in this study. If levetiracetam is contraindicated, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be administered.

Tabular overviews of the required assessments for the study (including the timing of each) can be found in Section 15. The Schedule of Assessments from Screening through the Apheresis Period is in Table 4, the Schedule of Assessments from Investigational Product Preparation and Testing through End of Gene Therapy Infusion is in Table 5, and the Schedule of Assessments for the Post-gene Therapy Infusion Follow-up Period (Study Week 1 [Day 1] through Study Week 52 [Day 365]) is in Table 6.

After study completion, consenting subjects will continue periodic safety and efficacy assessments for approximately 14 additional years (for a total of approximately 15 years post-gene therapy infusion follow-up) in a long-term follow-up study to AVRO-RD-02-201.

6.2 Discussion of Design and Control

6.2.1 Overall Study Design and Study Population

The open-label design was deemed appropriate for the early phase nature of the study. Approximately 8 to 16 switch-stable and treatment-naïve subjects with Type 1 Gaucher disease will be enrolled sequentially in this study to assess the safety and change-from-baseline efficacy of AVR-RD-02 in both subjects who have previously been on a stable ERT regimen and those who have not received ERT or SRT. This approach will allow for an early-phase assessment of engraftment in these two important populations, may allow for a greater understanding of the potential clinical benefit in these populations, and thereby may help inform future clinical development.

The study is designed to enable an assessment of safety and efficacy, in a risk-stratified manner, among adult subjects who have been stable on ERT and those naïve to ERT. Based on stopping rules and data collected from initial safety cohorts, an independent DMC review at pre-specified intervals will assess the appropriateness of the progressive introduction of additional cohorts and subjects.

Subjects with Type 1 Gaucher disease will be the focus of this Study AVRO-RD-02-201, as this is the most common form of Gaucher disease (Grabowski 2015). As this will be a study with no control arm, only an assessment of treatment response relative to baseline in Type 1 Gaucher disease will be performed. To facilitate the comparison to baseline, previous medical records will be requested for study subjects in order to establish a more robust estimate of pre-treatment measures of disease. The exclusion of subjects with neuronopathic forms of Gaucher disease (ie, Types 2 and 3 Gaucher disease) should prevent the potential introduction of confounding signals associated with these conditions, thereby allowing a better understanding and interpretability of this study's outcomes in relation to previous clinical study observations in Type 1 Gaucher disease (see Section 7.3).

In clinical studies of wild type GCase, velaglucerase alfa, immunogenicity was low for both switch-stable and treatment-naïve subjects (Pastores 2014; VPRIV PI).

Given the overall comparability of safety profiles among switch-stable and treatment-naïve subjects in these studies, AVRO-RD-02-201 aims to include both switch-stable and treatment-naïve subjects.

Once DMC review of the available data from a total of 2 safety cohorts of switch-stable subjects occurs, the DMC may determine that enrollment may expand to include additional switch-stable subjects in an unrestricted manner. In addition, the treatment-naïve study arm may be open to enrollment and will follow the same gated enrollment scheme as outlined above for the switch-stable arm (see Section 6.1).

The inclusion of subjects with ages ranging from ≥ 18 years to ≤ 50 years is based on several considerations. The age of onset/diagnosis of Type 1 Gaucher disease has been reported to range from the first to the ninth decade of life, is influenced by genotype, and varies among geographic regions. Diagnosis of Type 1 Gaucher disease has been initially reported prenatally and as late as the ninth decade (Charrow 2000). The median age of onset of Type 1 Gaucher disease is approximately 30 years among N370S homozygotes, with an earlier age of onset (75th percentile before the age of 30) observed with other genotypes. Age at diagnosis of younger than 10 years was reported in patients with N370S/84GG, L444P homozygotes, L444P/?, and N370S/IVS2+1 genotypes, all of which are often associated with severe symptomatology (Charrow 2000). All subjects will be required to be postpubertal, defined as having Tanner Stage 5 genital development and pubic hair distribution in males and breast development in females at the time of Screening. The requirement to have adult male (Tanner Stage 5) genital development or Tanner Stage 5 breast development was included as part of study eligibility to ensure subjects aged 18 years to 21 years are not continuing to undergo the metabolic and other physical changes associated with puberty. The requirement for subjects to be ≤ 50 years of age at Screening was included as rates of engraftment may decrease with increasing age. Therefore, safety and efficacy can best be assessed by capturing an age range in which most Type 1 Gaucher disease patients are diagnosed and limiting the age of the study population to individuals most likely to achieve successful engraftment (ie, ≥ 18 years and ≤ 50 years).

The Screening through Pre-gene Therapy Infusion Period could be as long as 21 weeks. To avoid any significant interruption of enzyme replacement for switch-stable subjects, ERT will be continued up to 2 weeks before the scheduled gene therapy infusion day. This 2-week timeframe should serve as an adequate washout period for the replacement enzyme before gene therapy infusion is initiated. This timeframe is based on the pharmacokinetics (PK) and clearance of commercially available ERTs and the corresponding dosing frequency recommendations (see the package inserts for imiglucerase, velaglucerase alfa, and taliglucerase alfa).

6.2.2 Stem Cell Mobilization and Conditioning Regimens

6.2.2.1 Stem Cell Mobilization

Both granulocyte-colony stimulating factor (G-CSF) and plerixafor are being used in the study for stem cell mobilization. Superiority of G-CSF and plerixafor over G-CSF alone in stem cell mobilization of subjects has been demonstrated (Baiamonte 2015; Goker 2015; Yannaki 2013); in combination with G-CSF, plerixafor has been shown to increase peripheral blood CD34+ cell counts, including increased frequency of more primitive CD34+ cell subtypes and capacity to produce colonies.

6.2.2.2 Conditioning

A 4-day conditioning regimen of IV busulfan once daily (QD) will be used in the study. Before the 4-day conditioning regimen begins, a weight-based test dose of busulfan 0.8 mg/kg IV will be administered on Day -6 to determine PK and the initial doses that will be given on Day -4 and Day -3 of the Conditioning phase. Busulfan PK samples may be drawn daily during the conditioning phase. The myeloablative cumulative target for busulfan exposure will be an area-under-the curve (AUC) of $90_{(\text{day 0-4})} \pm 2\%$ mg•hr/L. The United States Food and Drug Administration (US FDA), European Medicines Agency (EMA), and Health Canada recommend an initial IV busulfan weight-based dose of 0.8 mg/kg IV every 6 hours or 3.2 mg/kg IV daily for conditioning, with consideration for modified dosing strategies for patients with less than ideal body weight (Busulfan PI; Busulfan Product Monograph; Busulfan SmPC). However, Bartelink et al. (Bartelink 2012) investigated the covariates related to the PK of busulfan and determined that body weight was the most predictive covariate that explained variability in busulfan exposure between patients. The relationship between body weight and clearance was characterized by an allometric equation with a scaling component (Bartelink 2012). The scaling range for adolescents and adults was from 2.7 mg/kg/day to 4.1 mg/kg/day and allowed for individualized initial dosing based on body weight. However, further studies evaluating the PK of busulfan by Davis et al. (Davis 2019) and Weil et al. (Weil 2017) determined that administration of a busulfan test dose given before the 4-day myeloablative conditioning regimen allowed for the use of a personalized PK-guided dosing strategy based on the PK from the test dose and the patient's body weight, which resulted in significant improvement in target AUC attainment and less interpatient variability compared to using the weight-based dosing regimen alone as described by Bartelink et al. The personalized PK- and weight-based guided dosing strategy as suggested by Weil et al. and Davis et al. in addition therapeutic drug monitoring (TDM) of the PK of the test dose and during conditioning has resulted in more accurate cumulative AUC outcomes versus our previous weight-based and TDM-guided dosing without test dose.

This PK- and weight-based guided individualized dosing regimen of busulfan defines a once-daily regimen for myeloablative conditioning at an $AUC_{(\text{day 0-4})}$ of 90 mg•hr/L (5,400 µmol•min/day). Because busulfan PK display large interpatient and intrapatient variability and exposure, TDM of busulfan is routinely performed as standard clinical care in patients undergoing HSCT (Long-Boyle 2015).

The timing of the busulfan test dose and the approach to busulfan TDM at each site will be determined by the method used to perform TDM. The 2 options for performing busulfan TDM are as follows:

- Liquid chromatography-mass spectrometry (LC-MS): Busulfan TDM will be performed on the test dose on Conditioning Day -6 to inform the dose on Conditioning Days -4 and -3. The PK sampling may also be performed on Day -5 to support optimization of busulfan exposure. Busulfan TDM will also be performed on Conditioning Days -4 and -3 to inform the doses on Days -2 and -1, respectively. Collective information from TDM on all days during Conditioning (Day -4 to Day -1) will be used to calculate the cumulative AUC in Study AVRO-RD-02-201, with plasma samples taken at periodic intervals post-busulfan administration as described in Section 8.1.1.3 and in the Schedule

of Assessments (Table 5). All laboratories performing TDM will use validated methods to quantify busulfan in plasma, according to Good Laboratory Practice (GLP).

- An investigational, validated automated nanoparticle immunoassay kit (Saladax Biomedical, Inc.): Busulfan TDM will be performed on the test dose on Conditioning Day -5 to inform the dose on Conditioning Day -4. Busulfan TDM will be performed on Conditioning Day -4, and TDM PK results will be used to inform the dose on Conditioning Day -3 together with the TDM PK results from the test dose. Busulfan TDM will be performed on Conditioning Day -3, and TDM PK results will be used to inform the dose on Conditioning Day -2 together with the results from the test dose and Conditioning Day -4. Busulfan TDM will be performed on Conditioning Day -2, and TDM PK results will be used to inform the dose of Conditioning Day -1 together with the results from the test dose and Conditioning Days -4 and -3. Collective information from TDM on all days during Conditioning (Day -4 to Day -1) will be used to calculate the cumulative AUC in Study AVRO-RD-02-201, with plasma samples taken at periodic intervals post-busulfan administration as described in Section 8.1.1.3 and in the Schedule of Assessments (Table 5).

Further information regarding assessment of busulfan exposure can be found in the Laboratory Manual.

The busulfan dose-versus-concentration relationship over time will be evaluated to obtain an individualized dosing strategy that optimizes efficacy and minimizes toxicity. Optimizing the target for cumulative busulfan exposure has a significant impact on outcomes (Bartelink 2016). In transfusion-dependent beta-thalassemia (TDT) patients who underwent autologous HSCT for LV-mediated gene therapy, a 4-day myeloablative conditioning regimen, with an average busulfan daily dose range of 4,670 µmol•min/L to 5,212 µmol•min/L per day, was used (Thompson 2018). In 400 children and young adults who underwent allogeneic HSCT for non-oncology indications, a busulfan-AUC of 78 to 101 mg•hr/L was considered to be optimal for targeting a balance between acute toxicity and patient outcomes, including graft failure, or disease relapse (Bartelink 2016).

In the AVRO-RD-02-201 study, busulfan dosing will be determined using PK modeling software, as described in the Study Treatment Manual. Reports in the literature indicate that cumulative AUCs are sometimes calculated with methods that vary by institution, and thus, use of a PK modeling software program can provide a consistent, standardized approach in dosing strategies between sites (Bartelink 2016).

Busulfan crosses the blood-brain barrier and can cause neurotoxicity, including seizures, when administered at higher doses. Therefore, anticonvulsant medication is routinely prescribed for patients undergoing myeloablative therapy with busulfan in preparation for bone marrow transplant (BMT) or hematopoietic cell transplant (HCT). Consistent with this practice, anticonvulsant prophylaxis will be administered concurrent with busulfan administration for conditioning in this study. Levetiracetam will be administered in this study as described in Section 8.1.1.3. The dosing regimen selected for levetiracetam has been shown in literature to be effective at preventing seizures in adults receiving conditioning with busulfan in preparation for HSCT, and to have an acceptable safety profile when administered in combination with busulfan (Akiyama 2018). For subjects with contraindications to levetiracetam, an alternative anti-

convulsant medication (eg, benzodiazepines or valproic acid) may be used before and after busulfan treatment (Busulfan PI).

Busulfan treatment at the approved dose and schedule produces profound, transient myelosuppression in all patients. Hematologic changes have been observed in subjects receiving myeloablative busulfan conditioning regimens used in other autologous LV-mediated gene therapy studies for TDT, sickle cell disease (SCD), metachromatic leukodystrophy (MLD), leukocytes adhesion deficiency-1, and X-linked chronic granulomatous disease (Fumagalli 2022; Sessa 2016; Thompson 2018; Tisdale 2018; Kohn 2020; see also the Investigator's Brochure for AVR-RD-02). Similar outcomes were achieved by Mamcarz et al in LV-mediated gene therapy for X-linked severe combined immunodeficiency (SCID-X1) patients, albeit targeted busulfan was indicated at low dose (Mamcarz 2019).

While secondary malignancies have been reported among individuals exposed to busulfan during treatment for hematologic cancers, less is known about the magnitude of this risk among individuals exposed to short term busulfan conditioning in the management of non-malignant disorders (eg, gene therapy or BMT). To address this concern, AVROBIO conducted a literature review of busulfan conditioning in conjunction with BMT or HCT for non-malignant conditions (literature references available upon request). Similarly, AVROBIO reviewed the literature for busulfan conditioning before LV-mediated gene therapy for non-malignant conditions (literature references available upon request). With the exception of the one case of post therapy-related myelodysplastic syndrome (t-MDS) reported in an individual who underwent busulfan conditioning before LV-mediated gene therapy for SCD (Hsieh 2020), no other cases of t-MDS, leukemia (acute myeloid leukemia [AML]), cancer, or precancerous lesions were reported in over 700 exposures to busulfan conditioning for non-malignant conditions. Based on this literature review, the risk of t-MDS or leukemia due to busulfan conditioning before LV-mediated gene therapy for non-malignant conditions appears to be low. Nevertheless, deoxyribonucleic acid (DNA) will be collected from bone marrow aspirate and peripheral blood samples at Baseline and stored for subsequent evaluation should t-MDS, leukemia (AML), hematologic cancer, or a precancerous condition be observed.

The impact of the busulfan conditioning regimen used in this study on fertility is not fully understood. Data from studies of the use of conditioning agents in oncology indications suggest that these agents may be associated with transient or persistent infertility with a significant impact on QoL of transplanted patients (Faraci 2019; Busulfan PI; Busulfan Product Monograph; Busulfan SmPC).

The potential risk of infertility in the study will be mitigated by:

- Males will be offered sperm cryopreservation and females will be offered oocyte harvesting and cryopreservation.
- The impact of the conditioning regimen on the subjects' reproductive potential will be assessed during the study by evaluating the change from Baseline in sperm count, volume, sperm concentration, total motility, progressive motility, and morphology in males, and changes from Baseline in menstrual history, AMH, FSH, and LH (biomarkers of ovarian reserve) in females. For female subjects, the use of gonadotrophic-releasing hormone agonists (Lupron) for ovarian chemoprotection can be offered prior to gonadotoxic therapy such as busulfan.

- For both male and female subjects, additional assessments of reproductive potential and/or referral to a urologist (male subjects) or a reproductive endocrinologist (female subjects) is recommended.

Pregnancy outcomes, if any, will be monitored and recorded during this study.

6.2.3 Efficacy and Safety Measures

Measures of engraftment of gene-augmented HSCs will be evaluated by determining average VCN using qPCR and/or ddPCR in peripheral blood and bone marrow. To ensure subject safety, a CD34+ stem cell back-up of nontransduced cells will be collected and stored for use as rescue treatment in the unlikely event that there is prolonged bone marrow aplasia after AVR-RD-02 treatment (see Section 8.1.1.1).

Efficacy measures chosen for the planned AVRO-RD-02-201 study have been commonly used to measure treatment effects in trials of ERT and SRT for Gaucher disease. Efficacy and safety endpoints chosen, and the timing of these assessments, considered the following: the natural history of Gaucher disease, the clinical progression of signs/symptoms known to impact morbidity and mortality in subjects with the disease, and regulatory guidelines for follow-up of subjects receiving gene therapy products (ie, US Department of Health and Human Services [DHHS], FDA, Center for Biologics Evaluation and Research [CBER], Guidance for Industry, Long Term Follow-Up After Administration of Human Gene Therapy Products, January 2020 [FDA 2020a] US DHHS, FDA, CBER, Guidance for Industry, Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up, January 2020 [FDA 2020b] EMA, Guideline on Follow-up of Patients Administered with Gene Therapy Medicinal Products, October 2009 [EMA 2009]).

7 STUDY POPULATION

Individuals may be allowed to re-screen based on discussions with the PI and the Sponsor's medical representative or designee's review and approval.

Prospective approval of protocol deviations, but not limited to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

7.1 Inclusion Criteria

Common Inclusion Criteria for all Enrolled (Switch-stable and Treatment-naïve) Subjects:

Subjects must meet all of the following inclusion criteria for participation in this study:

1. Subject must be willing and able to provide written informed consent for the study in accordance with applicable regulations and guidelines and to comply with all study visits and procedures, including the use of any data collection device(s) that may be used to directly record subject data.
2. Subject is ≥ 18 years and ≤ 50 years old and postpubertal (Tanner Stage 5 genital development and pubic hair distribution in males and breast development in females). Guidelines for Tanner staging will be provided in the appropriate Study Manual.
3. Subject has a confirmed diagnosis of Type 1 Gaucher disease based on deficient GCase enzyme in blood (and/or documented historical deficiency of GCase activity, verified by the PI) and clinical phenotype (Gary 2018) at Screening. Deficient GCase activity results from DBS cards are acceptable for inclusion criteria at Screening (Miyamoto 2021).
    a. For switch-stable subjects, documentation of deficient GCase enzyme activity in peripheral blood prior to having been started on ERT is required. If GCase levels prior to ERT are not available, deficient trough GCase enzyme activity in peripheral blood at Screening is required.
4. Female subjects of reproductive potential will be counseled regarding the risks, benefits, limitations, and alternatives associated with female fertility preservation. Oocyte harvesting and cryopreservation will be offered.
5. Male subjects must be willing to refrain from donating sperm any time after receiving conditioning therapy. For subjects planning on (or for whom there is a possibility of) fathering children in the future, cryopreservation before undergoing conditioning will be recommended.
6. All subjects who have not undergone successful surgical sterilization (ie, vasectomy, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) must agree to remain sexually abstinent or use two effective methods of contraception while sexually active from the day of conditioning administration until 52 weeks post-gene therapy infusion (ie, the Week 52 study visit). Two methods of contraception are required even with documented medical assessment of surgical success of sterilization.
    a. For male subjects and for male spouses/partners of female subjects, condoms are an acceptable method of barrier contraception.
    b. For female subjects and for female spouses/partners of male subjects, acceptable methods of barrier contraception include diaphragm, cervical cap, or contraceptive sponge.

7. Male and female subjects must agree to refrain from donating sperm and eggs, respectively, after undergoing conditioning.
8. Subject must be willing to refrain from donating blood, organs, tissues, or cells for gene therapy infusion any time after AVR-RD-02 treatment.
9. Subject must be willing to receive blood or blood products transfusion to manage AEs.

Additional Inclusion Criteria for Switch-stable Subjects:

In addition to inclusion criteria 1 through 9, switch-stable subjects must meet all of the following inclusion criteria for participation in this study:

10. Subject has undergone a stable dose (within 75% to 125% of the prescribed dose) of ERT ≥ 15 U/kg and ≤ 60 U/kg every other week (or equivalent; ie, any combination of infusions resulting in a total monthly ERT dose of ≥ 30 U/kg and ≤ 120 U/kg) for ≥ 24 consecutive months with no significant interruptions, in the opinion of the Investigator, in dosing over the last 6 months prior to Screening.
11. Subject has normal or near-normal hematologic values at Screening defined as one or more of the following:
    a. Hemoglobin concentration ≥ 10g/dL
    b. Platelet count ≥ 80 × 109/L
12. Subject has stable Gaucher disease during the 6 months immediately preceding Screening defined by:
    a. Stable hemoglobin concentration (ie, within a range of ± 2 g/dL of the Screening value) based on documented historical clinical laboratory results

    and

Stable platelet count (within ± 20% of the Screening value) based on documented historical clinical laboratory results

13. Subject has not received SRT for Gaucher disease during the 12 months immediately preceding Screening.

Additional Inclusion Criteria for Treatment-naïve Subjects:

In addition to inclusion criteria 1 through 9, treatment-naïve subjects must meet the following inclusion criteria for participation in this study:

14. Subject has never received either ERT or SRT for Gaucher disease or has not received either ERT or SRT for Gaucher disease within 12 months of Screening.
15. Subject has a hemoglobin level ≤ 2 g/dL below the lower limit of normal (LLN) for age and sex at Screening and at least one of the following at Screening:
    a. Platelet count < 120×10^9/L

Enlarged liver by palpation, confirmed on abdominal MRI
Moderate splenomegaly by palpation, confirmed on abdominal MRI

16. For any subject who is treatment-naïve, ERT peri-procedurally (from the Screening Period through to Gene Therapy Infusion Period) will be considered in consultation with the PI and Sponsor Medical Monitor.

7.2 Exclusion Criteria

Subjects will be excluded from participation in this study if they meet any of the following exclusion criteria:

1. Subject has Type 2 or 3 Gaucher disease, has severe neurological signs and symptoms, defined as complete ocular paralysis, overt myoclonus or history of seizures, characteristic of neuronopathic Gaucher disease, or has a tremor, peripheral neuropathy or symptoms of Parkinson's disease.
2. Subject has any one of the following:
    a. Hemoglobin value < 9.0 g/dL, or
    b. Platelet count < 70×10^9/L, or
    c. Spleen volume > 10 × normal, or
    d. Pulmonary hypertension
3. Subject has experienced a prior anaphylactic or anaphylactoid reaction (of any severity) to ERT.
4. Treatment-naïve subject has history of clinically significant (CS) anti-GCase antibodies.
5. Subject has a contraindication to ERT, in the opinion of the Investigator.
6. Subject has a contraindication to HSC transplantation, in the opinion of the Investigator.
7. Subject presents with iron, folic acid, and/or vitamin B12 deficiency sustained anemia during Screening.
8. Subject has idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), thrombocytopenia, anemia, hepatomegaly, splenomegaly, and/or osteoporosis unrelated to Gaucher disease, in the opinion of the Investigator.
9. Subject has a clinical co-morbidity such as neurologic, cardiovascular, pulmonary, hepatic, gastrointestinal, renal, hematologic, endocrine, metabolic, genetic, immunologic, neoplastic, or psychiatric disease, other medical condition(s), or intercurrent illnesses that may confound the study results or, in the opinion of the Investigator, may preclude participation in the study.
10. Subject is a pregnant and/or lactating female.
11. Subject is unable to understand the nature, scope, and possible consequences of the study.
12. Subject has diabetes mellitus (Type 1 or Type 2).
13. Subject has active, progressive bone necrosis.
14. Subject has an active chronic infection during the Screening, Baseline, or Pre-gene Therapy Infusion Period of the study.
15. Subject has an active uncontrolled acute bacterial, viral, fungal, parasitic, or prion-associated infection during the Screening, Baseline, or Pre-gene Therapy Infusion Period of the study.
16. Subject has a history of (or current) tuberculosis.
17. Subject tests positive for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV, Type 1 or 2), human T-cell lymphotropic virus (HTLV)-1, HTLV-2, and/or syphilis on Venereal Disease Research Laboratory (VDRL) test, chemiluminescent microplate immunoassay (CMIA), or enzyme immunosorbent assay (EIA) at Screening.

18. Subject has a prior history of (or current) cancer or precancerous lesion or has a known genetic predisposition to cancer. The one exception is a prior history of resected squamous cell carcinoma.
19. Subject has any other medical condition that predisposes him/her to (or conveys increased risk of) malignancy, in the opinion of the Investigator, including history of (or current) monoclonal gammopathy of undetermined significance (MGUS).
20. Subject has a history of alcohol or illicit drug abuse, according to the Investigator's judgment.
21. Subject has undergone, or is scheduled to undergo, BMT, HSC, and/or solid organ transplant. NOTE: Subjects who are otherwise eligible for the study but are scheduled for bone marrow or HSC transplant to treat Type 1 Gaucher disease may be enrolled in the study (instead of receiving an allogeneic transplant) and undergo gene therapy infusion with AVR-RD-02.
22. Subject has white blood cell (WBC) count $< 3.0 \times 10^9$/L and/or uncorrected bleeding disorder from enrollment (ie, signing of informed consent at Screening) through the Gene Therapy Infusion Period of the study (ie, the day of AVR-RD-02 gene therapy infusion).
23. Subject has CS immunosuppressive disease or condition, in the opinion of the Investigator, at Screening.
24. Subject is on (or requires treatment with) cytotoxic or immunosuppressive agents from 60 days prior to signing informed consent at Screening (ie, study enrollment) through the Week 52 study visit; the one exception is treatment with cytotoxic or immunosuppressive agents required per protocol for stem cell transplant.
25. Subject is on (or requires treatment with) red blood cell (RBC) growth factor (eg, erythropoietin) from 6 months prior to enrollment (ie, signing of informed consent at Screening) through the Week 52 study visit.
26. Subject has any condition that makes it impossible to perform MRI studies.
27. Subject has medical condition(s) and/or is receiving medication(s) that would contraindicate ability to undergo mobilization (including contraindication to G-CSF and/or plerixafor), apheresis, or conditioning.
28. Busulfan is contraindicated for the subject.
29. Subject has previously received treatment with AVR-RD-02 or any other gene therapy.
30. Subject is participating in (or plans to participate in) any other investigational drug trial, or plans to be exposed to any other investigational agent, device, and/or procedure, from 30 days prior to enrollment (ie, signing of informed consent at Screening) through study completion.

7.3 Rationale for Inclusion or Exclusion of Certain Study Candidates

AVR-RD-02 is an *ex vivo* LV, autologous gene therapy intended for the durable provision of functional GCase in subjects with Gaucher disease. Enrollment is restricted to subjects with Type 1 Gaucher disease for this Phase 1/2 study to reduce between-subject symptom, and thus, endpoint variability. The most robust assessment of safety and efficacy with AVR-RD-02 treatment would be generated in subjects with Type 1 Gaucher disease who are switch-stable (as defined in this protocol) and in treatment-naïve subjects with Type 1 Gaucher disease. While it is generally recognized that individuals with Types 2 and 3 Gaucher disease exhibit significant neurologic features and markedly reduced life spans, the natural history of these conditions,

along with reports of treatment response, are not well understood. Whereas clinical study data on the safety and efficacy of treatments for Types 2 and 3 Gaucher disease have not been published, there is an extensive body of literature that has arisen from the numerous clinical studies conducted in Type 1 Gaucher disease, thereby allowing for more meaningful interpretation of outcomes in comparison to previous observations (Barton 1991; Ben Turkia 2013; Cox 2012; Cox 2015; Elstein 2015; Gonzalez 2013; Grabowski 1995; Mistry 2015; Pastores 2014; Zimran 2011).

Exclusion of subjects with Types 2 and 3 Gaucher disease prevents the potential introduction of confounding safety signals (see Section 6.2). Thus:

- Inclusion criterion 3 identifies the Type 1 Gaucher disease study population; and,
- Exclusion criterion 1 eliminates subjects who have, or are suspected of having, Types 2 or 3 Gaucher disease.

The requirement that subjects be postpubertal (defined as Tanner Stage 5 genital development and pubic hair distribution in males and breast development in females) at Screening was included to ensure that subjects 18 years to 21 years of age are not continuing to undergo the metabolic and other physical changes associated with puberty. The requirement for subjects to be ≤ 50 years of age at Screening was included as rates of engraftment may decrease with increasing age (see Section 6.2). Thus:

- Inclusion criterion 2 identifies eligible subjects as ≥ 18 years and ≤ 50 years old and postpubertal, defined as Tanner Stage 5 genital development and pubic hair distribution in males and breast development in females.

As the impact of the planned conditioning regimen on infertility and primary ovarian insufficiency in this study population is not fully understood, the entry criteria include items to off-set the potential risk of infertility to female and male subjects (see Section 6.2.2):

- Inclusion criterion 4 mitigates the female infertility risk associated with this protocol by offering reproductive counseling and oocyte harvesting and cryopreservation.
- Inclusion criterion 7 prohibits male and female subjects from donating sperm or egg, respectively, after undergoing conditioning, and offers male subjects the option of sperm cryopreservation prior to conditioning.

Inclusion criteria 10 through 13 define the switch-stable subjects, and inclusion criteria 14 and 15 define the treatment-naïve subjects.

Individuals may be allowed to re-screen based on discussions with the PI and the Sponsor's medical representative or designee's review and approval.

Other exclusion criteria are related to the subjects' fitness for the conditioning regimen and gene therapy infusion. In addition, to most accurately assess the effects of AVRO-RD-201, subjects with medical conditions that can potentially confound measures of safety and efficacy will also be excluded.

7.4 Discontinuations

7.4.1 Discontinuation of Subjects

The criteria for enrollment must be followed explicitly. If it is discovered that a subject who did not meet entry criteria was inadvertently enrolled prior to AVR-RD-02 gene therapy infusion, the subject will be discontinued from the study. If the subject underwent any study-related procedures and then experienced an AE, they will be followed for 30 days or until the AE resolves, whichever is longer (see Section 9.2). If it is discovered that a subject who did not meet entry criteria underwent gene therapy infusion, the subject will not be discontinued and will be followed as per protocol.

Subjects who discontinue from the study prior to gene therapy infusion of AVR-RD-02 may be replaced.

In addition, subjects may be discontinued from the study in the following circumstances:

- The subject or the subject's legal representative (ie, legal guardian) requests to be withdrawn from the study.
- Sponsor decision
  - The Sponsor or its designee discontinues the study or discontinues the subject's participation in the study for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and Good Clinical Practice (GCP).
  - The Sponsor or its designee discontinues the clinical study at a particular investigative site.
- AE
  - If the Investigator decides that the subject should be withdrawn because of an SAE or a CS laboratory value, study-related procedures are to be discontinued and appropriate safety measures taken. The Sponsor or its designee is to be alerted immediately. In cases where the subject has received gene therapy infusion, the subject will be provided the opportunity to participate in the long-term follow-up study.

7.4.2 Discontinuation of Study Sites/Site Terminated by Sponsor

Study site participation may be discontinued if the Sponsor or its designee, the Investigator, or the Institutional Review Board/Independent Ethics Committee (IRB/IEC) of the study site judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP. Subjects would be provided the opportunity to transfer to an eligible site.

7.4.3 Discontinuation of the Study/Study Terminated by Sponsor

The study will be discontinued if the Sponsor or its designee judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP. Subjects who have received gene therapy infusion will be provided the opportunity to participate in the long-term follow-up study.

7.4.4 Stopping Rules

7.4.4.1 Stopping Rules for AVR-RD-02

If the following occurs, gene therapy infusion procedures in additional subjects will be temporarily suspended, and a safety review meeting will take place with the DMC and the Sponsor:

- Death of a subject that is attributed as possibly, probably, or definitely related to AVR-RD-02.

Gene therapy infusion procedures may resume if appropriate changes to study conduct are identified and implemented to reduce any risks to subjects going forward.

7.4.4.2 Enrollment Pause Criteria

- Enrollment in this study may be paused at any time for safety reasons.
- In the event enrollment is paused, subjects who have already been treated with AVR-RD-02 Drug Product will continue in the study.
- The following are examples of events that may result in enrollment pause.
  - Death of a subject that is attributed as possibly, probably, or definitely related to AVR-RD-02.
  - Leukemia/lymphoma that is attributed to an AVRO-RD-02-201 study procedure.
  - Any individual subject shows no or sub-optimal evidence of engraftment of AVR-RD-02 gene modified cells (based on average VCN in blood and bone marrow samples or GCase enzyme activity in blood) at or after Week 26 assessments.
  - Detection of leukemia/lymphoma due to vector-mediated oncogenesis by insertional mutagenesis.
  - Failure to achieve reconstitution with transduced cells in at least one subject, necessitating the use of rescue cells.
  - Unexpected, CS, AVRO-RD-02-related Grade 3 or Grade 4 toxicities in > 2 subjects.
- If enrollment pause occurs, gene therapy infusion procedures in additional subjects will be temporarily paused, and a safety review meeting will take place with the DMC and the Sponsor.
- A determination will be made as to whether the trial should continue without change, continue with specific changes, or terminate.
- The Sponsor will inform the regulatory authorities and the Investigators, and each site's IRB/IEC and other appropriate institutional regulatory bodies will be promptly notified if a decision to pause enrollment is made.

Study enrollment may resume if appropriate changes to study conduct are identified and implemented to reduce any risks to subjects going forward.

7.4.5 Engraftment Failure

Engraftment failure is defined as failure to achieve an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L by Day 42 post-gene therapy infusion of AVR-RD-02 Drug Product. In the event of an engraftment failure:

- The subject should receive the back-up rescue cells.
- Based on the Investigator's discretion, the subject may receive additional evaluations and/or treatments that are outside the scope of this protocol to monitor for and manage engraftment failure.

7.4.6 Enzyme Replacement Therapy

Subjects will be routinely monitored throughout the post-gene therapy infusion period for changes from Baseline values in clinical parameters. Subjects may be started on standard of care ERT in accordance with prespecified ERT starting rules that have been established for time periods of 0 to 5 months and 6 months to 12 months after gene therapy infusion as outlined below.

7.4.6.1 Enzyme Replacement Therapy Initiation Rules for Switch-Stable Subjects Only: 0-5 Months After Gene Therapy Infusion

Between 0 and 5 months after gene therapy infusion, clinical parameters including hemoglobin concentration and platelet counts will be monitored monthly between the Week 5 (Day 30) and Week 26 (Day 180) visits. Levels of GCase activity will be monitored between the Week 9 (Day 60) and Week 26 (Day 180) visits. Subjects with GCase activity above the range for Gaucher disease (ie, > 15% of the lower limit of the range observed in healthy individuals; Pastores and Hughes 2020) in samples collected at 2 months or any of the subsequent months following gene therapy infusion will not receive ERT. At Week 13 (Month 3), subjects who have not received post-gene therapy infusion ERT and have laboratory values that fall within the following clinical parameters: GCase activity in blood > 15% of the lower limit of the range observed in healthy individuals and hemoglobin concentration and platelet count ≥ 80% of the baseline value, will not be required to undergo clinical assessments or GCase activity testing at Week 18 (Month 4) or Week 22 (Month 5). These subjects will resume their clinical assessments at the 6-month post-gene therapy infusion visit.

Subjects with GCase activity that falls within the diagnostic range for Gaucher disease (ie, GCase activity ≤ 15% of the lower limit of the range observed in healthy individuals) for two consecutive monthly visits at the Week 9, 13, 18, or 22 visits will be evaluated for starting ERT. All subjects whose GCase activity falls below 15% of the lower limit of the range observed in healthy individuals at Week 9, 13, 18, or 22 visits will be monitored. The GCase activity value will be distributed to the PI and the Sponsor's medical representative or designee. The determination to initiate post-gene therapy infusion ERT and the ERT dose will be based on this result and will be at the discretion of the PI and the Sponsor's medical representative or designee. Prior to initiation of ERT and to post-gene therapy infusion discontinuation of ERT that was restarted, blood and bone marrow samples will be collected to assist with evaluating the impact of restarting ERT.

7.4.6.2 Enzyme Replacement Therapy Starting Rules: 6-12 Months After Gene Therapy Infusion

The following only applies to subjects who have not been started on ERT between post-gene therapy infusion Weeks 9 to 26.

Switch-stable Subjects

Beginning with the Week 26 (Day 180/Month 6) visit, if two or more of the clinical parameters listed below are met over two consecutive visits, and if the observed clinical deterioration is, in the Investigator's judgment, directly related to the subject's Gaucher disease, the subject may be restarted on ERT at a dose of 15 U/kg every other week (or equivalent):

- Decrease from Baseline in hemoglobin concentration of > 2g/dL
- Platelet count of $< 35 \times 10^9$/L
- Increase in liver volume (as indicated by organ palpation) confirmed to be 20% greater than baseline (as measured by MRI)
- Increase in spleen volume (as indicated by organ palpation) confirmed to be 25% greater than baseline (as measured by MRI)
- New onset pulmonary hypertension

If the clinical parameters that prompted ERT dosing do not return to baseline values within 3 months, the Investigator has the option of increasing the ERT dose by additional increments of 15 U/kg every other week (or the equivalent). The Investigator will discontinue ERT once the affected clinical parameters return to baseline values.

If, after ERT re-initiation, ERT has again been discontinued and any of the clinical parameters outlined above are again observed and confirmed, and are, in the Investigator's judgment, directly related to the subject's Gaucher disease, blood and bone marrow samples will be collected to assist with evaluating no or sub-optimal engraftment of AVR-RD-02 gene modified cells. Consequently, if any individual subject shows no or sub-optimal evidence of engraftment of AVR-RD-02 gene modified cells (based on average VCN in blood and bone marrow samples or GCase enzyme activity in blood) at or after Week 26 assessments, the subject will continue the historical standard of care or the treatment that is recommended by the Investigator.

Treatment-naïve Subjects

Beginning with the Week 26 visit, if any of the clinical parameters outlined below are observed and confirmed, and are, in the Investigator's judgment, directly related to the subject's Gaucher disease, ERT therapy may be initiated:

1. Hemoglobin value < 7.0 g/dL
2. Platelet count $< 35 \times 10^9$/L
3. New onset pulmonary hypertension

The Investigator will discontinue ERT once the affected clinical parameters return to baseline values.

If, after the initiation of ERT, ERT has been discontinued and any of the clinical parameters outlined above are again observed and confirmed, and are, in the Investigator's judgment, directly related to the subject's Gaucher disease, the subject's ERT therapy may be re-introduced. The Investigator may again discontinue ERT once the affected clinical parameters return to baseline values. Prior to initiation of ERT and to post-gene therapy infusion discontinuation of ERT that was restarted, blood and bone marrow samples will be collected to assist with evaluating the impact of restarting ERT.

Sponsor intends to follow subjects enrolled in this study for approximately 14 additional years (for a total of approximately 15 years post-gene therapy infusion follow-up) by way of a follow-up study to AVRO-RD-02-201.

8 TREATMENT

8.1 Treatments Administered

The Sponsor identifier for the investigational product is AVR-RD-02. The active substance in AVR-RD-02 is autologous CD34+ enriched HSCs that have been genetically modified *ex vivo* with an LV containing an RNA transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the human genome, encodes for functional human GCase. The finished investigational product is presented as a cryopreserved cell suspension in infusion bag(s) in vapor-phase liquid nitrogen, which must be thawed prior to use. The investigational product is intended for one-time IV infusion of between 3×10^6/kg and 10×10^6/kg body weight autologous CD34+ enriched HSCs that have been genetically modified.

In addition to AVR-RD-02, subjects will receive the following medications as part of the autologous transplant procedure in this study:

- Between 4 and 8 G-CSF injections and 2 plerixafor injections for HSC mobilization before AVR-RD-02 gene therapy infusion.
  - Additional G-CSF injections will be considered beginning Day +30 after treatment with AVR-RD-02 gene therapy infusion if subject's ANC $< 0.5 \times 10^9$/L. G-CSF may be considered earlier if clinically needed in consultation with the PI and Sponsor Medical Monitor.
- Five IV infusions of busulfan, including 1 test dose to inform the conditioning dose and 4 doses for myeloablative conditioning before treatment with AVR-RD-02.
- Anticonvulsant medication administration, in conjunction with busulfan administration, for seizure prophylaxis.
  - Levetiracetam will be administered. If levetiracetam is contraindicated, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used.

Further details of treatment administration (including dosing regimens) are described in Sections 8.1.1 through 8.1.3. Additional details can also be found in the Study Treatment Manual.

8.1.1 Pre-gene Therapy Infusion Period (Approximately 8 Weeks to 10 Weeks)

In some instances, the Pre-gene Therapy Infusion Period may be extended beyond 10 weeks due to subject and/or site availability to initiate conditioning.

8.1.1.1 Mobilization and Apheresis

Mobilization of the subject's HSC will take place at the participating investigational site based upon standard institutional procedures. On Mobilization Days 1 through 4, subjects meeting eligibility criteria for study participation will receive a daily G-CSF dose of 10 µg/kg body weight administered IV or subcutaneously (SC) (for sites with only prefilled syringes, a G-CSF daily dose from 10 µg/kg body weight up to 12 µg/kg body weight, administered in either a single or divided twice a day [BID] dose may be administered). On the evening of Mobilization

Day 4, subjects will receive an injection of plerixafor (0.24 mg/kg) 8 hours to 11 hours prior to the first apheresis.

On the morning of Apheresis Day 1, mononuclear cells enriched with CD34+ stem cells will be harvested from the subject's peripheral blood by apheresis 8 hours to 11 hours after the first plerixafor administration on Mobilization Day 4, using standard institutional procedures at the participating investigational study site. The apheresis unit obtained on Apheresis Day 1 will be shipped to the manufacturing site for cell transduction and processing (see Section 8.1.1.2). Refer to the Apheresis Day 1 section of the Study Treatment Manual for further details regarding Day 1 of apheresis.

On the evening of Apheresis Day 1, subjects will receive another injection of plerixafor (0.24 mg/kg) 8 hours to 11 hours prior to the second apheresis. On the morning of Apheresis Day 2, subjects will undergo an additional harvest of mononuclear cells enriched with CD34+ stem cells from the subject's peripheral blood by apheresis, using standard institutional procedures at the participating investigational study site. Refer to the Apheresis Day 2 section of the Study Treatment Manual for further details regarding Day 2 of apheresis. The apheresis unit obtained on Apheresis Day 2 will be retained and stored at the investigational site for use as rescue treatment in the unlikely event that there is prolonged bone marrow aplasia after treatment with AVR-RD-02.

If plerixafor cannot be administered within 8 hours to 11 hours prior to the first and the second apheresis in the outpatient setting, the subject should stay in a facility with night staffing (eg, inpatient unit or partial hospital) to ensure that plerixafor is administered within window. If hospital admission is not feasible, plerixafor can be administered up to 14 hours prior to apheresis to achieve a peak CD34+ cell count (Mozobil PI).

After each apheresis (ie, on Apheresis Days 1 and 2), total cell count (per µL) and CD34+ cell enumeration by flow cytometry will be performed at the participating investigational study site to ensure an adequate number of cells have been procured for CD34+ cell selection. The target number of cells for the Apheresis Day 1 collection is 6.5×10^{10} total cells and 6.5×10^{8} CD34+ cells (shipped to the manufacturing site for transduction); the target number of cells for the Apheresis Day 2 collection is at least 3×10^{6} CD34+ cells/kg body weight (retained at the site).

If the quality of the cells is not optimum and affects transduction efficiency, additional round(s) of apheresis may be required. Similarly, if the AVR-RD-02 Drug Product manufactured does not meet release criteria for subject infusion, the Sponsor will immediately notify the Investigator, and an additional mobilization and apheresis cycle may be considered.

Details on timing for mobilization and apheresis are outlined in the Schedule of Assessments in Table 5, and details on the processes may be found in the Study Treatment Manual and Apheresis Operations Manual.

8.1.1.2 Investigational Product Preparation/Testing for Release

Following harvest on Apheresis Day 1, the apheresis unit harvested on that day will be transported under temperature-controlled conditions (2°C to 8ºC) to a manufacturing site for further processing. The manufacturing for the AVR-RD-02 investigational product is compliant with applicable current Good Manufacturing Practice (cGMP).

AVR-RD-02 will be tested for identity, potency, purity, and safety prior to release for clinical use. Once the release testing is complete, AVR-RD-02 investigational product will be dispositioned for clinical use and sent to the investigational site in dry shipper with a Certificate of Analysis(es), documenting that all release testing specifications for clinical use have been met, and that the investigational product can be used in humans. AVR-RD-02 is stable for up to 12 months when stored in vapor-phase liquid nitrogen.

8.1.1.3 Conditioning

After the site receives the investigational product dispositioned for clinical use, the subject will enter the Conditioning phase.

During this phase of the study, subjects will receive a QD, 4-day myeloablative conditioning regimen of IV busulfan that is informed by a weight-based test dose of 0.8 mg/kg administered on Conditioning Day -6. The test dose will serve to establish the predicted PK for each individual subject, as part of the TDM. The test dose of busulfan will be administered on Conditioning Day -6 for sites using LC-MS to perform busulfan TDM and on Conditioning Day -5 for sites using the immunoassay kit to perform busulfan TDM.

For Conditioning Days -4, -3, -2, and -1, a dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC, as described in the Study Treatment Manual. The myeloablative cumulative target for busulfan exposure in the AVRO-RD-02-201 study will be an AUC of $90_{(\text{day 0-4})} \pm 2\%$ mg•hr/L.

Once-daily dosing of IV busulfan with TDM will be administered as follows for sites using LC-MS to perform TDM (also see Table 1):

- On Conditioning Days -4 and -3, a PK- and weight-based guided IV busulfan dose, based on TDM PK of the test dose on Day -6 and the respective calculated AUC generated from the web-based dose simulation program, will be administered.
- For Conditioning Day -2, the dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC based on TDM PK of the test dose and Day -4.
- For Conditioning Day -1, the dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC based on TDM PK of the test dose, Day -4, and Day -3.
- If AUC of the previous dose is outside of the AUC_{target} determined by the web-based dose simulation program, the busulfan dose will be adjusted accordingly.

Table 1: PK- and Weight-Based Guided Busulfan Conditioning Regimen for Sites Using LC-MS to Perform Busulfan TDM

Dosing Day	Busulfan Dose (mg/kg/day)	TDM PK Schedule for Busulfan Dose Determination
Day -6	Test dose of 0.8 mg/kg	PK sampling at 1, 3, 5, and 7 hours post dose; TDM PK results will be used to inform the dose on Day -4 and Day -3
Day -4	Dose based on TDM PK of test dose	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the dose on Day -2 together with TDM PK results from the test dose

Day -3	Dose based on TDM PK of test dose	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the dose on Day -1 together with TDM PK results from the test dose and Day -4
Day -2	Dose based on TDM PK of test dose and Day -4	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the cumulative AUC
Day -1	Dose based on TDM PK of test dose, Day -4, and Day -3	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the cumulative AUC

Abbreviations: AUC = area-under-the curve; LC-MS = liquid chromatography-mass spectrometry; PK = pharmacokinetics; TDM = therapeutic drug monitoring.

Once-daily dosing of IV busulfan with TDM will be administered as follows for sites using the immunoassay kit to perform TDM (see also Table 2):

- On Conditioning Day -4, a PK- and weight-based guided IV busulfan dose, based on TDM PK of the test dose on Day -5 and the respective calculated AUC generated from the web-based dose simulation program, will be administered.
- For Conditioning Day -3, the web-based dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC based on TDM PK of the test dose and Day -4.
- For Conditioning Day -2, the web-based dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC based on TDM PK of the test dose, Day -4, and Day -3.
- For Conditioning Day -1, the web-based dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC based on TDM PK of the test dose, Day -4, Day -3, and Day -2.
- If AUC of the previous dose is outside of the AUC_{target} determined by the web-based dose simulation program, the busulfan dose will be adjusted accordingly.

Table 2: PK- and Weight-Based Guided Busulfan Conditioning Regimen for Sites Using the Immunoassay Kit to Perform Busulfan TDM

Dosing Day	Busulfan Dose (mg/kg/day)	TDM PK Schedule for Busulfan Dose Determination
Day -5	Test dose of 0.8 mg/kg	PK sampling at 1, 3, 5, and 7 hours post dose; TDM PK results will be used to inform the dose on Day -4
Day -4	Dose based on TDM PK of test dose	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the dose on Day -3 together with TDM PK results from the test dose
Day -3	Dose based on TDM PK of test dose and Day -4	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the dose on Day -2 together with TDM PK results from the test dose and Day -4
Day -2	Dose based on TDM PK of test dose, Day -4, and Day -3	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the dose on Day -1 together with TDM PK results from the test dose, Day -4, and Day -3
Day -1	Dose based on TDM PK of test dose, Day -4, Day -3, and Day -2	PK sampling at 1, 3, and 5 hours post dose; TDM PK results will be used to inform the cumulative AUC

Abbreviations: AUC = area-under-the curve; PK = pharmacokinetics; TDM = therapeutic drug monitoring.

Pharmacokinetic sampling may also be performed on Day -5 to support optimization of busulfan exposure.

Anticonvulsant medication will be administered for seizure prophylaxis in conjunction with busulfan administration. Levetiracetam will be administered at a dose of 1,000 mg per day from Conditioning Day -8 (ie, 2 days before administration of the busulfan test dose) until 1 day after the administration of the 4-day busulfan conditioning regimen. For subjects with contraindication to levetiracetam, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used.

After the 4-day conditioning regimen is administered, a busulfan washout period after the end of the last busulfan infusion for a minimum of 24 hours and up to a maximum of 48 hours is required prior to AVR-RD-02 administration.

Timing for the conditioning procedures is outlined in the Schedule of Assessments in Table 5, with further information included in the Study Treatment Manual.

8.1.2 Gene Therapy Infusion Period (1 Day)

After completion of the busulfan washout period (ie, a minimum of 24 hours up to a maximum of 48 hours after the end of the last busulfan infusion), AVR-RD-02 will be administered once by IV infusion. Prior to IV infusion, subject identifiers on AVR-RD-02 investigational product must be checked against the intended recipient, and the investigational product must be thawed at 37ºC prior to use. Further details on the gene therapy infusion process may be found in the Study Treatment Manual.

8.1.3 Post-gene Therapy Infusion Follow-up Period (Approximately 52 Weeks)

After AVR-RD-02 infusion, the subject will enter the Post-gene Therapy InfusionFollow-up Period, during which time periodic safety and efficacy assessments will be performed to monitor safety post-gene therapy infusion, and to assess measures of engraftment and clinical response.

In particular, safety laboratory assessments will be performed daily for the first 7 days post-gene therapy infusion. G-CSF injections will be considered in case a subject's ANC is $< 0.5 \times 10^9$/L at Day +30 post-gene therapy infusion. G-CSF may be considered earlier if clinically needed in consultation with the PI and Sponsor Medical Monitor.

8.2 Materials and Supplies

AVR-RD-02

The finished investigational product is presented as a cryopreserved product, which is thawed at 37°C immediately prior to use. The thawed investigational product is intended for one-time IV infusion of between 3×10^6/kg and 10×10^6/kg body weight autologous CD34+ enriched HSCs that have been genetically modified.

Upon receipt at the investigational site, cryopreserved investigational product must be maintained cryopreserved in infusion bag(s) in vapor-phase liquid nitrogen until ready to thaw for clinical use. Thawed investigational product maintained at controlled room temperature in cryopreservation media is stable for up to 3 hours.

Traceability from the initiation of autologous HSC procurement through the completion of AVR-RD-02 gene therapy infusion must be ensured through labeling and documentation control at each step. Investigational sites are required to maintain labeling control and documentation to support these steps. The exact volume infused, and the time of gene therapy infusion start, and completion will be documented in the subject's electronic case report form (eCRF).

Any unused AVR-RD-02 investigational product should be disposed of in accordance with local biosafety requirements.

Further details may be found in the Study Treatment Manual.

Other Study Treatments

For storage requirements, handling, and stability of other study treatments (ie, pre-gene therapy infusion injections), see the manufacturers packaging.

8.3 Dosing Considerations

8.3.1 Rationale for Selection of Dose in the Study

The goal of the gene therapy infusion is to provide as many CD34+ transduced cells as possible. Due to variables in individual apheresis results, the actual number of infused transduced cells will vary per subject.

- The appropriateness of proceeding with myeloablation in any given subject will be based on the results of that subject's corresponding Drug Product release test, including the number of cells and cell viability.
- The AVR-RD-02 dose range to be administered (ie, between 3×10^6/kg and 10×10^6/kg body weight autologous CD34+ enriched HSCs that have been genetically modified) is based upon well-established and accepted safe practices commonly used in autologous HSCT and has been shown to safely achieve rapid hematopoietic reconstitution along with long-term engraftment. The safety and potential efficacy of dosing using accepted autologous HSCT practice is highlighted by the demonstrated safety and benefit of autologous HSCT in the treatment of the visceral manifestation of Gaucher disease (Ito and Barrett 2013).
- The upper dose limit of 10×10^6 CD34+ cells/kg is supported by preclinical safety studies that demonstrated tolerability at twice the planned maximum dose.
- There is general consensus that a minimum dose of 2.5×10^6 cells/kg of mobilized CD34+ cells is required for favorable engraftment (Jillella and Ustun 2004). However, at lower cell counts, recovery of platelet and neutrophil counts may be delayed. Therefore, the lowest dose of AVR-RD-02 selected for the study is 3.0×10^6 cells/kg.
- Data collected during the course of this trial (eg, Drug Product testing results, cell number, cell viability, average VCN per cell, total dose administered along with clinical safety, engraftment, % transduction, and efficacy outcomes, etc.) may help inform future dosing considerations.

8.3.2 Other Important Treatment Considerations

As AVR-RD-02 is an autologously-derived product, it is not tested for transmissible infectious agents. Healthcare professionals handling AVR-RD-02 should therefore take appropriate

precautions per local guidelines to avoid potential transmission of infectious diseases. Additionally, as this is an autologous drug product pre-medications as mitigation of infusion reactions are not required routinely.

A summary of precautions required and key toxicities associated with G-CSF, plerixafor, busulfan, levetiracetam (or other anticonvulsant medication used) can be found in the Investigator's Brochure for AVR-RD-02. Investigators should refer to the most current local prescribing information for details regarding precautions required and a complete list of toxicities associated with G-CSF, plerixafor, busulfan, levetiracetam (or other anticonvulsant medication used).

Precautions should be taken during apheresis to avoid conditions such as hypotension, electrolyte abnormalities, citrate toxicity, thrombocytopenia, and coagulation abnormalities. Stem cell infusion can cause tightness in chest, hypotension, coughing, chest pain, decreased urine output, weakness, hypersensitivity reactions, electrolyte disturbances, and rarely, engraftment syndrome (fever, rash, and non-cardiogenic pulmonary edema). Subjects should be cautioned on these and other potential side effects of stem cell infusion.

Complete details regarding study treatments are contained in the Study Treatment Manual.

8.4 Blinding

This is an open-label study.

9 STUDY ASSESSMENTS

Tabular overviews of the required assessments for the study (including the timing of each) can be found in Section 15.1. The Schedule of Assessments from Screening through the Apheresis Period is in Table 4, the Schedule of Assessments from Investigational Product Preparation and Testing through End of Infusion is in Table 5, and the Schedule of Assessments for the Post-infusion Follow-up Period (Study Week 1 [Day 1] through Study Week 52 [Day 365]) is in Table 6.

9.1 Safety Assessments

9.1.1 Physical Examinations

A physical examination will be performed at the times specified in Table 4, Table 5, and Table 6. Each examination will include the following assessments: general appearance; skin; head, ears, eyes, nose, mouth, and throat; neck; lymph nodes; chest; heart; abdomen; limbs; central nervous system; and musculoskeletal system.

If CS worsening from Screening are noted for any assessments, the change will be documented as an AE in the AE eCRF. Conditions present at the time informed consent is signed and will be documented in the Medical History eCRF. Clinical significance is defined as any worsening in physical findings that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the subject until the parameter returns to baseline or until the Investigator determines that follow-up is no longer medically necessary.

9.1.2 Vital Signs

The following vital signs will be recorded at the times specified in Table 4, Table 5, and Table 6: heart rate (beats/minute), systolic and diastolic blood pressure (mmHg), respiratory rate (breaths/minute), and temperature (°C or °F). Vital signs will be obtained after the subject has been supine or seated. On Days 1 and 2 of the Apheresis phase of the Pre-gene Therapy Infusion Period, vital signs must be taken prior to apheresis. On Day 0 of the Gene Therapy Infusion Period (ie, the day of AVR-RD-02 infusion), vital signs must be taken pre-infusion (window -10 minutes) and post-infusion (window +10 minutes). Additional vital signs may be taken if clinically indicated and/or mandated per local institution practice for gene therapy infusion.

If CS worsening in vital signs as compared to Screening is noted, the change will be documented as an AE in the AE eCRF. Clinical significance is defined as any variation in vital signs that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the subject until the parameter returns to baseline or until the Investigator determines that follow-up is no longer medically necessary.

9.1.3 Serum Chemistry, Hematology, and Urinalysis

Serum chemistry, hematology, and urinalysis will be performed at the times specified in Table 4, Table 5, and Table 6. In particular, safety laboratory assessments will be performed daily for the first 7 days post-gene therapy infusion (see Section 8.1.3).

Serum chemistry includes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), amylase, lipase, alkaline phosphatase (ALP), lactate

dehydrogenase (LDH), bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. Electrolytes include sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), and serum creatinine. Hematology includes hematocrit, hemoglobin, platelet count, WBC count (total and differential), RBC count, ANC, and flow cytometric analysis of T and B cell counts as well as peripheral blood lymphocyte subsets (CD3, CD4, CD8, CD19, and CD16/56). Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated).

Laboratory assessments for safety will be tested locally.

It is anticipated that some laboratory values may be outside of the normal value range due to the underlying disease or recovery from conditioning. As in routine practice, the Investigators should use their medical judgment when assessing clinical significance. Clinical significance is defined as any worsening in laboratory measurements which has medical relevance and which results in a change in medical care. If any CS laboratory worsening from Baseline is noted, the changes will be documented as AEs in the AE eCRF. The Investigator will also assess the relationship to study treatment (see Section 9.2.1 for guidance on reporting, recording, and assessing AEs) for all CS out of range values. The Investigator will continue to monitor the subject with additional laboratory assessments until (1) values have reached normal range and/or baseline, or (2) in the judgment of the Investigator, out of range values are not related to the administration of study drug or other protocol-specific procedures.

9.1.4 Electrocardiograms

For each subject, 12-lead digital ECGs will be collected at the times specified in Table 4, Table 5, and Table 6 as single ECGs for over-read. Subjects must be supine for approximately 5 to 10 minutes before ECG collection and remain supine but awake during ECG collection.

Electrocardiograms may be obtained at additional times when deemed clinically necessary. Collection of more ECGs than expected at a given time point is allowed when needed to ensure high quality records.

Electrocardiograms will be interpreted by a qualified physician (the Investigator or qualified designee) at the site as soon after the time of ECG collection as possible, and ideally while the subject is still present to determine whether the subject meets entry criteria and for immediate subject management should any clinically relevant findings be identified.

9.1.5 Reproductive Potential

For male subjects, sperm samples for volume, sperm count, sperm concentration, total motility, progressive motility, and morphology will be collected at the times specified in Table 4, Table 5, and Table 6 and will be tested locally.

For female subjects, the use of gonadotrophic-releasing hormone agonists (Lupron) for ovarian chemoprotection can be offered prior to gonadotoxic therapy such as busulfan. A recall history of menstrual cycles for the year preceding enrollment will also be collected at Baseline. Monthly menstrual reports will be provided by female subjects for all menstrual cycles beginning at the Baseline visit through the end of the study. In addition, a blood sample for AMH, FSH, and LH will be collected at the times specified in Table 4, Table 5, and Table 6 and will be tested locally.

If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology in male subjects; abnormal AMH levels in female subjects), investigational sites should follow institutional guidelines for further evaluation. For both male and female subjects, additional assessments of reproductive potential and/or referral to a urologist (male subjects) or a reproductive endocrinologist (female subjects) may be recommended.

Reproductive outcomes, if any, will be monitored and recorded during this study.

9.1.6 Immunogenicity

Blood samples for immunogenicity testing will be collected at the times specified in Table 4, Table 5, and Table 6 to determine antibody production against GCase. Immunogenicity will be assessed by a central laboratory using validated assays designed to detect IgG, IgA, and IgM. Blood samples for immunogenicity testing may be obtained at additional times, including when an allergic reaction to investigational product is suspected and/or when otherwise deemed clinically necessary.

9.1.7 Replication-Competent Lentivirus

Peripheral blood samples for replication-competent lentivirus (RCL) testing will be collected at the times specified in Table 4 and Table 6 to detect the emergence of RCL.

Blood samples will be tested centrally. Details on blood sample collection, processing, and shipment will be included in the Laboratory Manual.

9.1.8 Insertional Site Analysis

For subjects with an average VCN in PBLs ≥ 0.01 as determined by the central laboratory, suggesting $\geq 1\%$ of the sampled blood cells are vector-positive with an average of 1 copy/diploid genome (c/dg), testing for vector ISA will also take place at the times specified in Table 4, Table 5, and Table 6.

Blood samples will be tested centrally. Details on blood sample collection, processing, and shipment will be included in the Laboratory Manual.

Additional blood sample collection for ISA may also take place at any time, if clinically indicated.

9.1.9 Samples to be Archived for Possible Future Testing

The DNA from peripheral blood and bone marrow aspirate samples will be collected at Baseline, as specified in Table 4, and will be archived for possible future testing should a subject develop hematologic cancer.

As knowledge of Type 1 Gaucher disease pathophysiology and related biomarkers continues to evolve, back-up plasma and urine samples will be collected and archived for possible future non-genomic and genomic biomarker testing either at specialized laboratories or a Biorepository site. These samples will be destroyed 5 years after the Clinical Study Report (CSR) is finalized.

9.1.10 COVID-19 Guidance: Sign and Symptom Assessment

During the period where there remains transmission risk of coronavirus disease 2019 (COVID-19), site staff will monitor the subject's temperature and perform a COVID-19 symptom assessment prior to study procedures, assessments, and visits per the individual site's guidelines. If there is no institution specific guideline, the site staff will perform a PCR test to check for current SARS-CoV-2/COVID-19 infection.

The COVID-19 test prior to study procedures, including mobilization, apheresis, conditioning and AVR-RD-02 gene therapy infusion, will be conducted no later than 5 days prior to the planned procedure.

Most recent updates on COVID-19 infection can be addressed in the latest version of the Public Health Surveillance for COVID-19, Interim Guidance (World Health Organization 2020).

9.2 Safety Monitoring

Investigators are responsible for monitoring the safety of subjects who have entered this study and for alerting the Sponsor or its designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the subject.

The Investigator is responsible for the appropriate medical care of subjects during the study.

If an SAE that is considered related to a study procedure or the study causes a subject to discontinue before completing the study, the Investigator remains responsible for following the event through an appropriate health care option. The subject should be followed for 30 days or until the event is resolved, whichever is longer. Frequency of follow-up evaluation is left to the discretion of the Investigator.

The Sponsor's medical representative or designee will monitor safety data throughout the course of the study.

The Sponsor's medical representative or designee will review SAEs within time frames mandated by company procedures. The Sponsor's medical representative or designee will, as appropriate, periodically review the following:

- Trends in safety data
- Laboratory analytes
- AEs, including monitoring of infections and malignancies

If a subject experiences elevated ALT or AST > 3×the upper limit of the normal range or elevated total bilirubin > 2×the upper limit of the normal range, clinical and laboratory monitoring should be initiated by the Investigator. Details for hepatic monitoring depend upon the severity and persistence of observed laboratory test abnormalities. To ensure subject safety and comply with regulatory guidance, the Investigator is to consult with the Sponsor's medical representative or designee regarding collection of specific recommended clinical information and follow-up laboratory tests.

9.2.1 Adverse Events

An AE is any untoward medical occurrence in a clinical study subject who has been administered a medicinal product and that does not necessarily have a causal relationship with

this treatment. An AE can therefore be any unfavorable or unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to this medicinal product.

For the purposes of this study, the definition of an AE also covers:

- Medication errors and uses outside of what is foreseen in the protocol, including accidental overdose, only if an AE results from the error
- Any Grade 3 or higher platelet count, Grade 3 or higher WBC count, and any Grade 2 or higher neutrophil count (grade determined by the Common Terminology Criteria for Adverse Events [CTCAE] v4.03; see Section 9.2.1.2) regardless of whether considered CS by Investigator report
- Fertility-related events, considered an AE of special interest (AESI) for the study

The following are not considered AEs:

- Situations where an untoward medical occurrence did not occur (eg, planned and/or elective admission to a hospital)
- Anticipated day-to-day fluctuations of pre-existing disease(s)
- Condition(s) present or detected at the start of the study that do not worsen

The Sponsor has standards for reporting AEs that are to be followed regardless of applicable regulatory requirements that may be less stringent.

Lack of drug effect is not an AE in clinical studies because the purpose of the clinical study is to establish drug effect.

Cases of pregnancy that occur in female subjects or partners of male subjects during exposure to study treatment are to be reported (see Section 9.2.2). Data on fetal outcome and breast-feeding are collected for regulatory reporting and drug safety evaluation.

Study site personnel will record the occurrence and nature of each subject's pre-existing conditions, including CS signs and symptoms of the disease under treatment in the study in Medical History eCRF.

After the informed consent form (ICF) is signed, site personnel will record any change in the condition(s) and the occurrence and nature of any AEs. Adverse events will be recorded in the eCRF according to the study period in which they occurred (ie, Screening, Baseline, Pre-gene Therapy Infusion [Mobilization, Apheresis, or Conditioning], Gene Therapy Infusion, or Post-gene Therapy Infusion Follow-up).

9.2.1.1 Exemptions to Adverse Event Recording/Reporting

For the purposes of this study, the following will not be considered AEs:

- Situations where an untoward medical occurrence did not occur (eg, social and/or convenience admission to a hospital, inpatient busulfan conditioning and management, etc.), and 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 over the course of the study
- Lack of drug effect (because one of the objectives of this study is to establish the effect of AVR-RD-02 treatment)

9.2.1.2 Severity Assessment

Adverse event severity will be graded using the CTCAE v4.03. The CTCAE is a descriptive terminology utilized for AE reporting. A grading (severity) scale is provided for each AE term (Each CTCAE v4.03 term is a Medical Dictionary for Regulatory Activities [MedDRA] Lowest Level Term).

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).[a]

Grade 3 Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.[b]

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

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

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

Change in severity of an AE should be documented based on specific guidelines in the eCRF completion guidelines.

Severity and seriousness must be differentiated; severity describes the intensity of an AE while seriousness refers to an AE that has met the criteria for an SAE (see Section 9.2.1.5).

9.2.1.3 Causality Assessment

Investigators will be instructed to report to the Sponsor or its designee their assessment of the potential relatedness of each AE (both serious and non-serious) to protocol procedures and/or study treatment. Causality assessments will be provided on the eCRF (and any additional forms as appropriate) according to the study period in which it occurred (ie, Screening, Baseline, Pre-gene Therapy Infusion [Mobilization, Apheresis, or Conditioning], Gene Therapy Infusion, or Post-gene therapy infusion Follow-up). Causality relationship will be classified according to the definitions in Table 3.

Table 3: Causality Definitions

Definite	• Event or laboratory test abnormality with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically) • Event definitive pharmacologically or phenomenologically (ie, an objective and specific medical disorder or a recognized pharmacological phenomenon) • Rechallenge satisfactory, if necessary

Probable	• Event or laboratory test abnormality with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	• Event or laboratory test abnormality with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal may be lacking or unclear
Unlikely	• Event or laboratory test abnormality with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations
Unrelated	• Event or laboratory test abnormality is plausibly related to the subject's clinical state, underlying disease, or the study procedure/conditions • Time relationship to drug intake makes a relationship unreasonable • Other obvious causes for event or laboratory test abnormality exist
Unknown	• Report suggests an AE; however, cannot be judged at this time because information is insufficient or contradictory • More data for proper assessment is needed, or additional data is under examination
N/A	• Report suggests an AE; however, the need to assess causality is not practical or of value due to the event itself or to the circumstances surrounding the event

If a subject's gene therapy infusion is cancelled or discontinued as a result of a protocol procedure- or pre-transfusion-related AE prior to the gene therapy infusion, study site personnel must clearly report to the Sponsor or its designee via eCRF the circumstances and data leading to any such cancellation or discontinuation of treatment. Please see the eCRF guidelines for additional details.

9.2.1.4 Outcome Assessment

Investigators will be instructed to report to the Sponsor or its designee their assessment of the outcome of each AE (both serious and non-serious). Definitions for possible AE outcomes include:

- Recovered/Resolved: the event has improved or the patient recuperated
- Recovered/Resolved with Sequelae: the patient recuperated but retained pathological conditions directly resulting from the disease or injury
- Recovering/Resolving: the event is improving
- Not Recovered/Not Resolved: the event has not improved or the patient has not recuperated
- Fatal: termination of life as a result of an AE. There should be only one AE marked with this outcome
- Unknown: not known, not observed, not recorded, or refused

9.2.1.5 Serious Adverse Events

The SAE collection begins after the subject has signed informed consent. Elective or planned procedures and/or hospitalizations that were scheduled or anticipated prior to entry into the study should not be reported as SAEs. During the study, elective, anticipated, or planned procedures/hospitalizations should not be reported as SAEs unless the underlying medical condition has worsened over the course of the study. For SAEs that are due to worsening underlying medical conditions, the SAE form should be marked as "due to underlying medical condition."

Study site personnel must alert the Sponsor or its designee of any SAE within 24 hours of Investigator awareness of the event via a Sponsor-approved method. This 24-hour notification requirement refers to the initial SAE information.

An SAE is any AE from this study that results in 1 or more of the following outcomes:

- Results in death
- Requires or prolongs hospitalization
- Is life-threatening (that is, immediate risk of dying)
- Persistent or significant disability/incapacity
- Congenital anomaly or birth defect
- Other medically important serious event

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, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

The SAEs occurring up to and including the subject's last study visit will be collected regardless of the Investigator's opinion of causation.

If an Investigator becomes aware of SAEs occurring to a subject after the subject's participation in the study has ended (including any protocol-required post-treatment follow-up), the Investigator should report the SAEs to the Sponsor regardless of the Investigator's opinion of causation.

Information on SAEs expected in the study population independent of study treatment and that will be assessed by the Sponsor in aggregate periodically during the course of the study may be found in the Reference Safety Information section of the Investigator's Brochure.

9.2.1.6 Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the Investigator's Brochure and that are identified as related to study treatment. United States 21 Code of Federal Regulations (CFR) 312.32 and European Union Clinical Trial Directive 2001/20/EC and the associated detailed guidances or national regulatory requirements in participating countries require the reporting of SUSARs. The Sponsor has procedures that will be followed for the recording and expedited reporting of SUSARs that are consistent with global regulations and the associated detailed guidances.

9.2.1.7 Reporting Adverse Events

All non-serious AEs must be recorded in the eCRF upon awareness.

All SAEs must be reported to the to Sponsor or designee, via the SAE contact in the Safety Management Plan, within 24 hours of the Investigator or their staff becoming aware of them. The Investigator must complete, sign, and date the SAE pages, verify the accuracy of the information recorded on the SAE pages with the corresponding source documents, and send a copy via electronic submission to the safety database or e-mail. Facsimile transmission may be used in the event of electronic submission or e-mail failure.

When further information becomes available, the SAE Report Form should be updated with the new information and reported immediately via the same methods as the initial information.

Additional follow-up information, if required or available, should be reported to the Sponsor or designee within 24 hours of the Investigator or their staff becoming aware of this additional information via same manner as above.

Follow-up information should be recorded on a follow-up SAE Report Form and placed with the original SAE information and kept with the appropriate section of the SAE Report Form and/or study file.

These timelines for reporting information to the Sponsor must be followed for all initial SAE reports and for all follow-up versions of the initial reports.

All SAEs (related and unrelated) will be recorded from the signing of informed consent until the Week 52 (or Early Termination) Visit.

For all SAEs the Investigator must provide the following:

- Appropriate and requested follow-up information in the time frame detailed above
- Causality of the SAE(s)
- Outcome of the SAE(s)
- Redacted medical records and laboratory/diagnostic information

9.2.1.8 Sponsor Reporting Requirements

The Sponsor or its legal representative is responsible for notifying the relevant regulatory authorities of SAEs meeting the reporting criteria. This protocol will use the current Investigator's Brochure as the Reference Safety Document. The expectedness and reporting criteria of an SAE will be determined by the Sponsor from the Reference Safety Document.

9.2.1.9 Investigator Reporting Requirements

The Investigator must fulfill all local regulatory obligations required for the study Investigators. It is the PI's responsibility to notify the IRB/IEC of all SAEs that occur at his or her site. Investigators will also be notified of all SUSAR events that occur during the clinical study. Each site is responsible for notifying its IRB/IEC of these additional SAEs according to local requirements.

9.2.2 Exposure During Pregnancy and/or Lactation

Pregnancy data will be collected during this study for all subjects.

Pregnancy is permissible during the study provided that the gestational carrier is not a subject in the study and the pregnancy is the result of either:

a. A fertilization involving the subject's gametes that occurred before the subject's busulfan exposure, or
b. A fertilization that involves gametes collected from the subject before busulfan exposure, or
c. A fertilization using donor sperm or egg in lieu of the subject's gametes.

Exposure during pregnancy must be recorded and the subject followed until the outcome of the pregnancy is known (spontaneous miscarriage, therapeutic abortion, elective termination, normal birth, congenital abnormality, or ongoing), even if the subject discontinues from the study.

If a female subject or a male subject's partner becomes pregnant while treated or exposed to study treatment, the Investigator must submit a pregnancy form to the Sponsor or designee via the same method as SAE reporting (see Section 9.2.1.7). When the outcome of the pregnancy becomes known (ie, spontaneous miscarriage, elective termination, normal birth, or congenital abnormality), the pregnancy form must be completed and returned to the Sponsor or designee. If additional follow-up is required, the Investigator will be requested to provide the information.

For any partner pregnancies reported during the study, the pregnancy must be followed until the outcome of the pregnancy is known, even if the subject discontinues from the study.

Pregnancy in itself is not regarded as an AE unless there is a suspicion that study treatment may have interfered with the effectiveness of a contraceptive medication. However, complications of pregnancy and abnormal outcomes of pregnancy are AEs, and many may meet criteria for an SAE (such as ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly). Elective abortions without complications should not be reported as AEs.

9.3 Engraftment and Efficacy Measures

9.3.1 Vector Copy Number

Blood samples for measurement of average VCN in PBLs and bone marrow aspirate by qPCR and/or ddPCR analysis will be collected at times shown in Table 4, Table 5, and Table 6. Samples will be tested at a central laboratory.

9.3.2 GCase Enzyme Activity in Peripheral Blood

Blood samples for measurement of GCase enzyme activity in peripheral blood will be collected at times shown in Table 4, Table 5, and Table 6. Samples will be tested at a central laboratory. Blood collection on DBS cards for GCase activity measurement will also occur at the indicated study time points in mitigation of unforeseen circumstances, which could ruin blood samples arriving out of the sample stability period at the central laboratory (Gill 2022; Miyamoto 2021).

9.3.3 Enzyme Replacement Therapy

Investigators will record the rationale for initiating standard of care ERT as well as gene therapy infusion dates and dose.

9.3.4 Primary Efficacy Measures

The following primary efficacy measures will be collected at the times shown in Table 4, Table 5, and Table 6:

- Spleen and liver volumes assessed by abdominal MRI. Scans will be performed locally and interpreted by a blinded central reader (local interpretation will be used to determine study eligibility). Details will be included in the Imaging (or other relevant) Manual for the study.
- Hemoglobin concentration, hematocrit percentage, and platelet count samples collected for efficacy measures will be tested at a central laboratory. Details regarding sample collection, processing, and shipment will be included in the Laboratory Manual.
- Biomarkers for Gaucher disease (ie, chitotriosidase enzyme activity and lyso-Gb1 plasma levels) will be analyzed in blood samples by a central laboratory. Details regarding sample collection, processing, and shipment will be included in the Laboratory Manual.

9.3.5 Secondary Efficacy Measures

The following secondary efficacy measures will be collected at the times shown in Table 4, Table 5, and Table 6:

- BMB will be assessed by bone MRI (DeMayo 2008; Maas 2003; Robertson 2007). Scans will be performed locally and scored by a blinded central reader. Details will be included in the Imaging (or other relevant) Manual for the study.
- BMD assessed by DXA. Scans will be performed and interpreted locally.

9.3.6 Exploratory Efficacy Measures

The following exploratory efficacy measures will be collected at the times shown in Table 4 and Table 6:

- Functional status assessed by the SF-36: The SF-36 is a 36-item, subject-reported measure yielding 8 subscale scores (Vitality, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Role Functioning, Emotional Role Functioning, Social Role Functioning, and Mental Health) and 2 component summary scores (Physical Functioning and Mental Functioning).
- Pain assessed by the BPI-SF questionnaire: The BPI-SF is a 9-item self-administered questionnaire used to evaluate the severity of a subject's pain and the impact of this pain on the subject's daily functioning on an 11-point scale (ie, 0 = "no pain" to 10 = "pain as bad as you can imagine").
- Exploratory biomarkers for Gaucher disease in serum, plasma, PBLs, and urine: As knowledge of Gaucher disease pathophysiology and related biomarkers continues to evolve, serum, urine, plasma and PBL samples will be collected and archived for possible future non-genomic and genomic biomarker testing. Samples will be destroyed 5 years after the CSR is finalized.
- To assess engraftment dynamics, average VCN will be determined, and ISA performed, in whole blood and bone marrow cells at time points specified in Table 4; Table 5; and Table 6. Details on whole blood and bone marrow sample collection, processing, and shipment will be included in the Laboratory Manual for the study.

9.4 Appropriateness of Measurements

Efficacy and safety measures chosen for this study have commonly been used to measure treatment effects in studies of Gaucher disease (Barton 1991; Ben Turkia 2013; Elstein 2011; Elstein 2015; Gonzalez 2013; Grabowski 1995; Kamath 2014; Lukina 2010; Pastores 2016; Pastores 2004; Rolfs 2013; Zimran 2010; Zimran 2011; Zimran 2013; Zimran 2015; Miyamoto 2021).

10 DATA QUALITY ASSURANCE

To ensure accurate, complete, and reliable data, the Sponsor or its representatives will do the following:

- Provide instructional material to the study sites, as appropriate
- Provide training to instruct the Investigators and Study Coordinators. This training will give instruction on the protocol, the completion of the eCRFs, and the study procedures.
- Conduct monitoring visits at the study site
- Be available for consultation and stay in contact with the study site personnel
- Review data/data listings on an ongoing basis, and use standard computer edits to detect errors in data collection
- Conduct a quality review of the database

In addition, the Sponsor or its representatives will periodically check a sample of the subject data recorded against source documents at the study site. The study and/or study site may be audited by the Sponsor or its representatives and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

To ensure the safety of subjects in the study and to ensure accurate, complete, and reliable data, the Investigator will keep records of laboratory tests, clinical notes, and subject medical records in the subject files as original source documents for the study. If requested, the Investigator will provide the Sponsor, applicable regulatory agencies, and applicable IRBs/IECs with direct access to original source documents.

10.1 Data Collection and Storage

All clinical raw data will be recorded promptly, accurately, and legibly either directly into a data capture system as e-source data or indelibly on paper (eg, ECG readings). A detailed list of the type (electronic or paper) and location for all source data will be included in the Trial Master File. When recorded electronically, CRFs will be electronically generated. All raw data will be preserved in order to maintain data integrity. The Investigator or designee will assume the responsibility of ensuring the completeness, accuracy, and timeliness of the clinical data.

At each scheduled monitoring visit, the Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of data capture.

The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE and concomitant medication reporting, and raw data collection forms) designed to record all observations and other pertinent data for each subject.

The Investigator will allow Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the IRB/IEC to have direct access to all documents pertaining to the study.

11 STATISTICAL METHODS AND PLANNED ANALYSES

11.1 General Considerations

This first-in-human study is designed to assess both safety and early efficacy in a limited number of switch-stable subjects and treatment-naïve subjects. This is not a pivotal study. The results of this Phase 1/2 study will help inform the appropriateness and design of future clinical studies that evaluate AVR-RD-02.

In this open-label study, after AVR-RD-02 gene therapy infusion, measure of engraftment, clinical response, and safety are analyzed for each individual subject on an ongoing basis. Considering this, a discrete interim analysis of data collected in this study will not be performed. Final analysis for the study will be performed after all enrolled subjects complete the Week 52 assessments (or prematurely discontinue the study). Prior to the analysis of the study data, a detailed statistical analysis plan (SAP) will be written describing all analyses to be performed. The SAP may contain modifications to the analysis plan described below and will supersede the statistical sections of this protocol. Any changes to the statistical plans will be described and justified in the CSR.

The study aims to assess the safety and tolerability of AVR-RD-02 as well as to explore the possibility of any indications of an efficacy response signal. As such, all continuous safety, tolerability, and efficacy endpoints will be summarized descriptively at each protocol-scheduled time point and as change from Baseline. Continuous data will be summarized using descriptive summary statistics (number of non-missing observations, mean, standard deviation, median, minimum, and maximum). Categorical data will be summarized as frequency counts and percentages at each protocol-scheduled time point.

For all safety endpoints, baseline will be defined as the last available, non-missing observation prior to first study treatment administration unless specifically mentioned otherwise. For all efficacy endpoints, baseline will be defined as the last available, non-missing observation prior to AVR-RD-02 administration. In general, Unknown, Not Done, Not Applicable, and other classifications of missing data will not be considered when calculating baseline observations. However, valid categorical observations will be considered for baseline calculations. In addition, non-missing results from unscheduled assessments prior to first study drug administration may also be considered in the calculation of baseline observations.

Only data from nominal protocol-scheduled visits will be included in summary tables. Data from unscheduled visits will be included in listings.

No formal hypothesis tests or statistical models are planned.

11.2 Determination of Sample Size

The sample size of approximately 8 to 16 subjects chosen for this study was selected without statistical considerations but was determined based on prior Phase 1/2 study designs in Gaucher disease (Barton 1991; Zimran 2010). Because this study is designed to minimize risk, the study size may be adjusted based on safety and/or efficacy assessments performed during the course of the study and/or recommendations of the independent DMC and/or regulatory agencies.

11.3 Analysis Sets

Subject inclusion into each population defined below will be determined.

Intent-to-Treat (ITT) Population: All enrolled subjects who initiate any study procedures, beginning with mobilization by G-CSF and plerixafor. This is the primary safety and efficacy population.

Treatment Population (TP): All enrolled subjects who receive AVR-RD-XX gene therapy infusion.

Per Protocol Population: All enrolled subjects who complete the study without major (ie, important) protocol deviations.

11.4 Demographics and Baseline Characteristics

Demographic (age, race, ethnicity, height, weight, and BMI) and baseline data, including disease history and characteristics will be summarized. Medical and surgical history will be coded using the most current MedDRA and summarized by System Organ Class and Preferred Term. All other data recorded at the Screening assessment will be listed only.

11.5 Subject Disposition

The total number of subjects enrolled and treated in each analysis population will be summarized. The number of subjects discontinuing the study early, along with the reason for early study discontinuation, will also be summarized.

11.6 Concomitant Medications and Non-Pharmacologic Therapies and Procedures

Medications used in this study will be coded by using the most current World Health Organization (WHO) Drug Dictionary Enhanced.

The start dates of medications will be used to assign medications into the following categories:

- Prior medication: any medication that started before initial dosing of study treatment regardless of when it ended
- Concomitant medication: medication continued or newly received at or after initial dosing of study treatment

Medications will be classified as a prior medication and/or a concomitant medication. If a medication has a missing or partial missing start/end date or time and it cannot be determined whether it was taken before initial treatment or concomitantly, it will be considered as prior and concomitant. Prior and concomitant medications will be summarized by Anatomical Therapeutic Chemical (ATC) classification and drug preferred term; the summary tables will show the number and percentage of subjects taking each medication by ATC classification and preferred term. Subjects who take the same medication (in terms of the ATC classification and preferred term) more than once will be counted only once for that medication.

Prior medication and concomitant medication will be listed and summarized separately.

11.6.1 Prophylaxis

Medications routinely administered as prophylaxis against infectious disease should be administered per institutional guidelines.

11.6.2 Re-Vaccination

Re-vaccination schedule after AVR-RD-02 gene therapy infusion will occur upon at least the partial reconstitution of the subject's adaptive immunity after busulfan myeloablative conditioning regimen. Investigational sites therefore are encouraged to follow their current internal bone marrow transplantation re-vaccination guidelines in alignment with the study Investigator and the Transplant Physician.

11.7 Treatment Compliance

All study treatment administration data will be listed. As all study treatment will be administered at the investigational site, treatment compliance will not be determined for this study.

11.8 Safety Analyses

All safety analyses will be conducted on the Safety Population.

11.8.1 Physical Examination and Vital Signs

All individual vital signs results will be presented in data listings. Observed vital signs parameter values and changes from Baseline will be summarized at each protocol-scheduled time point.

Physical examination data will be listed only.

11.8.2 Clinical Laboratory Tests

All individual clinical laboratory results will be presented in data listings. Values outside the laboratory reference range will be flagged (low-L, high-H).

Observed values and changes from Baseline in clinical laboratory data will be summarized at each protocol-scheduled time point. Frequency tabulations of the number of normal and abnormal (low and high) records, as well as the number of CS and not clinically significant (NCS) records, will also be summarized at each protocol-scheduled time point. Urinalysis results will be summarized at each protocol-scheduled time point using frequency tabulations, and any available microscopic urinalysis results will be listed.

In addition, shift tables will be produced per CTCAE grading.

11.8.3 Adverse Events

Adverse events will be coded using MedDRA. Adverse events will be grouped by System Organ Class and Preferred Term and summarized. The summary tables will present the frequency and percentage of total subjects by System Organ Class and Preferred Term.

For the summaries of AEs, subjects who experience the same AE (in terms of the MedDRA Preferred Term) more than once will be counted only once for that event in the number of subjects; however, all occurrences of the same event will be counted in the number of events.

Adverse event summaries will be restricted to treatment-emergent AEs (TEAEs) only. Treatment-emergent adverse events are defined as AEs that commence on or after the time of first study treatment administration. AEs without an onset date or time will be defined as TEAEs except where an incomplete date (eg, month and year) clearly indicates that the event started prior to start of first study treatment administration or if the AE stop date indicates that the event started and/or stopped prior to start of first study treatment administration.

Incidence of TEAEs as well as duration, severity, relationship to study treatment, outcome, and actions taken will be listed for each subject. In addition, listings of TEAEs leading to discontinuation of the study, SAEs, and deaths will be provided if applicable.

The following TEAE summaries will be provided:

- Overall summary of TEAEs:
  - Any TEAE
  - Any severe / ≥ CTCAE Grade 3 TEAE
  - Any TEAE considered as related to any study treatment (assessed by the Investigator as being possibly related, probably related, or definitely related)
  - Any TEAE leading to discontinuation from the study
  - Any serious TEAE
  - Any life-threatening TEAE
  - Any TEAE leading to death
- TEAEs overall and by System Organ Class and Preferred Term
- TEAEs related to any study treatment overall and by System Organ Class and Preferred Term (a TEAE related to study treatment is defined as any TEAE assessed by the Investigator as being possibly related, probably related, or definitely related to any study treatment)
- TEAEs by severity/CTCAE Grade overall and by System Organ Class and Preferred Term
- TEAEs leading to study discontinuation overall and by System Organ Class and Preferred Term
- Serious TEAEs overall and by System Organ Class and Preferred Term
- TEAEs of interest (related TEAEs, infections, infertility, and malignancies)

Treatment-emergent adverse events will be presented by decreasing frequency across all subjects, broken out by switch-stable and treatment-naïve study arms.

11.8.4 Reproductive Potential

Reproductive potential for male subjects will be evaluated based on sperm count, volume, sperm concentration, total motility, progressive motility, and morphology. Reproductive potential for female subjects will be evaluated based on changes in menstrual history and ovarian reserve (based on AMH, FSH, and LH levels).

All individual results will be presented in data listings. Values outside the laboratory reference range will be flagged (low-L, high-H).

Observed values and changes from Baseline in reproductive endpoints will be summarized at each protocol-scheduled time point. Frequency tabulations of the number of normal and abnormal (low and high) records, as well as the number of CS and NCS records, will also be summarized at each protocol-scheduled time point.

Pregnancy outcomes, if any, will be monitored and recorded during this study.

11.8.5 Other Safety Assessments

All individual ECG parameter results and the clinical assessment of the ECG will be presented in data listings. Observed ECG parameter values and changes from Baseline will be summarized at each protocol-scheduled time point. Clinical assessment of the ECG (Normal, Abnormal NCS, Abnormal CS) will be summarized using frequency tabulations.

The anti-GCase antibody data will also be summarized. All other safety data will be listed only.

11.9 Analysis of Immunogenicity

Immunogenicity testing will be conducted in enrolled subjects at Baseline and Weeks 5, 13, 26, 39, and 52. Change from Baseline in anti-GCase antibody isotypes and titers will be summarized.

11.10 Analysis of Engraftment

All engraftment analyses will be conducted on the Engraftment Population. Measurement of average VCN in peripheral blood at Baseline and Weeks 13, 26, 39, and 52, and bone marrow aspirates at Baseline and Weeks 26 and 52, will be summarized. Changes from Baseline will also be summarized.

11.11 Analysis of Enzyme Activity

All GCase enzyme activity analyses will be conducted on the Enzyme Activity Population. Observed values and changes from Baseline in anti-GCase enzyme activity level at Weeks 13, 26, 39, and 52 will be summarized.

11.12 Analysis of Standard of Care ERT

The analyses for standard of care ERT will be conducted on the Enzyme Activity Population. The rationale, frequency, and dose of ERT between Weeks 26 through 52, inclusive, will be summarized. Changes from Baseline will also be summarized.

11.13 Efficacy Analyses

All efficacy analyses will be conducted on the Efficacy Population. All raw and derived efficacy data will be listed.

11.13.1 Secondary Efficacy Analysis

For categorical endpoints, the number and percentage of subjects will be summarized using frequency tabulations. For continuous endpoints, observed values and changes from Baseline will be summarized at each protocol-scheduled time point.

11.13.2 Exploratory Efficacy Analyses

For categorical endpoints, the number and percentage of subjects will be summarized using frequency tabulations. For continuous endpoints, observed values and changes from Baseline will be summarized at each protocol-scheduled time point.

For questionnaire data, the appropriate scores will be calculated, and data will be summarized overall and for each domain where relevant.

11.14 Subgroup Analyses

Subgroups will be defined in the SAP for this study.

11.15 Missing or Invalid Data

For subjects who are withdrawn from the study prior to study completion, all data compiled up to the point of discontinuation will be used for analysis. All withdrawals will be included in all analyses up to the time of withdrawal. Any imputations for missing data will be detailed in the SAP.

12 DATA COLLECTION AND QUALITY CONTROL

Data collection is the responsibility of the staff at the study site under the supervision of the Investigator. Permission must be secured to obtain retrospective and prospective data corresponding to endpoints/data collected in this trial from the medical charts of the subjects. The designated study site staff will enter the data required by the protocol into the eCRFs. The eCRF is the primary data collection instrument for the study. The eCRFs have been built using a fully validated, secure, web-enabled software that conforms to CFR Title 21 Part 11 requirements. The Investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported in the eCRFs and all other required reports. Data reported on the eCRF that are derived from source documents should be consistent with the source documents, or the discrepancies should be explained. All data requested on the eCRF must be recorded. Any missing data must be explained. For eCRFs, an audit trail will be maintained by the system to capture data changes. Study site staff will not be given access to the electronic data capture system until they have been trained. Automatic validation programs check for data discrepancies in the eCRFs and allow modification or verification of the entered data by the study site staff.

The Investigator is responsible for assuring that the data recorded on eCRFs are complete, accurate, and that the entry and updates are performed in a timely manner.

12.1 Database Management and Data Quality Control

The Sponsor's medical representative or designee will review the data entered by the investigational staff for completeness and accuracy. Electronic data queries stating the nature of the problem and requesting clarification will be created for discrepancies and missing values by generating appropriate error messages. In addition, the outsourced vendor data management staff will review the data using validation programs and database listings and will enter electronic data queries for discrepancies allowing modification or verification of the entered data by the designated study staff.

12.2 Data Monitoring Committee

An independent DMC will be established for the study. The DMC will consist of clinical study experts in stem cell transplantation, gene therapy, and Gaucher disease independent of the study. The DMC will monitor the safety aspects of the trial, including review of all SAEs and data, after subjects complete the Week 5 (Day 30) follow-up visit. The proposed DMC schedule as subjects are enrolled in the study is shown in Table 7. Additional meetings may be scheduled as necessary.

The specific responsibilities of the DMC are described in the DMC Charter, which is maintained as a separate document.

12.3 Subject Confidentiality and Data Privacy

In order to maintain subject confidentiality, each subject will be assigned a unique subject identifier. This subject identifier will be used in place of the subject's name and initials for the purpose of data analysis and reporting. Medical record number or other local reference identifiers will not be collected as part of the study database. The subject's date of birth will be requested;

however, only the subject's birth year will be collected in the study database to document age eligibility requirements.

All parties will ensure protection of subject personal data and will not include subject names or initials on any study forms, reports, publications, or in any other documents, except where required by law. An exception to this will be any identifying information required by the site and the manufacturer for purposes of traceability of apheresed stem cells throughout the extraction, transportation, manufacturing and re-delivery process to ensure that the correct stem cells are administered to the study subject.

In accordance with local regulations in each of the study countries, subjects will be informed about data handling procedures (including, as applicable, the lawful basis for the processing of data) and asked for their consent. All parties will conduct the study in accordance with applicable laws and regulations governing privacy and data protection, such as the Health Insurance Portability and Accountability Act of 1996, the General Data Protection Regulation, and the United Kingdom Data Protection Act of 2018. Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing subject data. Every effort will be made to protect subject confidentiality in accordance with local regulations.

12.4 Study Record Retention

Study documentation includes, but is not limited to, all eCRFs, data correction forms or queries, images, laboratory reports, source documents, monitoring logs/letters, training records, curriculum vitaes, and regulatory documents (eg, protocol and amendments, IRB/IEC correspondence and approval, and signed participant consent/permission/assent forms).

Government agency regulations and directives require that the study Investigator must retain all study documentation pertaining to the conduct of a clinical study. It is expected that all study documentation will be retained for 2 years following the last anticipated regulatory approval or up to 25 years from study completion, whichever is longer. However, the length of storage of study documentation varies by country and stage of clinical development in any region in the world. Therefore, no study documentation will be destroyed or moved to a new location without prior written approval from the Sponsor. If offsite archiving is used, all records must be readily retrievable and made available for review at the time of an audit or regulatory authority inspection.

13 ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS

13.1 Informed Consent

The Investigator is responsible for ensuring that the subject understands the potential risks, benefits, limitations, and alternatives to participating in the study, including answering any questions the subject may have throughout the study and sharing in a timely manner any new information that may be relevant to the subject's willingness to continue his or her participation in the study.

The ICF will be used to explain the potential risks and benefits of study participation to the subject in simple terms before the subject is entered into the study, and to document that the subject is satisfied with his or her understanding of the risks, benefits, limitations, and alternatives to participating in the study and desires to participate in the study.

The Investigator is responsible for ensuring that informed consent is given by each subject or legal representative. This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of investigational product.

As used in this protocol, the term "informed consent" includes all consent given by subjects or their legal representatives.

13.2 Ethical Conduct of the Study

The Sponsor or its representatives must approve all ICFs before they are used at investigative site(s). All ICFs must be compliant with the International Council for Harmonisation (ICH) guideline on GCP.

Documentation of IRB/IEC approval of the protocol and the ICF must be provided to the Sponsor before the study may begin at the investigative site(s). The IRBs/IECs will review the protocol as required.

13.3 Regulatory Considerations

This study will be conducted in accordance with:

1. US CFR
2. The ICH GCP Guideline (E6 [R2])
3. Declaration of Helsinki ("Recommendations Guiding Physicians in Biomedical Research Involving Human Patients") and all its accepted amendments to date concerning medical research in humans
4. Consensus ethics principles derived from international ethics guidelines, including the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
5. Applicable laws and regulations

13.3.1 Investigator Information

One PI will oversee the trial at each investigative site. The PI undertakes to perform the study in accordance with this clinical study protocol, the ICH GCP guidelines, and the applicable national regulations and local IRB/IEC requirements.

The PI may appoint other individuals as he/she deems appropriate to assist in the conduct of the study. All appointed designees will be listed in the site delegation log. The appointed designates will perform their duties in accordance with ICH GCP guidelines and will be supervised by and under the responsibility of the PI.

13.3.2 Protocol Signatures

The Sponsor's medical representative or designee will approve the protocol, confirming that, to the best of his or her knowledge, the protocol accurately describes the planned design and conduct of the study.

After reading the protocol, each PI will sign the protocol signature page and send a copy of the signed page to a Sponsor representative.

13.3.3 Final Report Signature

The CSR-coordinating Investigator will sign the final CSR for this study, indicating agreement that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

The Sponsor's medical representative or designee and statistician will approve the final CSR for this study, confirming that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

13.4 Publication Policy

The full terms for publication are included in the clinical study agreement between the Sponsor and the Investigator.

14 LIST OF REFERENCES

Akiyama 2018

Akiyama K, Kume T, Fukaya M, Shiki I, Enami T, Tatara R, et al. Comparison of levetiracetam with phenytoin for the prevention of intravenous busulfan-induced seizures in hematopoietic cell transplantation recipients. *Cancer Chemother Pharmacol*. 2018;82(4):717-21.

Baiamonte 2015

Baiamonte E, Barone R, Di Stefano R, Lo Iacono M, Spina B, Contino F, et al. Hematopoietic Stem Cell Mobilization for Gene Therapy: The Combination of G-CSF+Plerixafor in Patients with Beta-Thalassemia Major Provides High Yields of CD34+ Cells with Primitive Signatures. *Blood*. 2015;126(23):4412.

Bartelink 2016

Bartelink IH, Lalmohamed A, van Reij EM, Dvorak CC, Savic RM, Zwaveling J, et al. Association of busulfan exposure with survival and toxicity after haemopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis. Lancet Haematol. 2016;3(11):e526-e536.

Bartelink 2012

Bartelink IH, van Kesteren C, Boelens JJ, Egberts TC, Bierings MB, Cuvelier GD, et al. Predictive performance of a busulfan pharmacokinetic model in children and young adults. *Ther Drug Monit*. 2012;34(5):574-83.

Barton 1991

Barton NW, Brady RO, Dambrosia JM, Di Bisceglie AM, Doppelt SH, Hill SC, et al. Replacement therapy for inherited enzyme deficiency--macrophage-targeted glucocerebrosidase for Gaucher's disease. *N Engl J Med*. 1991;324(21):1464-70.

Becker-Cohen 2022

Becker-Cohen M, Revel-Vilk S, Frydman D, Istaiti M, Dinur T, Cozma C, et al. An 18-month report on the safety and efficacy of rapid intravenous velaglucerase alfa infusions in naïve patients with Gaucher disease. *Mol Gen and Met*; 135(2):S22, 2022.

Ben Turkia 2013

Ben Turkia H, Gonzalez DE, Barton NW, Zimran A, Kabra M, Lukina EA, et al. Velaglucerase alfa enzyme replacement therapy compared with imiglucerase in patients with Gaucher disease. *Am J Hematol*. 2013;88(3):179-84.

Bennett 2018

Bennett L and Fellner C. Pharmacotherapy of Gaucher Disease: current and future options. *P&T*. 2018;43:274-309.

Biffi 2016

Biffi A. Gene therapy for lysosomal storage disorders: a good start. *Hum Mol Genet*. 2016;25(R1):R65-75.

Busulfan PI

Busulfan. Prescribing Information: Available at: https://dailymed.nlm.nih.gov/dailymed/search.cfm?labeltype=all&query=busulfan.

Busulfan Product Monograph

Busulfan. Product Monograph: BC Cancer Drug Manual; 2018 http://www.bccancer.bc.ca/drug-database-site/Drug%20Index/Busulfan%20monograph.pdf.

Busulfan SmPC

Busulfan. SmPC: Accord Healthcare Limited, North Harrow, Middlesex, UK; 2018 https://www.medicines.org.uk/emc/product/2160/smpc.

CERDELGA PI

CERDELGA. Prescribing Information: Sanofi Genzyme. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=819f828a-b888-4e46-83fc-94d774a28a83. Accessed 01 July 2019.

CEREZYME PI

Cerezyme. Prescribing Information: Sanofi Genzyme. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=df60f030-866b-4374-a31f-8ae3f6b45c38. Accessed 01 July 2019.

Charrow 2000

Charrow J, Andersson H, Kaplan P, Kolodny E, Mistry P, Pastores G, et al. The Gaucher registry: demographics, and disease characteristics of 1698 patients with Gaucher disease. Arch Intern Med. 2000; 160: 2835-43.

Charrow and Scott 2015

Charrow J and Scott CR. Long-term treatment outcomes in Gaucher disease. *Am J Hematol*. 2015;90 Suppl 1:S19-24.

Cox 2012

Cox TM, Amato D, Hollak CE, Luzy C, Silkey M, Giorgino R, et al. Evaluation of miglustat as maintenance therapy after enzyme therapy in adults with stable type 1 Gaucher disease: a prospective, open-label non-inferiority study. *Orphanet J Rare Dis*. 2012;7:102.

Cox 2015

Cox TM, Drelichman G, Cravo R, Balwani M, Burrow TA, Martins AM, et al. Eliglustat compared with imiglucerase in patients with Gaucher's disease type 1 stabilised on enzyme replacement therapy: a phase 3, randomised, open-label, non-inferiority trial. *Lancet*. 2015;385(9985):2355-62.

Dahl 2015

Dahl M, Doyle A, Olsson K, Mansson JE, Marques ARA, Mirzaian M, et al. Lentiviral gene therapy using cellular promoters cures type 1 Gaucher disease in mice. *Mol Ther*. 2015;23(5):835-44.

Davis 2019

Davis JM, Ivanova A, Chung Y, Shaw JR, Rao KV, Ptachcinski JR, et al. Evaluation of a Test Dose Strategy for Pharmacokinetically-Guided Busulfan Dosing for Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant*. 2019;25(2):391-7.

DeMayo 2008

DeMayo RF, Haims AH, McRae MC, Yang R, and Mistry PK. Correlation of MRI-Based bone marrow burden score with genotype and spleen status in Gaucher's disease. *AJR Am J Roentgenol.* 2008;191(1):115-23.

ELELYSO PI

ELELYSO. Prescribing Information: Pfizer Inc. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=fa3cbd5d-677c-4b19-9032-d9182cb69a83. Accessed 01 July 2019.

Elstein 2015

Elstein D, Mehta A, Hughes DA, Giraldo P, Charrow J, Smith L, et al. Safety and efficacy results of switch from imiglucerase to velaglucerase alfa treatment in patients with type 1 Gaucher disease. *Am J Hematol*. 2015;90(7):592-7.

Elstein 2011

Elstein D, Foldes AJ, Zahrieh D, Cohn GM, Djordjevic M, Brutaru C, et al. Significant and continuous improvement in bone mineral density among type 1 Gaucher disease patients treated with velaglucerase alfa: 69-month experience, including dose reduction. *Blood Cells Mol Dis*. 2011;47(1):56-61.

Faraci 2019

Faraci M, Diesch T, Labopin M, Dalissier A, Lankaster A, Gennery A, et al. Gonadal function after busulfan compared with treosulfan in children and adolescents undergoing allogeneic hematopoietic stem cell transplant. Biol Blood Marrow Transplant. 2019; 25: 1786 – 91.

EMA 2009

EMA. Guideline on Follow-up of Patients Administered with Gene Therapy Medicinal Products. 2009. https://www.ema.europa.eu/documents/scientific-guideline/guideline-follow-patients-administered-gene-therapy-medicinal-products_en.pdf.

FACT Eigth Edition

FACT-JACIE International Standards for Hematopoietic Cecllular Therapy Product Collection, Processing, and Administration. Eigth Edition. Version 8.1. 14 December 2021.

FDA 2020a

FDA. Guidance for Industry: Long Term Follow-Up After Administration of Human Gene Therapy Products. 2020a. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/long-term-follow-after-administration-human-gene-therapy-products.

FDA 2020b

FDA. Guidance for Industry: Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up. 2020b. https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM610800.pdf.

Fumagalli 2022

Fumagalli, F, Calbi V, Sora M, Baldoli C, Rancolta P, Ciotti F, et al. Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: long-term results from a non-randomised, open-label, phase 1/2 trial and expanded access. Lancet. 2022; 399: 372-83.

Gary 2018

Gary SE, Ryan E, Steward AM, and Sidransky E. Recent advances in the diagnosis and management of Gaucher disease. *Expert Rev Endocrinol Metab*. 2018;13(2):107-18.

Gill 2022

Gill EL, Patel K, Dickerson JA, Dulik MC, Grant RP, Heaney DL, et al. Alternative Sample Matrices Supporting Remote Sample Collection during the Pandemic and Beyond. *Clin Chem*. 2022;68(2):269-75.

Goker 2015

Goker H, Etgul S, and Buyukasik Y. Optimizing mobilization strategies in difficult-to-mobilize patients: The role of plerixafor. *Transfus Apher Sci*. 2015;53(1):23-9.

Gonzalez 2013

Gonzalez DE, Turkia HB, Lukina EA, Kisinovsky I, Dridi MF, Elstein D, et al. Enzyme replacement therapy with velaglucerase alfa in Gaucher disease: Results from a randomized, double-blind, multinational, Phase 3 study. *Am J Hematol*. 2013;88(3):166-71.

Grabowski 2015

Grabowski GA, Zimran A, and Ida H. Gaucher disease types 1 and 3: Phenotypic characterization of large populations from the ICGG Gaucher Registry. *Am J Hematol*. 2015;90 Suppl 1:S12-8.

Grabowski 1995

Grabowski GA, Barton NW, Pastores G, Dambrosia JM, Banerjee TK, McKee MA, et al. Enzyme therapy in type 1 Gaucher disease: comparative efficacy of mannose-terminated glucocerebrosidase from natural and recombinant sources. *Ann Intern Med*. 1995;122(1):33-9.

Hsieh 2020

Hsieh MM, Bonner M, Pierciey FJ, Uchida N, Rottman J, Demopoulos L, et al. Myelodysplastic syndrome unrelated to lentiviral vector in a patient treated with gene therapy for sickle cell disease. *Blood Adv*. 2020;4(9):2058-63.

Ito and Barrett 2013

Ito S and Barrett AJ. Gauchers disease—A Reappraisal of Hematopoietic Stem Cell Transplantation. *Ped Hematol Oncol*. 2013;30(2):61-70.

Jillella and Ustun 2004

Jillella AP and Ustun C. What is the optimum number of CD34+ peripheral blood stem cells for an autologous transplant? *Stem Cells Dev*. 2004;13(6):598-606.

Kamath 2014

Kamath RS, Lukina E, Watman N, Dragosky M, Pastores GM, Arreguin EA, et al. Skeletal improvement in patients with Gaucher disease type 1: a phase 2 trial of oral eliglustat. *Skeletal Radiol*. 2014;43(10):1353-60.

Kohn 2020

Kohn DB, Booth C, Kang EM, Pai SY, Shaw KL, Santilli G, et al. Lentiviral gene therapy for X-linked chronic granulomatous disease. *Nat Med.* 2020;26:200-6.

Long-Boyle 2015

Long-Boyle JR, Savic R, Yan S, Bartelink I, Musick L, French D, et al. Population pharmacokinetics of busulfan in pediatric and young adult patients undergoing hematopoietic cell transplant: a model-based dosing algorithm for personalized therapy and implementation into routine clinical use. *Ther Drug Monit*. 2015;37(2):236-45.

Lukina 2010

Lukina E, Watman N, Arreguin EA, Banikazemi M, Dragosky M, Iastrebner M, et al. A phase 2 study of eliglustat tartrate (Genz-112638), an oral substrate reduction therapy for Gaucher disease type 1. *Blood*. 2010;116(6):893-9.

Maas 2003

Maas M, van Kuijk C, Stoker J, Hollak CE, Akkerman EM, Aerts JF, et al. Quantification of bone involvement in Gaucher disease: MR imaging bone marrow burden score as an alternative to Dixon quantitative chemical shift MR imaging--initial experience. *Radiology*. 2003;229(2):554-61.

Mamcarz 2019

Mamcarz E, Zhou S, Lockey T, Abdelsamed H, Cross S, Kang G, et al. Lentiviral Gene Therapy Combined with Low-Dose Busulfan in Infants with SCID-X1. N Engl J Med. 2019;380:1525-34.

Mistry 2015

Mistry PK, Lukina E, Ben Turkia H, Amato D, Baris H, Dasouki M, et al. Effect of oral eliglustat on splenomegaly in patients with Gaucher disease type 1: the ENGAGE randomized clinical trial. *JAMA*. 2015;313(7):695-706.

Miyamoto 2021

Miyamoto T, Iino M, Komorizome Y, Kiguchi T, Furukawa M, Otsuka M, et al. Screening for Gaucher Disease using dried blood spots tests: A Japanese multicenter, cross-sectional survey. *Intern. Med.* 2021;60:699-707.

Mozobil PI

Mozobil. Prescribing Information: Sanofi. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022311s001lbl.pdf. Accessed 13 May 2022.

Pastores and Hughes 2020

Pastores GM, and Hughes DA. Gaucher Disease. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. 2020;Seattle, Washington:University of Washington; 1993-2019. URL: https://www.ncbi.nlm.nih.gov/books/NBK1269/.

Pastores 2016

Pastores GM, Turkia HB, Gonzalez DE, Ida H, Tantawy AA, Qin Y, et al. Development of anti-velaglucerase alfa antibodies in clinical trial-treated patients with Gaucher disease. *Blood Cells Mol Dis*. 2016;59:37-43.

Pastores 2014

Pastores GM, Rosenbloom B, Weinreb N, Goker-Alpan O, Grabowski G, Cohn GM, et al. A multicenter open-label treatment protocol (HGT-GCB-058) of velaglucerase alfa enzyme replacement therapy in patients with Gaucher disease type 1: safety and tolerability. *Genet Med*. 2014;16(5):359-66.

Pastores 2004

Pastores GM, Weinreb NJ, Aerts H, Andria G, Cox TM, Giralt M, et al. Therapeutic goals in the treatment of Gaucher disease. *Semin Hematol*. 2004;41(4 Suppl 5):4-14.

Robertson 2007

Robertson PL, Maas M, and Goldblatt J. Semiquantitative assessment of skeletal response to enzyme replacement therapy for Gaucher's disease using the bone marrow burden score. *AJR Am J Roentgenol*. 2007;188(6):1521-8.

Rolfs 2013

Rolfs A, Giese AK, Grittner U, Mascher D, Elstein D, Zimran A, et al. Glucosylsphingosine is a highly sensitive and specific biomarker for primary diagnostic and follow-up monitoring in Gaucher disease in a non-Jewish, Caucasian cohort of Gaucher disease patients. *PLoS One*. 2013;8(11):e79732.

Sessa 2016

Sessa M, Lorioli L, Fumagalli F, Acquati S, Redaelli D, Baldoli C, et al. Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. *Lancet*. 2016;388:476–87.

Stirnemann 2017

Stirnemann J, Belmatoug N, Camou F, Serratrice C, Froissart R, Caillaud C, et al. A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. *Int J Mol Sci*. 2017;18(2):10.3390/ijms18020441.

Thompson 2018

Thompson AA, Walters MC, Kwiatkowski J, Rasko JEJ, Ribeil JA, Hongeng S, et al. Gene Therapy in Patients with Transfusion-Dependent beta-Thalassemia. *N Engl J Med*. 2018;378(16):1479-93.

Tisdale 2018

Tisdale JF, Kanter J, Mapara MY, Kwiatkowski JL, Krishnamurti L, Schmidt M, et al. Oral Presentation: Current Results of Lentiglobin Gene Therapy in Patients with Severe Sickle Cell Disease Treated Under a Refined Protocol in the Phase 1 Hgb-206 Study. *American Society of Hematology Annual Meeting*. 2018.

VPRIV PI

VPRIV. Prescribing Information: Shire Human Genetic Therapies, Inc. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=ca02f7a4-ae4f-43c1-a06a-259fe4fcf9cf. Accessed 01 July 2019.

Weil 2017

Weil E, Zook F, Oxencis C, Canadeo A, Urmanski A, Waggoner M, et al. Evaluation of the Pharmacokinetics and Efficacy of a Busulfan Test Dose in Adult Patients Undergoing Myeloablative Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant*. 2017;23(6):952-7.

Weinreb 2021

Weinreb N, Camelo J, Charrow J, McClain M, Mistry P, Belmatoug for the International Collaborative Gaucher Group (ICGG) Gaucher Registry (NCT00358943) investigators. Gaucher disease type 1 patients from the ICGG Gaucher Registry sustain initial clinical improvements during twenty years of imiglucerase treatment. *Mol. Genet. Metab.* 2021;132:100-11.

World Health Organization 2020

World Health Organization. Public health surveillance for COVID-19: interim guidance, 7 August 2020. World Health Organization.

Yannaki 2013

Yannaki E, Karponi G, Zervou F, Constantinou V, Bouinta A, Tachynopoulou V, et al. Hematopoietic stem cell mobilization for gene therapy: superior mobilization by the combination of granulocyte-colony stimulating factor plus plerixafor in patients with beta-thalassemia major. *Hum Gene Ther*. 2013;24(10):852-60.

ZAVESCA PI

ZAVESCA. Prescribing Information: Actelion Pharmaceuticals US, Inc. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=817892d1-ee12-4632-85fc-57ccdf16d7b8. Accessed 01 July 2019.

Zimran 2015

Zimran A, Gonzalez-Rodriguez DE, Abrahamov A, Elstein D, Paz A, Brill-Almon E, et al. Safety and efficacy of two dose levels of taliglucerase alfa in pediatric patients with Gaucher disease. Blood Cells Mol Dis. 2015;54(1):9-16.

Zimran 2013

Zimran A, Pastores GM, Tylki-Szymanska A, Hughes DA, Elstein D, Mardach R, et al. Safety and efficacy of velaglucerase alfa in Gaucher disease type 1 patients previously treated with imiglucerase. Am J Hematol. 2013;88(3):172-8.

Zimran 2011

Zimran A, Brill-Almon E, Chertkoff R, Petakov M, Blanco-Favela F, Munoz ET, et al. Pivotal trial with plant cell-expressed recombinant glucocerebrosidase, taliglucerase alfa, a novel enzyme replacement therapy for Gaucher disease. *Blood*. 2011;118(22):5767-73.

Zimran 2010

Zimran A, Altarescu G, Philips M, Attias D, Jmoudiak M, Deeb M, et al. Phase 1/2 and extension study of velaglucerase alfa replacement therapy in adults with type 1 Gaucher disease: 48-month experience. *Blood*. 2010;115(23):4651-6.

15 APPENDICES

15.1 Schedules of Assessments: Screening through the Apheresis Period

Table 4: Schedule of Assessments from Screening through the Apheresis Period

	Study Period								
			Pre-gene Therapy Infusion						
			Mobilization[c]				Apheresis		Early Termination / Premature Discontinuation[d]
Study Assessment	**Screening[a]**	**Baseline[b]**							
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---
Study Week [W]	---	---	--	---	---	---	---	---	---
Assessment Window	**-60D**		--	---	---	---	---	---	---
Informed consent/assent	X								
Demographics	X								
General- and Gaucher-specific medical and surgical history[e]	X								
COVID-19 testing[ee]						X			
Transmittable disease testing[f]	X								
Blood group (type) and screen			X						
PTT and INR	X		X						
Efficacy Assessments									
Blood sample collection for biomarkers for Gaucher disease (ie, chitotriosidase enzyme activity, lyso-Gb1 plasma levels, and GCase activity in plasma)[g]	X	X							X
Abdominal MRI[h]	X								X
Bone MRI[h]	X								X
Hemoglobin concentration, hematocrit percentage, and platelet count[i]	X	X							X

Study Assessment	Study Period								
			Pre-gene Therapy Infusion						Early Termination / Premature Discontinuation[d]
	Screening[a]	Baseline[b]	Mobilization[c]				Apheresis		
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---
Study Week [W]	---	---	--	---	---	---	---	---	---
Assessment Window	**-60D**		--	---	---	---	---	---	---
Blood sample collection for GCase enzyme activity in PBLs[j]	X	X							X
Blood sample collection for GCase enzyme activity in DBS cards	X	X							X
DXA for BMD determination[k]		X							X
Leukocytes, plasma, and urine sample collection for exploratory biomarker analysis[l]		X							X
SF-36[m]	X	X							X
BPI-SF[m]	X	X							X
Blood sample collection for average VCN determination[g]		X							X
Bone marrow aspirate collection for average VCN determination[g]		X							
Safety Assessments									
Physical examination	X	X							X
Weight		X							X
Height		X							
Vital signs[n]	X	X	X	X	X	X	X	X	X
12-lead ECG	X	X							X
CBC with differential[o]	X	X	X	X	X	X	X	X	X

Study Assessment	Study Period								
			Pre-gene Therapy Infusion						
			Mobilization[c]				Apheresis		Early Termination / Premature Discontinuation[d]
	Screening[a]	Baseline[b]							
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---
Study Week [W]	---	---	--	---	---	---	---	---	---
Assessment Window	**-60D**		--	---	---	---	---	---	---
Blood sample collection for T and B cell counts[p]	X	X							X
Serum chemistry[q]	X	X							X
Electrolytes, BUN, and serum creatinine[q]	X	X	X						X
Urinalysis[r]	X	X							X
Blood sample collection for immunogenicity testing[g,s,t]	X	X							X
Peripheral blood sample collection for RCL testing[g,s]		X							X
Blood sample collection for ISA[g,s]		X							X
Peripheral blood sample collection for DNA storage[u]		X							
Bone marrow aspirate collection for DNA storage[u]		X							
Measures of reproductive potential (males)[v]		X							X
Serum pregnancy test (females)[w]	X	X							
Monthly menstrual calendar collection (females)[x]		X							X
Measures of reproductive potential (females)[y]		X							X

Study Assessment	Study Period								
			Pre-gene Therapy Infusion						Early Termination / Premature Discontinuation[d]
	Screening[a]	Baseline[b]	Mobilization[c]				Apheresis		
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---
Study Week [W]	---	---	---	---	---	---	---	---	---
Assessment Window	-60D		---	---	---	---	---	---	---
AVR-RD-02 Drug Product Preparation Procedures for Gene Therapy Infusion									
G-CSF injection[z]			X	X	X	X			
Plerixafor administration[aa]						X	X		
Apheresis for stem cell harvesting[bb]							X	X	
CD34+ cell count/µL							X	X	
CD34+ cell enumeration by flow cytometry							X	X	
Continuous Monitoring Procedures									
Prior and concomitant medications and therapies[cc]	Continuous monitoring								X
Adverse events[dd]	Continuous monitoring								X

Abbreviations: A = apheresis; Ab = antibody; AEs = adverse events; Ag = antigen; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AMH = anti-Müllerian hormone; ANC = absolute neutrophil count; anti-HBc = antibodies (IgG and IgM) to hepatitis B core antigen; AST = aspartate aminotransferase; B = Baseline; BID = twice a day; BMD = bone mineral density; BPI-SF = Brief Pain Inventory-Short Form; BUN = blood urea nitrogen; CBC = complete blood cell count; CDC = Centers for Disease Control; CMIA = chemiluminescent microplate immunoassay; CMV = cytomegalovirus; COVID-19 = coronavirus disease 2019; D = day; DBS = dried blood spot; DNA = deoxyribonucleic acid; DXA = dual-energy X-ray absorptiometry; ECG = electrocardiogram; EIA = enzyme immunosorbent assay; ERT = enzyme replacement therapy; FSH = follicle-stimulating hormone; GCase = glucocerebrosidase; G-CSF = granulocyte-colony stimulating factor; GGT = gamma-glutamyl transferase; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; HSV = herpes simplex virus; HTLV = human T-cell lymphotropic virus; IgG = immunoglobulin G; IgM = immunoglobulin M; INR = international normalized ratio; ISA = insertional site analysis; IV = intravenously; LDH = lactate dehydrogenase; lyso-Gb1 = glucosylsphingosine; LH = luteinizing hormone; M = Mobilization; MRI = magnetic resonance imaging; NAT = nucleic acid-amplification testing; PBL = peripheral blood leukocyte; PCR = polymerase chain reaction; PTT = partial thromboplastin time; RBC = red blood cell; RCL = replication-competent lentivirus; SAEs = serious adverse events; SC = subcutaneously; SF-36 = 36-Item Short Form Health Survey;

SRT = substrate reduction therapy; VCN = vector copy number; VDRL = Venereal Disease Research Laboratory; VSV = vesicular stomatitis virus; W = week; WBC = white blood cell.

[a] Screening assessments must be performed within 60 days prior to the Baseline visit.

[b] The Baseline visit may be conducted over the course of up to 7 days.

[c] Mobilization is to be initiated the day following the last Baseline assessment.

[d] Assessments required for those subjects who prematurely discontinue the study after initiation of the Pre-gene Therapy Infusion Period. Assessments should be completed as soon as feasible after the decision to discontinue the study has been made. If logistic or medical constraints result in delays, the assessments must be completed within 3 months following the decision to discontinue. After discontinuation, every effort should be made to continue to follow the subject for safety monitoring (including any indication[s] of loss of gene therapy effect).

[e] Medical history will include retrospective collection from medical records of Gaucher disease-specific medical and surgical history, including historical results of GCase enzyme activity testing and previous use of ERT and SRTs. Copies of medical records will be retained in the source documents.

[f] Transmittable disease testing includes serological analysis for HIV Ag/Ab, HIV NAT, HBsAg, anti-HBc, HBV NAT, anti-HCV antibody, HCV NAT, syphilis test (VDRL, CMIA, or EIA), CMV IgG Ab, CMV IgM Ab, HTLV-1 Ab, HTLV-2 Ab, HSV-1 IgG Ab, HSV-2 IgG Ab, and, if applicable, VSV IgG Ab. If applicable, testing for West Nile virus by PCR must also be performed. To determine if a viral test is applicable to the subject, investigational clinical sites should consult the CDC website for up-to-date guidance. Subjects who test positive for HBV, HCV, HIV (Type 1 or 2), HTLV-1, HTLV-2, and/or syphilis on VDRL, CMIA, or EIA will be screen failures. Consult the Study Treatment Manual for a list of applicable testing for your region. Similarly, to determine if a viral test is applicable for the region, investigational clinical sites should consult the CDC website for up-to-date guidance. Testing should be completed within 30 days prior to apheresis.

[g] Blood sample(s) and bone marrow aspirate will be analyzed by a central laboratory. Additional blood sample and bone marrow aspirate must also be collected before initiation and cessation of ERT, when applicable. Additional blood samples will be used for the following tests: GCase enzyme activity, biomarkers for Gaucher disease, VCN, ISA, immunogenicity, and RCL. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[h] Abdominal and bone MRI scans will be performed locally and will be interpreted by a blinded central reader. The abdominal MRI performed at Screening will also be interpreted locally to determine study eligibility. Blinded central reader interpretation will be used for final efficacy analysis. Details on collection, processing, and shipment will be included in an Imaging (or other relevant) Manual for the study.

[i] Hemoglobin, hematocrit, and platelet assessments collected for efficacy analysis will be tested at a central laboratory. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[j] Two tubes for GCase in PBLs.

[k] DXA will be performed and interpreted locally.

[l] Samples to be collected and stored for possible future testing. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[m] The SF-36 and BPI-SF should be completed prior to any other required assessments at the study visit.

[n] Vital signs include heart rate, blood pressure, respiratory rate, and temperature. Vital signs will be obtained with the subject supine or seated. On Days 1 and 2 of the Apheresis phase of the Pre-gene therapy infusion Period, vital signs must be taken prior to apheresis. On Day 0 of the Gene Therapy Infusion Period (ie, the day of AVR-RD-02 gene therapy infusion), vital signs must be taken pre-gene therapy infusion (window -10 minutes) and post-gene therapy infusion (window +10 minutes). Additional vital signs may be taken if clinically indicated and/or mandated per local institution practice for gene therapy infusion.

[o] CBC with differential performed locally includes the following: hemoglobin, hematocrit, platelet count, WBC count (total and differential), RBC count, and ANC count.

[p] Assessment will include routine flow cytometric analysis of T and B cell counts as well as peripheral blood lymphocyte subsets. Panel will include CD3, CD4, CD8, CD19, and CD16/56. Performed locally.

[q] Serum chemistry includes AST, ALT, GGT, amylase, lipase, ALP, LDH, bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. Electrolytes include sodium, potassium, chloride, bicarbonate, BUN, and serum creatinine. Performed locally.

[r] Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated). Performed locally.

[s] Additional blood sample collection for immunogenicity testing, RCL testing, and ISA may also take place at any time, if clinically indicated.

[t] Screening samples are to be collected for treatment-naïve subjects only. All subsequent samples are to be collected from all treatment-naïve and switch-stable subjects.

[u] Samples will be collected and stored for possible future testing should hematologic cancer develop. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[v] For measures of reproductive potential in male subjects, sperm samples for sperm count, volume, sperm concentration, total motility, progressive motility, and morphology will be collected. Samples should be collected after at least 5 days to 7 days of abstinence. Samples will be tested locally. For samples that yield abnormal results, an additional sample must be collected and tested to confirm the initial results. Percutaneous epididymal sperm aspiration and hormonal laboratory assessments (eg, testosterone) will also be offered to male subjects (optional assessments), which will be analyzed locally. If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology), investigational sites should follow institutional guidelines for further evaluation. Additional assessments of reproductive potential and/or referral to a urologist may be recommended. Early termination assessments of measures of reproductive potential are not required for male subjects who have had a vasectomy and sterility confirmed at the Baseline assessment.

[w] Results of the serum pregnancy test to be obtained at Screening (exclusion criterion) and prior to starting mobilization drugs and apheresis procedure (FACT Eigth Edition).

[x] For females, monthly menstrual calendar (for all menstrual cycles beginning at the Baseline visit) by subject report will be collected at the specified study visits. At Baseline, a recall of menstrual history over the previous 12 months will be collected.

[y] Measures of reproductive potential in females include AMH, FSH, and LH for assessment of ovarian reserve, which will be assessed locally. Early termination assessments of measures of reproductive potential are not required for female subjects who are confirmed as not of child-bearing potential at Baseline assessment.

[z] On Mobilization Days 1 through 4 of the Pre-gene Therapy Infusion Period, a daily G-CSF dose of 10 µg/kg body weight will be administered (for sites with only prefilled syringes, a G-CSF daily dose from 10 µg/kg body weight up to 12 µg/kg body weight administered in either a single or divided BID dose may be administered). The G-CSF dose may be administered SC or IV.

[aa] On the evening of Mobilization Day 4 and on the evening of Apheresis Day 1, plerixafor (0.24 mg/kg) will be administered SC.

[bb] On the morning of Apheresis Day 1 (ie, between 8 hours and 11 hours after the first plerixafor administration on Mobilization Day 4), cells will be harvested from the subject's peripheral blood; this apheresis unit will be shipped to the manufacturing site for cell transduction and processing. On the morning of Apheresis Day 2 (ie, between 8 hours and 11 hours after plerixafor administration on Apheresis Day 1), an additional harvest of cells will occur; cells harvested from this apheresis (nontransduced cells) will be retained and stored at the investigational site for use as rescue treatment (if needed). Standard institutional procedures at the participating investigational study site will be used for both apheresis procedures. If the quality of the cells is not optimum and affects transduction efficiency, additional round(s) of apheresis may be required.

[cc] Prior and concomitant medications and therapies will be recorded from the time of signing of informed consent until completion of the Week 52 assessments for the study.

[dd] All AEs (including SAEs and AEs of interest) will be recorded from the time of signing of informed consent until completion of the Week 52 assessments for the study.

[ee] The COVID-19 test prior to study procedures, including mobilization, apheresis, conditioning and AVR-RD-02 gene therapy infusion, will be conducted no later than 5 days prior to the planned procedure.

15.2 Schedules of Assessments: Investigational Product Preparation and Testing through End of Gene Therapy Infusion

Table 5: Schedule of Assessments from Investigational Product Preparation and Testing through End of Gene Therapy Infusion

Study Assessment	Study Period											
	Pre-gene Therapy Infusion											
	Investigational Product Preparation and Testing for Release for Gene Therapy Infusion[a]	Conditioning Phase (including washout period)[b]									Gene Therapy Infusion[c]	Early Termination / Premature Discontinuation[d]
Study Day [D]	---	C D-8	C D-7	C D-6	C D-5	C D-4	C D-3	C D-2	C D-1	Conditioning Washout Period	I D0	---
Study Week [W]	---	---	---	---	---	---	---	---	---		W1	---
Assessment Window		---	---	---	---	---	---	---	---	From min of 24 hours to max of 48 hours[b]	---	---
PTT and INR			X									
COVID-19 testing[x]										X		
Efficacy Assessments												
Blood sample collection for biomarkers for Gaucher disease (ie, chitotriosidase enzyme activity and lyso-Gb1 plasma levels)[e]												X
Hemoglobin concentration, hematocrit percentage, and platelet count[f]											X	X
Blood sample collection for GCase enzyme activity in DBS cards											X	X

Study Assessment	Study Period										
	Pre-gene Therapy Infusion										
	Investigational Product Preparation and Testing for Release for Gene Therapy Infusion[a]	Conditioning Phase (including washout period)[b]								Gene Therapy Infusion[c]	Early Termination / Premature Discontinuation[d]
Study Day [D]	---	C D-8	C D-7	C D-6	C D-5	C D-4	C D-3	C D-2	C D-1	Conditioning Washout Period	I D0	---
Study Week [W]	---	---	---	---	---	---	---	---	---		W1	---
Assessment Window		---	---	---	---	---	---	---	---	From min of 24 hours to max of 48 hours[b]	---	---
Blood sample collection for GCase enzyme activity in PBLs[e]											X	X
Blood sample collection for GCase enzyme activity in plasma (processed and frozen at the site)											X	X
Blood sample collection for average VCN determination[e]											X	X
Safety Assessments												
Physical examination			X								X	X
Weight			X	X							X	X
Vital signs[g]			X	X		X	X	X	X		X	X
12-lead ECG			X									
CBC with differential[h]			X				X	X	X	X[h]	X	X
Blood sample collection for T and B cell counts[i]			X									X
Serum chemistry[j]			X				X	X	X	X[j]	X	X

Study Assessment	**Study Period**											
	Pre-gene Therapy Infusion											
	Investigational Product Preparation and Testing for Release for Gene Therapy Infusion[a]	**Conditioning Phase (including washout period)[b]**									**Gene Therapy Infusion[c]**	**Early Termination / Premature Discontinuation[d]**
Study Day [D]	---	**C D-8**	**C D-7**	**C D-6**	**C D-5**	**C D-4**	**C D-3**	**C D-2**	**C D-1**	**Conditioning Washout Period**	**I D0**	---
Study Week [W]	---	---	---	---	---	---	---	---	---		**W1**	---
Assessment Window		---	---	---	---	---	---	---	---	**From min of 24 hours to max of 48 hours[b]**	---	---
Electrolytes, BUN, and serum creatinine[j]			X				X	X	X	X[j]	X	X
Urinalysis[k]												X
Blood sample collection for immunogenicity testing[e,l]												X
Blood sample collection for ISA[e,l]											X	X
Measures of reproductive potential (males)[m]												X
Serum pregnancy test (females)[n]		X										
Monthly menstrual calendar collection (females)[o]		X										X
Measures of reproductive potential (females)[p]												X
Levetiracetam administration for prophylaxis[q]		X	X	X	X	X	X	X	X	X[q]		

Study Assessment	**Study Period**											
	Pre-gene Therapy Infusion											
	Investigational Product Preparation and Testing for Release for Gene Therapy Infusion[a]	**Conditioning Phase (including washout period)[b]**									**Gene Therapy Infusion[c]**	**Early Termination / Premature Discontinuation[d]**
Study Day [D]	---	**C D-8**	**C D-7**	**C D-6**	**C D-5**	**C D-4**	**C D-3**	**C D-2**	**C D-1**	**Conditioning Washout Period**	**I D0**	---
Study Week [W]	---	---	---	---	---	---	---	---	---		**W1**	---
Assessment Window		---	---	---	---	---	---	---	---	**From min of 24 hours to max of 48 hours[b]**	---	---
IV busulfan administration for conditioning[r]				X (test dose)		X	X	X	X			
Serial blood sample collection for TDM (for busulfan dose determination and calculation of the cumulative AUC from Day -4 to Day -1)[s]				X[s]	X[s,t,w]	X	X	X	X	X		
ERT Washout Period												
Switch-stable subject discontinue ERT	At least 2 weeks before the scheduled gene therapy infusion day.											
Treatment-naïve subject discontinue ERT	At least 2 weeks before the scheduled gene therapy infusion day										X	
AVR-RD-02 gene therapy infusion											X	

Study Assessment	Study Period											
	Pre-gene Therapy Infusion											
	Investigational Product Preparation and Testing for Release for Gene Therapy Infusion[a]	Conditioning Phase (including washout period)[b]									Gene Therapy Infusion[c]	Early Termination / Premature Discontinuation[d]
Study Day [D]	---	C D-8	C D-7	C D-6	C D-5	C D-4	C D-3	C D-2	C D-1	Conditioning Washout Period	I D0	---
Study Week [W]	---	---	---	---	---	---	---	---	---		W1	---
Assessment Window		---	---	---	---	---	---	---	---	From min of 24 hours to max of 48 hours[b]	---	---
Continuous Monitoring Procedures												
Prior and concomitant medications and therapies[u]	Continuous monitoring											X
AE[v]	Continuous monitoring											X

Abbreviations: AEs = adverse events; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AMH = anti-Müllerian hormone; ANC = absolute neutrophil count; AST = aspartate aminotransferase; AUC = area-under-the curve; BUN = blood urea nitrogen; C = conditioning; CBC = complete blood cell count; COVID-19 = coronavirus disease 2019; D = day; DBS = dried blood spot; ECG = electrocardiogram; eCRF = electronic case report form; ERT = enzyme replacement therapy; FSH = follicle-stimulating hormone; GCase = glucocerebrosidase; GGT = gamma-glutamyl transferase; GLP = Good Laboratory Practice; I = infusion; INR = international normalized ratio; ISA = insertional site analysis; IV = intravenously; LC-MS = liquid chromatography-mass spectrometry; LDH = lactate dehydrogenase; LH = luteinizing hormone; lyso-Gb1 = glucosylsphingosine; max = maximum; min = minimum; PBL = peripheral blood leukocyte; PK = pharmacokinetics; PTT = partial thromboplastin time; RBC = red blood cell; RCL = replication-competent lentivirus; SAEs = serious adverse events; TDM = therapeutic drug monitoring; VCN = vector copy number; W = week; WBC = white blood cell.

[a] Between the Apheresis and Conditioning phases of the Pre-gene Therapy Infusion Period, there is an approximately 8- to 10-week period during which time AVR-RD-02 investigational product is tested for release for infusion.

[b] The Conditioning phase of the Pre-gene Therapy Infusion Period includes both the conditioning procedures and the conditioning washout period. The washout period must be a minimum of 24 hours and may be up to a maximum of 48 hours after the end of the last busulfan infusion and before AVR-RD-02 infusion. Study days during the Conditioning phase are relative to Day 0 in the Gene Therapy Infusion Period (ie, the day of AVR-RD-02 infusion).

[c] During the Gene Therapy Infusion Period (Day 0), all blood samples for clinical laboratory testing must be collected (and other required assessments performed) prior to AVR-RD-02 gene therapy infusion.

[d] Assessments required for those subjects who prematurely discontinue the study after initiation of the Pre-gene Therapy Infusion Period. Assessments should be completed as soon as feasible after the decision to discontinue the study has been made. If logistic or medical constraints result in delays, the assessments

must be completed within 3 months following the decision to discontinue. After discontinuation, every effort should be made to continue to follow the subject for safety monitoring (including any indication[s] of loss of gene therapy effect).

[e] Blood sample(s) will be analyzed by a central laboratory. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[f] Hemoglobin, hematocrit, and platelet assessments for the efficacy analysis will be tested at a central laboratory. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[g] Vital signs include heart rate, blood pressure, respiratory rate, and temperature. Vital signs will be obtained with the subject supine or seated. On Day 0 of the Gene Therapy Infusion Period (ie, the day of AVR-RD-02 gene therapy infusion), vital signs must be taken pre-gene therapy infusion (window -10 minutes) and post-gene therapy infusion (window +10 minutes). Additional vital signs may be taken if clinically indicated and/or mandated per local institution practice for gene therapy infusion.

[h] The CBC with differential, performed locally, includes the following: hemoglobin, hematocrit, platelet count, WBC count (total and differential), RBC count, and ANC. During the Conditioning washout period, testing should be performed daily. Samples will be tested locally.

[i] Assessment will include routine flow cytometric analysis of T and B cell counts as well as peripheral blood lymphocyte subsets. Panel will include: CD3, CD4, CD8, CD19, and CD16/56. Performed locally.

[j] Serum chemistry includes AST, ALT, GGT, amylase, lipase, ALP, LDH, bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. Electrolytes include sodium, potassium, chloride, bicarbonate, BUN, and serum creatinine. During the Conditioning washout period, testing should be performed daily. Samples will be tested locally.

[k] Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated). Performed locally.

[l] Additional blood sample collection for immunogenicity testing, RCL testing, and ISA may also take place at any time, if clinically indicated.

[m] For measures of reproductive potential in male subjects, sperm samples for sperm count, volume, sperm concentration, total motility, progressive motility, and morphology will be collected. Samples should be collected after at least 5 days to 7 days of abstinence. Samples will be tested locally. For samples that yield abnormal results, an additional sample must be collected and tested to confirm the initial results. Percutaneous epididymal sperm aspiration and hormonal laboratory assessments (eg. testosterone) will also be offered to male subjects (optional assessments), which will be analyzed locally. If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology), investigational sites should follow institutional guidelines for further evaluation. Additional assessments of reproductive potential and/or referral to a urologist is recommended. Early termination assessments of measures of reproductive potential are not required for male subjects who have had a vasectomy and sterility confirmed at the Baseline assessment.

[n] For female subjects, results of the serum pregnancy test on Conditioning Day -8 must be confirmed negative prior to the administration of the test dose of busulfan on Conditioning Day -6.

[o] For females, monthly menstrual calendar (for all menstrual cycles beginning at the Baseline visit) by subject report will be collected at the specified study visits.

[p] Measures of reproductive potential in females include AMH, FSH, and LH for assessment of ovarian reserve, which will be assessed locally. If test results indicate reduced ovarian reserve during the study, investigational sites should follow institutional guidelines for further evaluation of female subjects. Additional assessments of reproductive potential and/or referral to an endocrinologist is recommended.

[q] Levetiracetam will be administered at a dose of 1,000 mg per day beginning 2 days before administration of the busulfan test dose (ie, on Conditioning Day -8) and continuing until 1 day after administration of the 4-day busulfan administration conditioning regimen (ie, until 1 day into the Conditioning washout period). For subjects with a contraindication to levetiracetam, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used. Details on medication administered must be captured in the eCRF.

[r] A conditioning regimen of IV busulfan including a test dose of 0.8 mg/kg on Day -6 will be administered. For sites using LC-MS to perform busulfan TDM, the busulfan test dose will be administered on Day -6. The Day -4 and Day -3 busulfan dose will be PK and weight-guided based on the test dose and the

respective calculated AUC using a web-based dose simulation program. The busulfan doses for Conditioning Days -2 and -1 will be based on an AUC calculation generated from a web-based dose simulation program that uses measurements of busulfan in serial blood samples drawn over several hours (see footnote 's') on Conditioning Days -6 (test dose), -4 (for the Day -2 dose), and -3 (for the Day -1 dose). For sites using the investigational automated nanoparticle immunoassay kit (Saladax Biomedical, Inc.) to perform busulfan TDM, the busulfan test dose will be administered on Day -5. The Day -4 busulfan dose will be PK- and weight-guided based on the test dose and the respective calculated AUC using a web-based dosing simulation program. The busulfan doses for Day -3, Day -2, and Day -1 will be based on an AUC calculation generated from a web-based dose simulation program that will use measurements of busulfan in serial blood samples over several hours (see footnote 't') on Conditioning Day -5 (test dose) and all subsequent Conditioning days prior to that day's dose. If the $AUC_{previous\ dose}$ is outside of ± 0% of the AUC_{target} determined by the program, the busulfan dose will be adjusted accordingly. See Section 8.1.1.3 (and the Study Treatment Manual) for further details on busulfan dose determination.

[s] Blood samples are to be collected 1, 3, 5, and 7 hours after busulfan test dose administration for busulfan TDM and dose determination (see footnote 'r'). Blood samples will be collected 1, 3, and 5 hours after busulfan dose administration on Days -4, -3, -2, and -1 for busulfan TDM for dose determination (ie, Days -4 and -3) and actual cumulative AUC calculations (ie, Days -4, -3, -2 and -1; see also footnote 'r'). Laboratories performing TDM must use validated methods to quantify busulfan in plasma according to GLP.

[t] For sites using LC-MS to perform busulfan TDM, PK sampling may also be performed on Day -5 to support optimization of busulfan exposure.

[u] Prior and concomitant medications and therapies will be recorded from the time of signing of informed consent until completion of the Week 52 assessments for the study.

[v] All AEs (including SAEs and AEs of special interest) will be recorded from the time of signing of informed consent until completion of the Week 52 assessments for the study.

[w] Additional samples for determination of busulfan exposure may be collected.

[x] The COVID-19 test prior to study procedures, including mobilization, apheresis, conditioning and AVR-RD-02 gene therapy infusion, will be conducted no later than 5 days prior to the planned procedure.

15.3 Schedules of Assessments: Post-gene Therapy Infusion Follow-up Period (Study Week 1 [Day 1] through Study Week 52 [Day 365])

Table 6: Schedule of Assessments for the Post-gene Therapy Infusion Follow-up Period (Study Week 1 [Day 1] through Study Week 52 [Day 365])

Study Assessment	Study Period: Post-gene Therapy Infusion Follow-up[b]																	Early Termination / Premature Discontinuation[d]
Study Day [D][a]	D1	D2	D3	D4	D5	D6	D7	D10	D14	D30	D60	D90	D120	D150	D180	D270	D365[c]	---
Study Week [W][a]	W1	W1	W1	W1	W1	W1	W1	W2	W2	W5	W9	W13	W18[y]	W22[y]	W26	W39	W52[c]	---
Assessment Window (Days)	---	---	---	---	---	---	---	± 1D	± 1D	± 3D	± 3D	± 3D	± 3D	± 3D	± 7D	± 7D	±7D	---
Efficacy Assessments[b]																		
Blood sample collection for biomarkers for Gaucher disease (ie, chitotriosidase enzyme activity and lyso-Gb1 plasma level)[e]												X			X	X	X	X
Abdominal MRI[f]															X	X	X	X
Bone MRI[f]															X	X	X	X
Hemoglobin concentration, hematocrit percentage, and platelet count[g]										X	X	X			X	X	X	X
Blood sample collection for GCase enzyme activity in DBS cards										X	X	X	X	X	X	X	X	X
Blood sample collection for GCase enzyme activity in PBLs[e,h]											X[f]	X[f]	X[f]	X[f]	X	X	X	X

Study Assessment	Study Period																	Early Termination / Premature Discontinuation[d]
	Post-gene Therapy Infusion Follow-up[b]																	
Study Day [D][a]	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D10**	**D14**	**D30**	**D60**	**D90**	**D120**	**D150**	**D180**	**D270**	**D365[c]**	---
Study Week [W][a]	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W2**	**W2**	**W5**	**W9**	**W13**	**W18[y]**	**W22[y]**	**W26**	**W39**	**W52[c]**	---
Assessment Window (Days)	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**± 1D**	**± 1D**	**± 3D**	**± 3D**	**± 3D**	**± 3D**	**± 3D**	**± 7D**	**± 7D**	**±7D**	**---**
Blood sample collection for GCase enzyme activity in plasma (processed and frozen at the site)										X	X	X	X	X	X	X	X	X
DXA for BMD determination[i]																	X	X
Leukocytes, plasma, serum and urine sample collection for exploratory biomarker analysis[j]												X			X	X	X	X
SF-36[k]															X		X	X
BPI-SF[h,k]											X	X	X	X	X	X	X	X
Blood sample collection for average VCN determination[e]										X	X	X			X	X	X	X
Bone marrow aspirate collection for average VCN determination[e]																	X	

Study Assessment	Study Period																	Early Termination / Premature Discontinuation[d]
	Post-gene Therapy Infusion Follow-up[b]																	
Study Day [D][a]	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D10**	**D14**	**D30**	**D60**	**D90**	**D120**	**D150**	**D180**	**D270**	**D365[c]**	---
Study Week [W][a]	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W2**	**W2**	**W5**	**W9**	**W13**	**W18[y]**	**W22[y]**	**W26**	**W39**	**W52[c]**	---
Assessment Window (Days)	---	---	---	---	---	---	---	± 1D	± 1D	± 3D	± 3D	± 3D	± 3D	± 3D	± 7D	± 7D	±7D	---
Safety Assessments[b]																		
Physical examination	X[l]	X	X	X	X	X	X			X		X			X	X	X	X
Weight	X									X					X	X	X	X
Vital signs[m]	X[l]	X	X	X	X	X	X			X		X			X	X	X	X
12-lead ECG															X	X	X	X
CBC with differential[n]	X[m]	X	X	X	X	X	X	X	X	X	X	X[f]	X[f]	X[f]	X	X	X	X
G-CSF injection[o]										G-CSF if ANC is < 0.5×10^9/L								
Blood sample collection for T and B cell counts[p]									X	X	X	X			X	X	X	X
Serum chemistry[q]							X		X	X	X				X	X	X	X
Electrolytes, BUN, and serum creatinine[q]	X[m]	X	X	X	X	X	X		X	X	X	X			X	X	X	X
Urinalysis[r]										X	X				X	X	X	X
Blood sample collection for immunogenicity testing[e,s]										X		X			X	X	X	X
Peripheral blood sample collection for RCL testing[e,s]												X			X	X	X	X

Study Assessment	Study Period																	Early Termination / Premature Discontinuation[d]
	Post-gene Therapy Infusion Follow-up[b]																	
Study Day [D][a]	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D10**	**D14**	**D30**	**D60**	**D90**	**D120**	**D150**	**D180**	**D270**	**D365[c]**	---
Study Week [W][a]	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W2**	**W2**	**W5**	**W9**	**W13**	**W18[y]**	**W22[y]**	**W26**	**W39**	**W52[c]**	---
Assessment Window (Days)	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**± 1D**	**± 1D**	**± 3D**	**± 3D**	**± 3D**	**± 3D**	**± 3D**	**± 7D**	**± 7D**	**±7D**	**---**
Blood sample collection for ISA[e,s]										X	X	X			X	X	X	X
Measures of reproductive potential (males)[t]																	X	X
Monthly menstrual calendar collection (females)[u]										X	X	X			X	X	X	X
Measures of reproductive potential (females)[v]															X		X	X
Continuous Monitoring Procedures																		
Prior and concomitant medications and therapies[w]	Continuous monitoring																	X
Adverse events[x]	Continuous monitoring																	X

Abbreviations: AEs = adverse events; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AMH = anti-Müllerian hormone; ANC = absolute neutrophil count; AST = aspartate aminotransferase; BID = twice a day; BMD = bone mineral density; BPI-SF = Brief Pain Inventory-Short Form; BUN = blood urea nitrogen; CBC = complete blood cell count; D = Day; DXA = dual-energy X-ray absorptiometry; ECG = electrocardiogram; ERT = enzyme replacement therapy; FSH = follicle-stimulating hormone; GCase = glucocerebrosidase; G-CSF = granulocyte-colony stimulating factor; GGT = gamma-glutamyl transferase; ISA = insertional site analysis; LDH = lactate dehydrogenase; LH = luteinizing hormone; lyso-Gb1 = glucosylsphingosine; MRI = magnetic resonance imaging; PBL = peripheral blood leukocyte; RBC = red blood cell; RCL = replication-competent lentivirus; SAEs = serious adverse events; SF-36 = 36-Item Short Form Health Survey; VCN = vector copy number; W = week; WBC = white blood cell.

[a] In the Post-gene Therapy Infusion Follow-up Period, study days (and weeks) are relative to Day 0 in the Gene Therapy Infusion Period (ie, the day of AVR-RD-02 gene therapy infusion).

[b] During the Post-gene Therapy Infusion Follow-up Period, additional assessments may be performed at any time for clinical management or safety concerns at the discretion of the Investigator.

[c] The Week 52 (End of Study; Day 365) visit may be conducted over the course of up to 3 days.

[d] Assessments required for those subjects who prematurely discontinue the study. Assessments should be completed as soon as feasible after the decision to discontinue the study has been made. If logistic or medical constraints result in delays, the assessments must be completed within 3 months following the decision to discontinue. After discontinuation, every effort should be made to continue to follow the subject for safety monitoring (including any indication[s] of loss of gene therapy effect).

[e] Blood sample(s) and bone marrow aspirate will be analyzed by a central laboratory. Additional blood sample and bone marrow aspirate must also be collected before initiation and cessation of ERT, when applicable. Additional blood samples will be used for the following tests: GCase enzyme activity, biomarkers for Gaucher disease, VCN, ISA, when applicable, immunogenicity, and RCL. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[f] Abdominal and bone MRI scans will be performed locally and interpreted by a blinded central reader. Details on collection, processing, and shipment will be included in an Imaging (or other relevant) Manual for the study.

[g] Hemoglobin, hematocrit, and platelet assessments for efficacy analysis will be tested at a central laboratory. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[h] All subjects whose GCase activity falls 15% of the lower limit of the range observed in healthy individuals at Week 9 (Day 60 / Month 2), Week 13 (Day 90 / Month 3), Week 18 (Day 120 / Month 4), and Week 22 (Day 150 / Month 5) (see Section 7.4.6) will be monitored. At Week 13 (Month 3), subjects who have not restarted ERT and meet the following clinical parameters: blood GCase activity > 15% and hemoglobin concentration and platelet count ≥ 80% of the baseline value, will not be required to undergo clinical assessments at Week 18 (Month 4) or Week 22 (Month 5). These subjects will resume their clinical assessments with the 6-month post-gene therapy infusion visit.

[i] The DXA will be performed and interpreted locally.

[j] Samples to be collected and stored for possible future testing. Details on collection, processing, and shipment will be included in the Laboratory Manual for the study.

[k] The SF-36 and BPI-SF should be completed prior to any other required assessments at the study visit.

[l] At a minimum, physical examinations, vital signs collection, and blood sample collection for CBC with differential and electrolytes must occur daily through a minimum of 7 days post-gene therapy infusion (ie, until Day 7 of the Post-gene Therapy Infusion Follow-up Period). If clinically indicated and/or mandated per local institution practice post-gene therapy infusion, additional physical examinations, vital signs collection, and blood sample collection for CBC with differential and electrolytes should also take place.

[m] Vital signs include heart rate, blood pressure, respiratory rate, and temperature, and will be obtained with the subject supine or seated.

[n] CBC with differential performed locally includes the following: hematocrit, hemoglobin, platelet count, WBC count (total and differential), RBC count, and ANC count. If ANC is $\leq 1.5 \times 10^9$/L on Day 5 of the Post-gene Therapy Infusion Follow-up Period, blood sample for ANC should continue to be collected (and G-CSF injection administered daily [see footnote 'o']) until the subject achieves an ANC $> 1.5 \times 10^9$/L. During and after completion of daily testing for ANC post-gene therapy infusion, blood sample for hemoglobin and platelet count must continue to be collected in accordance with the Schedule of Assessments for these clinical laboratories collected for efficacy.

[o] A G-CSF injection of 5 µg/kg body weight (for sites with only prefilled syringes, a G-CSF dose from 4 µg/kg body weight up to 6 µg/kg body weight, administered in either a single or divided BID dose) will be considered in case a subject's ANC is $< 0.5 \times 10^9$/L at Day +30 post-gene therapy infusion and onwards. G-CSF may be considered earlier if clinically needed in consultation with the PI and Sponsor Medical Monitor.

[p] Assessment will include routine flow cytometric analysis of T and B cell counts as well as peripheral blood lymphocyte subsets. Panel will include: CD3, CD4, CD8, CD19, and CD16/56. Performed locally.

[q] Serum chemistry includes AST, ALT, GGT, amylase, lipase, ALP, LDH, bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. Electrolytes include sodium, potassium, chloride, bicarbonate, BUN, and serum creatinine. Performed locally.

[r] Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated). Performed locally.

[s] Additional blood sample collection for immunogenicity testing, RCL testing, and ISA may also take place at any time, if clinically indicated.

[t] For measures of reproductive potential in male subjects, sperm samples for sperm count, volume, sperm concentration, total motility, progressive motility, and morphology will be collected. Samples should be collected after at least 5 days to 7 days of abstinence. Samples will be tested locally. For samples that yield abnormal results, an additional sample must be collected and tested to confirm the initial results. Percutaneous epididymal sperm aspiration and hormonal laboratory assessments (eg, testosterone) will also be offered to male subjects (optional assessments), which will be analyzed locally. If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology), investigational sites should follow institutional guidelines for further evaluation. Additional assessments of reproductive potential and/or referral to a urologist may be recommended. End-of-treatment/early termination assessments of measures of reproductive potential are not required for male subjects who have had a vasectomy and sterility confirmed at the Baseline assessment.

[u] For females, monthly menstrual calendar (for all menstrual cycles beginning at the Baseline visit) by subject-report will be collected at the specified study visits.

[v] Measures of reproductive potential in females include AMH, FSH, and LH for assessment of ovarian reserve, which will be assessed locally. If test results indicate reduced ovarian reserve during the study, investigational sites should follow institutional guidelines for further evaluation of female subjects. Additional assessments of reproductive potential and/or referral to an endocrinologist is recommended.

[w] Prior and concomitant medications and therapies will be recorded from the time of signing of informed consent until completion of the Week 52 assessments for the study.

[x] All AEs (including SAEs and AEs of interest) will be recorded from the time of signing of informed consent until completion of the Week 52 assessments for the study.

[y] Remote visits may be conducted in lieu of clinic visits for the following protocol-scheduled visits: Week 18 (Day 120) and Week 22 (Day 150). The site will coordinate the collection of blood samples. If protocol-scheduled clinic visits are no longer possible, the Investigator must consult with the Sponsor Medical Monitor to determine the best course of action which could include remote visits.

15.4 Enrollment (and Data Monitoring Committee Review) Schedule

Table 7: Enrollment (and DMC Review) Schedule

Dosing Cohort[a]	No. of Subjects	Safety Review by DMC?[b]	Safety Data Reviewed by DMC	DMC Review Outcome and Study Impact
Switch-stable Subjects				
1	1	Yes	Safety data through the Week 5 (Day 30) follow-up assessments for 1 subject	If review of safety data by the DMC determines that enrollment may proceed, the mobilization therapy for a switch-stable subject enrolled in dosing cohort 2 may begin. If safety concerns are identified by the DMC, the DMC will provide recommendations to AVROBIO. Dosing and enrollment may proceed on the basis of these recommendations or following remediation as set forth in the DMC Charter.
2	1	Yes	Safety data through the Week 5 (Day 30) follow-up assessments for the subject in safety cohort 2 (all available data from the subject enrolled in dosing cohort 1 will also be provided)	If review of safety data by the DMC determines that enrollment may proceed, the mobilization therapy for switch-stable subjects enrolled in dosing in cohort 3 may begin without further restriction. In addition, mobilization therapy for a treatment-naïve subject enrolled in dosing cohort 4 may begin. If safety concerns are identified by the DMC, the DMC will provide recommendations to AVROBIO. Dosing and enrollment may proceed on the basis of these recommendations or following remediation as set forth in the DMC Charter.
Treatment-naïve Subjects				
4	1	Yes	Safety data through the Week 5 (Day 30) follow-up assessments for 1 subject	If review of safety data by the DMC determines that enrollment may proceed, the mobilization therapy for a treatment-naïve subject enrolled in dosing cohort 5 may begin. If safety concerns are identified by the DMC, the DMC will provide recommendations to AVROBIO. Dosing and enrollment may proceed on the basis of these recommendations or following remediation as set forth in the DMC Charter.

Dosing Cohort[a]	No. of Subjects	Safety Review by DMC?[b]	Safety Data Reviewed by DMC	DMC Review Outcome and Study Impact
5	1	Yes	Safety data through the Week 5 (Day 30) follow-up assessments for the subject in safety cohort 5 (all available data from the subject enrolled in dosing cohort 4 will also be provided)	If review of safety data by the DMC determines that enrollment may proceed, the mobilization therapy for treatment-naïve subjects enrolled in dosing in cohort 6 may begin without further restriction. If safety concerns are identified by the DMC, the DMC will provide recommendations to AVROBIO. Dosing and enrollment may proceed on the basis of these recommendations or following remediation as set forth in the DMC Charter.

Abbreviation: DMC = Data Monitoring Committee.

[a] Enrollment will continue up to the maximum planned enrollment for the study that considers both switch-stable and treatment-naïve subjects.

[b] The DMC reviews included are those planned for making continued dosing and study enrollment decisions. Additional DMC reviews may also take place in accordance with the protocol and DMC Charter established for the study.